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Integrated strategy to prevent mycotoxin risks (Inspyr)

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1. Abstract

Fusarium head blight (FHB) poses an increasing threat to wheat and barley crops in the UK. Under high disease pressure, current varieties are unable to restrict accumulation of deoxynivalenol (DON) mycotoxin to below EU thresholds. This project set out to facilitate incorporation of FHB resistance into UK varieties to reduce/eliminate the risk of trichothecene mycotoxins entering the food chain and enhance production efficiency. The project aimed to identify and characterise new sources of FHB resistance in wheat and barley and determine whether it is possible to break the association between the semi-dwarfing gene (*Rht2*) and susceptibility to FHB. The project also investigated the potential of an integrated approach combining host resistance with fungicides to further reduce the risk of mycotoxin accumulation on grain.

The presence of awns appears to increase FHB resistance. This finding is significant because almost all UK wheat varieties lack awns and this may, in part, account for the overall high level of susceptibility in UK wheat varieties.

Most UK varieties carry the semi dwarfing gene (*Rht2*). The presence of this gene has long been associated with susceptibility to FHB. The Inspyr project revealed that the effect is due, not to the Rht2 gene itself, but to a nearby gene. Using markers located near the susceptibility gene, it is now possible for breeders to select lines of the desired height for UK conditions but that lack the FHB susceptibility factor. This should provide a very rapid means to improve the overall FHB resistance of UK wheat varieties.

The Inspyr project demonstrated that an integrated approach of growing FHB resistant varieties and treating with appropriate fungicides at the time of flowering provides a means to reduce the risk of DON accumulation in grain exceeding EU thresholds. This offers an approach to maintain crop and consumer health, even under conditions of high disease pressure.

While in wheat, greater resistance to FHB is often associated with greater plant height this does not appear to be the case for barley. A potent FHB resistance was identified in the heritage barley variety Chevallier that functions independently of plant height.

The findings of the Inspyr project offer plant breeders and growers a number of ways in which to improve the overall FHB resistance of UK wheat and barley varieties and to reduce the risk of mycotoxin accumulation in grain in their crops, even when exposed to high disease pressure.

Further work will be required to find the optimum number and type of FHB resistances required to work alongside the available fungicides to eliminate the risk posed by FHB in most circumstances. Additional work will also be required to identify how the resistances interact with different fungicide chemistries to provide optimal control.

2. Key messages for Levy Payers

- This project focussed on gaining improved understanding of the basis of fusarium head blight resistance in wheat and barley in order to provide information to plant breeders to assist them in their efforts to improve the levels of FHB resistance in these crops. A parallel intention was to determine whether host resistance could combine with fungicide chemistry to provide a genuinely integrated approach to tackling the problem of FHB and associated contamination of grain with mycotoxins.

- New sources of resistance to FHB were identified in wheat and barley and selected sources were characterised to identify regions associated with reduced FHB and mycotoxin contamination.

- In wheat, a potent FHB resistance was identified that is associated with the presence of awns. This resistance appears to reduce the ability of the fungi to infect the heads. It is not known whether this effect is due to altered morphology which might affect the microclimate at the surface of the head. Further research will be required to establish whether this effect is due to the gene controlling the presence of awns or to nearby linked genes.

- FHB resistance in wheat is often associated with plant height and we examined whether this is also the case in barley. The heritage barley variety 'Chevallier' is tall and highly resistant to FHB whereas NSF Tipple is short and susceptible to FHB. Most of the difference in height is due to a gene on chromosome 3H whereas much of the effect on FHB resistance is due to gene(s) on chromosome 6H. With this knowledge breeders should be able to produce short barley varieties with high levels of FHB resistance.

- The *Rht2* semi-dwarfing gene (now known as *RhtD1b*) has been used extensively in production of UK varieties. Previous study had highlighted the link between the gene *Rht2* and susceptibility to FHB. Through careful study of the region around *Rht2* we determined that the susceptibility is not due to the *Rht2* gene itself but to a gene nearby (linkage). Molecular markers were produced to allow breeders to select lines carrying the desired *Rht2* gene but lacking the FHB susceptibility. This breakthrough should remove one of the major obstacles to the improvement of FHB resistance in UK wheat.

- Association genetic analysis of a large collection of two-row barley varieties revealed several regions associated with resistance to FHB. While some of these were effective in most environments, several appeared only to be effective in particular years or locations. The

distribution of loci across the barley genome should enable breeders to select and combine the most potent of the effects to significantly improve the overall FHB resistance of barley varieties.

- Application to wheat heads of fungicide with activity against FHB at an optimal timing was shown repeatedly to reduce mycotoxin content (DON) by approximately 50%. The effect of fungicide application complemented that of Type 2 resistance (resistance to spread) in the host while having little effect on wheat lines with higher levels of Type 1 resistance. Under conditions of high disease pressure, the application of fungicide to lines with high levels of Type 2 resistance (carrying multiple Type 2 resistances) could reduce DON content of grain to below the EU threshold. An integrated approach to FHB control through increased host resistance combined with application of appropriate fungicides offers the opportunity to significantly reduce losses due to DON contamination levels exceeding EU thresholds.

3. SUMMARY

3.1. Background

Fusarium head blight (FHB) of cereals poses an increasing threat to the UK wheat and barley crops. New *Fusarium* species have appeared and spread in the UK, the most significant of which are *Fusarium graminearum* and *F. langsethiae.* The appearance of the former is most probably due to the recent increase in maize production in the UK (West et al 2012a). *Fusarium graminearum* can infect maize and produces fungal structures (perithecia) from which it releases airborne conidia (ascospores) that contaminate subsequent wheat and barley crops. Future predicted climate changes are likely to exacerbate risks of epidemics in the UK (West et al 2012b). In addition to yield loss associated with FHB the disease is of particular concern because the *Fusarium* species produce a spectrum of trichothecene mycotoxins (DON, NIV, T2 and HT-2) within grain that are harmful for human and animal consumption.

FHB reached its highest recorded level in the UK in 2007 with 86% of samples from the Defra 'CropMonitor' project containing FHB pathogens. In 2008, although the percentage of samples containing FHB pathogens was reduced (64%) the delayed harvest due to rain promoted mycotoxin accumulation.

Yield loss relationships that were established during a previous AHDB Cereals & Oilseeds funded project on fusarium epidemiology can be used to calculate national losses due to FHB. In 2008, UK wheat production was 17.5 million tonnes. Additional losses are incurred through the contamination of grain with mycotoxins. Approximately 15% of wheat samples within the 2008 survey exceeded the EU limit for DON while 30% exceeded the limit for another *Fusarium* mycotoxin, zearalenone. These levels make the grain unsuitable for human consumption. For milling wheat in 2008 the DON contamination would potentially have led to 927,000 tonnes of grain being unsuitable for processing.

In an epidemic year, with an average of 5% ear disease nationally the expected losses would be in the region of £130 million for wheat at £150 per tonne. This does not include the cost of fungicide application or the losses due to reduced quality. The requirement to test for mycotoxins also incurs a very significant additional cost to producers and processors.

It is widely recognised that resistant varieties offer the best option to control FHB. All wheat and barley breeders consider it as a major but difficult target for resistance breeding. Incorporation of high levels of resistance to FHB into wheat and barley will be critical to prevent DON, T2, HT-2 and nivalenol (NIV) mycotoxin contamination of grain from becoming a major problem for all elements of the UK food and feed chains.

Two forms of fusarium head blight (FHB) resistance are well recognised: Type 1 (resistance to initial infection) and Type 2 (resistance to spread within the head) (Schroeder and Christensen, 1963). Evidence suggests that Type 2 resistance counters the effect of deoxynivalenol (DON) mycotoxin. Mutants of *F. graminearum* that cannot produce DON are still able to infect wheat heads but are not able to spread within the head (Bai et al., 2001). This suggests that DON is not required for pathogenicity but acts as a virulence factor to overcome Type 2 resistance of wheat and enhances the ability of the fungus to colonise the host.

Different trichothecene mycotoxins have significantly different toxicities towards wheat. Whereas DON is highly phytotoxic, others such as NIV, T-2 and HT-2 are much less potent against wheat (Eudes, 2000; Shimada, 1990). Different cereals also differ in susceptibility to these toxins. Barley is much more resistant to DON than wheat and exhibits inherently high levels of Type 2 resistance. Thus, differential FHB resistance among barley varieties is due to differences in Type 1 resistance.

A previous AHDB Cereals & Oilseeds-supported LINK project (Nicholson, 2008) showed that Type 2 resistance in wheat is of relevance mainly to DON-producing isolates of only some FHB-causing species. In contrast, Type 1 resistance should be effective against all FHB species including producers of T-2 and HT-2. Type 1 resistance is thus relevant to both wheat and barley and in relation to disease caused by all the species responsible for FHB. Integrated strategy to prevent mycotoxin risks (INSPYR) focussed on so-called Type 1 resistance that should provide resistance towards all *Fusarium* species producing different mycotoxins as well as non toxin-producing pathogens such as *Microdochium* species. The genetic analyses set out to identify, characterise and localise the FHB resistance genes in novel germplasm. The ongoing programme to identify new resistance sources provides valuable resources for future breeding and research.

Nicholson (2008) in REFAM project showed that most current UK wheat varieties are highly susceptible to FHB with little genetic variation for resistance. Much of the susceptibility of UK varieties may be due to the presence of the *Rht2* semi-dwarfing gene which was in almost all UK varieties at that time. This gene has frequently been associated with higher levels of susceptibility to FHB (Srinivasachary et al 2008, 2009; Lu et al 2011). It was not known whether this effect was due to a pleiotropic effect of the semi-dwarfing gene itself or due to linkage with a gene conferring

increased susceptibility to FHB. If it is the latter then this association must be broken to enable breeders to produce FHB resistant varieties with acceptable agronomic characters.

As stated above, UK wheat and barley varieties are highly or moderately susceptible to FHB. No commercially significant variety is sufficiently resistant to remove the need for fungicide application to control the species responsible for FHB under conditions conducive for infection. Timely application with appropriate triazole fungicides can restrict disease development and mycotoxin accumulation. Under moderate to high disease pressure, however, fungicide application often fails to reduce DON contamination to below EU legislative limits in susceptible varieties such as those currently grown in the UK.

An integrated approach, based on varieties with significantly enhanced resistance and appropriate fungicide application may provide the best means to achieve sustainable control of FHB and minimise the risk of mycotoxins entering the food and feed chains.

3.2. Project overall aim:

- To identify and characterise new sources of resistance in wheat and barley that act against all the species that cause FHB by preventing initial infection (type 1 resistance).
- To facilitate incorporation of FHB resistance into UK varieties to reduce/eliminate the risk of trichothecene mycotoxins (DON, NIV, T-2, HT-2) entering the food chain and enhance production efficiency.
- To develop an integrated and sustainable strategy to control FHB and minimise mycotoxin risks through the use of resistant varieties combined with appropriate fungicide application.

The project was divided into five work packages to reflect the specific objectives:

1) Identification of FHB resistance in wheat and barley with emphasis on Type 1 resistance.
2) Exploitation of synteny to break the linkage between FHB susceptibility and *Rht2* (*Rht-D1b*) locus.
3) Identification and mapping of FHB resistance quantitative trait loci (QTL) in barley by association genetics.
4) Identification of the optimal integration of host resistance and fungicides to control FHB and mycotoxin accumulation.
5) Fine mapping of the Type 1 FHB resistance on chromosome 4AS of *Triticum macha*.

3.3. Anticipated benefits:

The INSPYR project is intended to benefit the industry, through assisting breeders to more effectively develop varieties with resistance to all FHB species by expanding knowledge of the genetics of

resistance. Widespread cultivation of FHB resistant varieties will also reduce the need for highly expensive mycotoxin testing by millers and maltsters.

Growers will benefit through new highly resistant varieties with desirable agronomic characteristics (e.g. semi-dwarf). These will combine increased yield with reduced inputs to significantly improve efficiency and reduce the carbon footprint of the industry.

Most importantly, the INSPYR project will provide information on resistance that is relevant to both wheat and barley, to all FHB pathogens and to attempts to limit the accumulation of DON, T-2 and HT2 mycotoxins in grain.

This project will provide new knowledge about the genetics of resistance and sources of resistance that can be applied immediately in plant breeding programmes in the UK and Europe.

The project will also produce molecular markers to the region about *Rht2* allowing breeders to maintain this agronomically important allele in their breeding programmes while selecting against the putative linked FHB susceptibility factor.

This project will demonstrate how different forms of disease resistance (Type 1 and 2) interact with appropriate fungicide application to reduce disease and mycotoxin levels.

3.4. Work package 1: Identification of FHB resistance in wheat and barley with emphasis on Type 1 resistance

3.4.1. Background

Resistance of wheat to fusarium head blight (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 2 resistance (Anderson et al, 2001; Buestmayr et al, 2002; Shen et al, 2003a, 2003b) but relatively few studies have identified QTL for Type 1 resistance (Buerstmayr et al, 2002; Steiner et al, 2004; Steed et al, 2005). This may reflect a paucity of Type 1 resistance in the germplasm under study, but it is also probable that the need to infer Type 1 resistance hampers the identification of this form of resistance.

In a previous AHDB Cereals & Oilseeds supported LINK project (Project Report 432), a total of 300 lines from the FHB breeding programme of Dr. Maarten van Ginkel at the International Maize and Wheat Improvement Center (CIMMYT), Mexico were screened in field trials by inoculating with a DON-producing isolate of *F. graminearum* to identify lines carrying high levels of combined Type 1 and Type 2 resistance. Sixty of the most resistant lines were further characterised to determine the relative contribution of Type 1 and Type 2 resistances in each of them. This was achieved by spraying plants with either a DON producing isolate of *F. culmorum* or a NIV producing isolate of *F. graminearum* in field trials, and by point inoculation of spikelets using a DON producing isolate of *F. graminearum* in a polytunnel experiment (Nicholson et al. 2008). From these experiments three lines 'CIMMYT112' (TX90D9277/PB812), 'CIMMYT 186' (pedigree unknown) and 'CIMMYT 251' (Vorona x (Kauz x Vorona)) were determined to have very high levels of Type 1 and Type 2 FHB resistance. The relative resistances of the three selected CIMMYT lines are shown alongside those of the moderately FHB susceptible spring wheat cultivar Paragon and a 'synthetic wheat' line SHW144 (Figure 4.1). These three lines were crossed to Paragon the prior to the start of the project to produce F_1 seed from which to develop mapping populations through single seed decent.

FHB is also a major disease of barley where the mycotoxin-producing *Fusarium* species cause yield loss and pose a potential health risk to organisms that consume mycotoxin-contaminated grain. Like wheat, no single barley cultivar currently used in breeding programs demonstrates consistent resistance. In contrast to wheat, barley does not exhibit spread of symptoms through the head following infection. Barley has inherently high levels of Type 2 resistance and inter-varietal differences in susceptibility to FHB are due to differences in levels of Type 1 resistance. Previous study (Nicholson, unpublished) demonstrated that Chevallier, a heritage malting barley variety exhibits significant resistance to FHB in marked contrast to the relatively high FHB susceptibility of the modern malting variety NFC Tipple. Chevallier is a very tall variety in contrast to the short variety NFC Tipple. Resistance to FHB in both barley and wheat has been linked to height, with taller varieties generally being more resistant. However increased plant height can lead to lodging, creating a trade-off between disease resistance and agronomic traits. Our previous study also indicated that the variety Armelle, a moderately tall variety, exhibits a high level of FHB resistance. The relative resistances of Chevallier and Armelle are shown alongside those of the FHB susceptible spring barley cultivar and NFC Tipple in Figure 4.2.

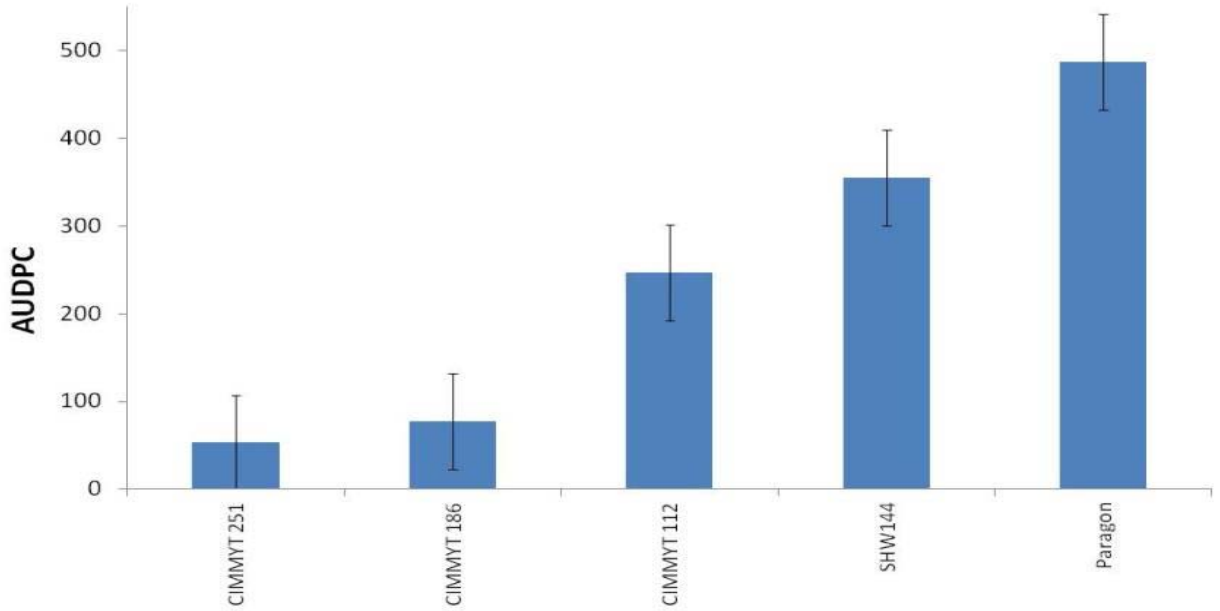

Figure 4.1 Fusarium head blight disease scores (area under disease progress curve) of the three CIMMYT lines selected for analysis alongside the moderately FHB susceptible parent variety Paragon.

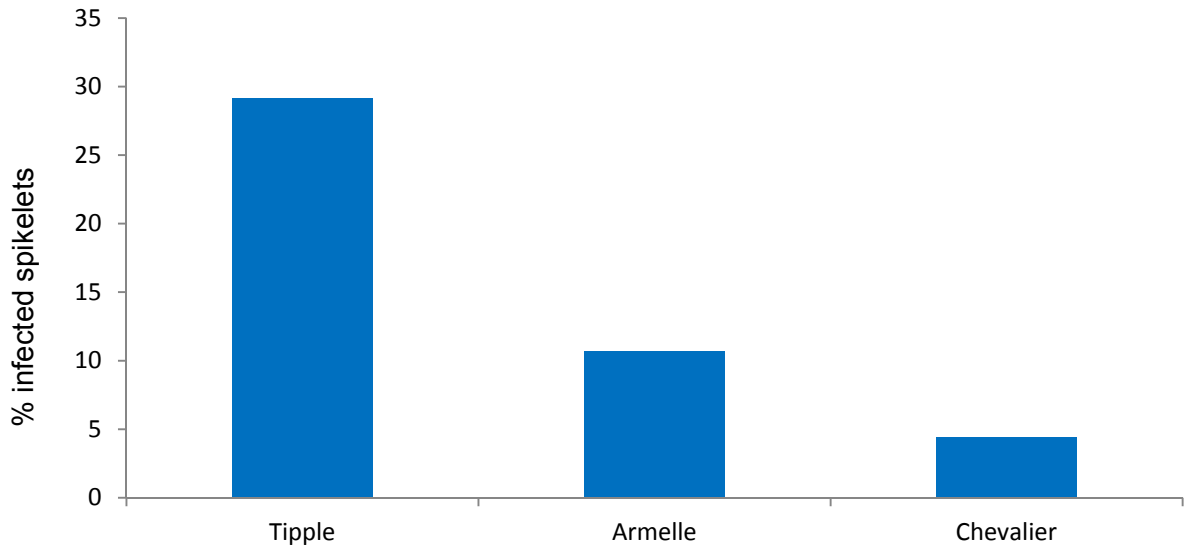

Figure 4.2 Fusarium head blight disease (% infected spikelets) on the FHB susceptible parent variety NFC Tipple alongside Armelle and Chevallier.

3.4.2. Population development

F_1 seed of the bi-parental cross materials for the five populations was provided to collaborating breeding companies for progression by single seed decent, to produce lines for screening of FHB disease resistance and evaluation of agronomic traits. Each company progressed material to produce seed stocks of the F_5 generation for field trials and FHB disease screening due to take place in the 2012–2013 growing season. Lantmännen SW Seed, Sweden produced almost 300 F_5 lines of

the CIMMYT251 x Paragon population (CIM251). Sejet Plantbreeding, Denmark produced in excess of 150 F_5 lines of the CIMMYT112 x Paragon population (CIM112). Secobra, France produced 197 F_5 lines of the CIMMYT186 x Paragon population (CIM186).

Bi-parental crosses were made between the short modern malting cultivar NFC Tipple and two tall older varieties to determine whether the FHB resistance of Chevallier and Armelle are associated with, or independent of, height. KWS UK Ltd. produced 233 F_5 lines of the Chevallier crossed to the Tipple population (CxT) and Syngenta Seeds Ltd produced 250 F_5 lines of the Armelle crossed to the Tipple population (AxT). Where sufficient seed was available lines were included in FHB disease trials screened in 2013.

3.4.3. FHB trials

The CIMMYT251 line exhibited the highest level of FHB resistance (Figure 4.1) and had greatest amount of seed produced of the three wheat populations. For this reason the field trials were focussed to obtain repeated observation of FHB resistance of this population across several environments (three sites).

The CIM251 recombinant inbred lines (RILs) (200 RILs) and the two parents were phenotyped in three experiments in three different environments in 2013: John Innes Centre, Norwich, UK (JIC); National Institute of Agricultural Botany, Cambridge, UK (NIAB), and Lantmännen SW Seeds, Laberweinting, Germany (SW). These experiments were all conducted in 2013. The trials at JIC and NIAB were both arranged in two blocks, each block containing one plot (1m x 3m) of each single seed decent (SSD line and two plots of each parent line. Both JIC and NIAB trials were inoculated with a highly virulent DON-producing *F. culmorum* isolate (Fu42) at mid-anthesis and mist irrigated to maintain high humidity. The SW trial was conducted in two blocks, each block containing one plot of each SSD line and eight plots of each of the parent lines. This trial was drilled in maize-stubble after minimum tillage in an historically disease conducive environment in southern Germany. No artificial inoculum or irrigation was applied to the SW trial. Disease was scored on a 1–9 scale on two occasions and a mean was taken across the two scoring dates. In the JIC trial, plant height (cm), time to mid-anthesis (days since drilling) and presence or absence of awns, were also recorded.

The 202 line F_5 CxT population developed by KWS was sown in a whole plot single replicate design (approx. 5–9g seed per plot), containing 18 randomised controls (NFC Tipple, Chevallier, Golden Promise, Paragon and Cadenza) at the John Innes Centre field trial site in 2013. The population was scored for phenotypic traits (height, heading date) and sprayed six to seven times from mid-anthesis with *F. culmorum* conidial suspension and scored for FHB at four time points. A further FHB trial containing the 202 CxT F_5 lines in a three row split plot design was sown in spring 2013 at the KWS, Cambridge site. Phenotype data on height and heading date was also collected.

A large proportion (198 lines) of the AxT population was sown at the National Institute of Agricultural Botany, Cambridge, UK (NIAB), in 2013. The trial was arranged in split-plots, each block containing one plot (1m x 3m) of each SSD line. Plants were inoculated and scored as described above for NIAB wheat trials using the DON-producing *F. culmorum* isolate (Fu42).

3.4.4. Marker analysis

The parent lines of the CIM251 population were screened using a wheat Single Nucleotide Polymorphism (SNP) panel of over 5,000 validated SNP assays (Allen et al., 2011; Allen et al., 2013). Polymorphic markers were selected to provide an even coverage of the 21 wheat chromosomes based on map positions in the Avalon x Cadenza and Savanah x Rialto populations (Allen et al., 2013; Wilkinson et al., 2012) and applied to the lines of the CIM251 RIL population by the JIC Genotyping Service.

The CxT and AxT populations were genotyped at the James Hutton Institute using the BeadXpress system. Genetic maps were constructed for the CIM251 population with KASP-SNP markers and for the CxT and AxT populations with the BeadXpress markers using the JoinMap software. The presence (CIMMYT 251) or absence (Paragon) of awns segregated 57%: 43% and was therefore included as a marker in the map construction.

3.4.5. FHB analyses and QTL identification in CIMMYT 251 and Paragon

A highly significant FHB resistance QTL originating from Paragon was detected on chromosome 2D in both the JIC trial and in the SW trial, but not in the NIAB trial. The JIC 2D QTL was located in a similar location to the SW 2D QTL suggesting that they represent the same genetic effect (Figure 3.3). The location of these QTL is coincident with a highly significant flowering time effect at the *Ppd-D1* locus, with early flowering originating from CIMMYT 251, which has the *Ppd-D1a* (early) allele. In addition, a weak height effect was identified in this region, also which may be due to the semi-dwarfing gene *Rht8*, which is linked to *Ppd-D1* on chromosome 2D (Gasperini et al., 2012).

An FHB resistance QTL of large effect was identified on the distal portion of chromosome 5AL in both JIC and NIAB trial with resistance conferred by CIMMYT 251. These effects were identified in similar genetic locations with the peaks only 3.4 cM apart suggesting that these represent the same QTL. This QTL was associated with the presence of awns but was not coincident with any other agronomic traits in the present study suggesting that this resistance is not conferred by a height or flowering time effect. However, it was not possible to detect this QTL in the SW trial.

As anticipated, a plant height QTL of large effect was identified on chromosome 4B and centred on the diagnostic marker for *RhtB1*. Reduced height originated from CIMMYT 251, which contains the *Rht1* (*RhtB1b*) semi-dwarf allele. No significant QTL for FHB traits were detected at this locus in any

of the three trials. Additional minor height QTL were detected on chromosomes 6A and 7D, but no coincident QTL were detected for FHB traits in these regions.

3.4.6. FHB analyses and QTL identification in Chevallier x NFC Tipple and Armelle x NFC Tipple populations

High levels of disease developed at the JIC site whereas much lower levels of disease were recorded at the KWS site. A QTL for FHB resistance of major effect was identified at the JIC site originating from Chevallier and located on the long arm of chromosome 6H. No QTL for FHB resistance were identified at the KWS site. A QTL identified at both JIC and KWS and originating from NFC tipple had a very large effect on both heading date and plant height. This QTL was located on chromosome 3H in the location of the *Denso* dwarfing gene (Laurie et al., 1993).

No QTL of major effect for FHB resistance were detected in the AxT population despite the large difference in FHB susceptibility of the two parents. A QTL for FHB resistance of minor effect was identified in the AxT population and was associated with chromosome 3H in the location of the *Denso* dwarfing gene with NFC Tipple contributing the more susceptible allele. This result suggests that, although no effect of this locus was observed in the CxT population where the effect of the 6H QTL was dominant, the *Denso* allele does confer a small negative effect on FHB resistance.

3.4.7. Discussion

The data from the CIM251 population indicates that the presence of awns contributes towards resistance to FHB. Interestingly, additional study at NIAB supported the view that the FHB resistance at this locus is of Type 1 as they determined that it was effective against *Microdochium majus*, a species that does not spread within the head (P. Howell, personal communication). It is not clear whether the effect on FHB resistance is due to the awns themselves or perhaps to a gene near the locus that controls awning. Some research (Meterhazy, 1995) indicates that the presence of awns might alter the microclimate about the wheat head and increase susceptibility to FHB through creating an environment more conducive to the growth of the fungus. Our data do not support this view. Many varieties that are highly susceptible to FHB have awns but it is not known whether their high susceptibility is due to the lack of genes conferring FHB resistance elsewhere in their genomes. Further research is required to clarify whether the gene responsible for the presence of awns is itself contributing towards FHB resistance or whether the effect is due to closely linked genes nearby. Our current results, however, suggest that the selection of lines with awns may enhance FHB resistance levels in UK varieties but this contradicts previous understanding that awns increase FHB susceptibility (Mesterhazy, 1995)).

The second locus associated with FHB resistance coincided with that for flowering time with the early flowering lines appearing more susceptible to FHB than later flowering ones. This effect is most probably due to an interaction with the environment where conditions were more conducive to

infection and/or subsequent fungal colonisation at the time of mid-anthesis (and inoculation) of the early flowering lines.

Barley has inherently high levels of Type 2 resistance and so varietal differences in susceptibility are due to differential Type 1 resistance. A potent FHB resistance was identified on chromosome 6H of the heritage variety Chevallier. Chevallier is a very tall variety but, significantly, this resistance was independent of any height effect. This demonstrates that the introduction of this resistance into modern short cultivars should be possible without compromising plant height characteristics. The data obtained for Armelle indicates that most of the differential between this variety and NFC Tipple is probably due to a large number of genes of small effect, making it difficult to introgress and track individual FHB QTL.

3.5. Work package 2: Exploitation of synteny to break the linkage between FHB susceptibility and *Rht2* (*Rht-D1*) locus.

3.5.1. Background

The previous project (PR432) showed that most current UK wheat varieties are highly susceptible to FHB with little genetic variation for resistance. Much of the susceptibility of UK varieties was associated with the *Rht2* semi-dwarfing gene which was in almost all UK varieties at that time. We have shown that *Rht1* and *Rht2* have differing effects on susceptibility to FHB with the latter conferring a much greater increase in FHB susceptibility than the former (Srinivasachary et al., 2009). It was not known whether this effect was due to a pleiotropic effect of the semi-dwarfing gene itself or due to linkage with a gene conferring increased susceptibility to FHB. Subsequent study (Saville et al 2011) indicated that *Rht* has a negative effect on resistance to fungi that grow biotrophically and increases Type 1 susceptibility (Fusarium grows biotrophically during initial infection). However *Rht* has a positive effect on fungi growing necrotrophically as in later stages of infection (during the spreading phase when fusarium is producing DON), increasing Type 2 resistance (Saville et al 2011). Wheat lines carrying *Rht2* do not exhibit this positive effect on Type 2 resistance. Our accumulated data indicate that either *Rht2* acts in a unique fashion or that the negative effect on Type 2 resistance is not due to the *Rht2* gene itself but to a gene nearby. If it is the latter then this association could be broken to enable breeders to produce FHB resistant varieties with desirable agronomic height characters.

3.5.2. Population development and mapping

Maris Huntsman was crossed to a Maris Huntsman near-isogenic line (NIL) carrying *Rht2* and resulting F_1 seed was used to generate a large F_2 population for screening to identify lines recombined at the *Rht2* (*RhtD1* locus).

All mapping relies upon genetic differences (polymorphisms) between the parents. The polyploid wheat genome consists of three independent genomes (A, B and D). Modern so-called hexaploid wheat only came into existence approximately 10,000 years ago when the D genome donor (*Aegilops tauschii*) crossed with emmer wheat, *Triticum dicoccoides* (A and B genome donor). The recent introduction of the D genome results in very little polymorphism in the D genome, making any mapping in this genome particularly challenging. The absence of a genome sequence for wheat is also a significant hindrance to mapping in this species. Gene order, however, is partially conserved between grass species, making it possible to infer the identity of the genes adjacent to *Rht* by comparison with grass species such as rice, sorghum and *Brachypodium distachyon,* for which genome sequences are available. The genomic regions of these species at the *Rht* locus were compared and the information used to develop DNA markers to characterise the *Rht2* introgression in the Maris Huntsman NIL and identify recombinants in the progeny of the cross between this line and Maris Huntsman. A very large number of F_2 progeny (1,352) were screened using DNA markers to the *Rht2* region to determine the size of the introgressed segment carrying *Rht2* and to identify lines recombined in this region. Recombinant lines were selfed and homozygous recombinant lines selected to produce sufficient seed for use in FHB trials. Almost 50 recombinant lines were identified and seed bulked for use in disease trials.

3.5.3. Type 2 FHB resistance screening

Following point inoculation to assess Type 2 resistance, the presence of *Rht1* (*RhtB1b*) increases resistance relative to the tall parent Maris Huntsman. The negative effect of *Rht2* (*Rht D1b*) is most manifest as a failure to increase resistance to symptom development following point inoculation. A very large replicated trial was established in a polytunnel at JIC in the summer of 2013. Almost 2,750 individual wheat heads were inoculated using a syringe to inject conidia of *F. culmorum* into the cavity of a spikelet at mid anthesis. The plant material tested included the control lines Maris Huntsman, Maris Huntsman *Rht1* (*RhtB1b*), Maris Huntsman *Rht2* (*RhtD1b*) along with the recombinant lines. Disease was monitored at numerous time-points post inoculation to produce over 16,500 data points. As anticipated, while the presence of *Rht1* (*RhtB1b*) increased resistance to spreading symptoms (greater Type 2 resistance) the presence of *Rht2* (*RhtD1b*) did not increase resistance relative to that of the parent line Maris Huntsman which carries the ‘tall’ version of both genes (Figure 4.3).

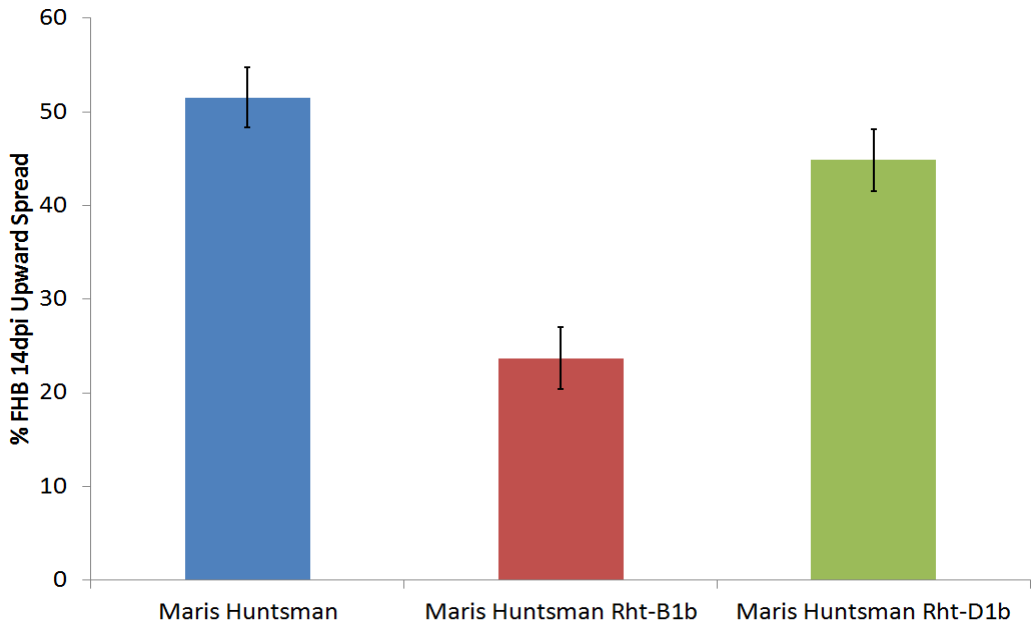

Figure 4.3 Disease symptoms 14 days after inoculation on Maris Huntsman (tall) and two semi-dwarf near-isogenic lines of Maris Huntsman carrying either *RhtB1b* (*Rht1*) or *RhtD1b* (*Rht2*).

The disease levels on the recombinant lines varied between those of the 'tall' line Maris Huntsman and the *Rht2* Maris huntsman NIL. The relationship between disease levels and the 'tall' and '*Rht2*' allele of each of the molecular markers was assessed. Lines in which the *Rht2* gene was isolated from the rest of the segment that had been introgressed in the original *Rht2* NIL showed significantly less disease than the origin parent line. These lines exhibited the increase in Type 2 resistance that was observed for lines containing *Rht1* (*RhtB1b*) i.e. they now behaved as expected for *Rht* semi-dwarfing alleles. In contrast, recombinant lines that had lost the *Rht2* gene but still carried a portion of the introgressed segment exhibited significantly greater susceptibility than their 'tall' equivalent. This result clearly demonstrated that the apparent negative effect of *Rht2* on Type 2 FHB resistance is not due to the *Rht2* (*RhtD1b*) allele itself, but is due to the action of nearby gene(s) that were introduced into UK varieties inadvertently when the first crosses were produced. These genes became fixed along with *Rht2* in the majority of UK varieties produced following the Green Revolution.

3.5.4. Discussion

The semi-dwarfing genes *Rht1* (*RhtB1b*) and *Rht2* (*RhtD1b*) are members of the so-called DELLA family that regulate response to gibberellin (GA) plant growth hormones. Extensive study in wheat and barley had demonstrated the effect of the mutation of these genes to decrease response to GA (Saville et al 2011). Decreased responsiveness to GA through mutation of *Rht* increases susceptibility to fungi growing biotrophically (requiring living host tissue) while increasing resistance to fungi when growing necrotrophically (killing host tissue). The *Rht2* mutation, however, did not

behave according to this scheme. This may be due to the mutation itself behaving differently with respect to host resistance or because of the effects of a nearby gene carried along with *Rht2* when breeders select for short stature plants. The current project clearly demonstrated that the negative effect on FHB is due to a nearby gene rather than the *Rht2* gene itself. Knowledge that the negative effect on Type 2 FHB resistance is due to a linked gene is a significant advance for plant breeders, who can use this information along with the molecular markers developed within the Inspyr project, to select wheat lines carrying *Rht2* to provide the desired plant height while removing the gene(s) nearby that cause increased susceptibility to FHB.

3.6. Workpackage 3: Identification and mapping of FHB resistance QTL in barley by association genetics.

3.6.1. Background

Conventional genetic mapping generally involves the production of homozygous (or near homozygous) lines from a cross between parents differing in the trait of interest. This was the approach used in Work package 1 to identify QTL associated with FHB resistance in wheat and barley. While potentially highly informative this approach suffers a number of significant drawbacks. It takes a considerable length of time to produce appropriate populations for study. The number of recombination events present within the population is relatively limited because recombination occurs infrequently, being in the order of one to two events per chromosome per generation. Furthermore, the parents reflect only a small part of the variation present within a species and, for many crop plants, very little polymorphism is present between varieties making it difficult to map some regions of the genome.

Genome-wide association studies (GWAS) overcome many of these barriers. The approach requires the production of a high density marker map such as can be achieved using modern DNA sequencing technologies. The markers are based upon single nucleotide polymorphisms (SNPs) that can be assessed in high throughput formats. The GWAS panel consists of a large collection of diverse varieties and takes advantage of the high number of recombination events that have occurred throughout the generation of these varieties to increase the resolution of trait-marker associations. The panel of barley varieties used in the Inspyr project originated from the set used within a previous project AGOUEB (http://www.agoueb.org/) (HGCA, 2014) along with the associated marker set.

3.6.2. FHB screening and GWAS

Four field trials were carried out to determine the relative FHB resistance of barley varieties in the AGOUEB set. In 2011 single plot trials were established at JIC and NIAB for 200 varieties. The trials

were repeated and extended in 2012 to include 300 varieties at each site. Plots were inoculated as described above in Work package 1 and disease scored in a similar manner.

The original set of varieties included a small number of six-row types (Larker, Manchurian, Pirkka, Quinn, Stander, Sudan, Thule, Tifang and Tradition) and these were removed from subsequent analysis because of their very small number and the known association between six-row type and susceptibility to FHB. Full phenotype and genotype data was available for 220 two-row varieties. The data was analysed using GenStat 14th Edition (VSN International, 2011). The associations were identified using QTL data command lines and population structure controlled using the eigen analysis method which employs principal components analysis to identify population structure. Disease was scored at JIC and NIAB on a subset of 200 genotypes in 2011 and all 220 genotypes in 2012, associations were identified using a single combined data score and as individual environments (NIAB, 2011; NIAB, 2012; JIC, 2011; JIC, 2012). Significant associations were identified when -log 10(P) was greater than 3 (although this was reduced 2.5 for the NIAB 2012 dataset) and effects of the minor allele identified using standard errors.

3.6.3. Single combined score

The data from the four FHB trials was combined to produce a single overall score for each line. Using this approach six QTLs were identified; 1H at 90cM and 138cM; 3H at 123.7cM; 5H at 133cM and 7H at 55cM and 107cM as well as several unmapped associated loci. The frequency of the two allelic forms at each marker can be used to establish whether the less frequent allele is conferring increased resistance or increased susceptibility. For the QTL on 1H and 3H the minor allele confers a reduction in disease incidence. In contrast those accessions with the minor allele on 5H and both 7H regions were associated with increased susceptibility.

3.6.4. Separate environments

NIAB 2011 & 2012

The most significant associations at the NIAB site were observed in 2011 even though full genotype and phenotype data was available for only a subset of lines (137 accessions). Disease levels in 2012 were lower than those observed in 2011 and only SNPs on 7H reached the threshold of –log10(P) of 3 in 2012. As stated above the threshold for assigning a positive effect was reduced 2.5 for NIAB 2012 dataset to take this into consideration. In both years, three regions of the genome identified QTLs associated with disease resistance. The QTL at 130cM on 5H and at 107.2cM on 7H were in similar locations to those observed using the combined data set. Again, the more commonly occurring allele at both 5H and 7H was associated with greater FHB resistance indicating that most varieties already possess the more desirable allele at this locus. For example, in 2011, varieties carrying the minor allele had average disease levels of 7.8 whereas those with the major allele had

levels of 4.5 while in 2012 the disease levels associated with the minor and major alleles were 12.2 and 10.4, respectively).

A second QTL was detected on 2H at 120cM along the chromosome. Unexpectedly, the region on 2H had contrasting effects on disease at NIAB in the two years. In 2011 the minor allele (present in approximately 28% of varieties) had a large negative effect on FHB resistance with a mean disease value of 7.9 compared to 4.2 for the common allele. In 2012, however, the minor allele in the same region of 2H had a small but significant positive effect on FHB resistance with a mean value of 9.7 compared to 11.2 for the common allele.

JIC 2011 and JIC 2012

Significant associations were found on 1H in both years of trials at JIC although they were at different locations on the chromosome. The regions at 90cM and 138cM were associated with differing FHB susceptibility in 2011 while that at 56cM was associated in 2012. The less frequent allele contributed greater FHB resistance in all cases. On chromosome 3H the region at 129cM had a significant effect on FHB resistance in both years and again the less frequent allele was associated with greater FHB resistance. In 2012 only, several regions distributed along chromosome 2H (39, 74, 112, 122cM) were associated with differing levels of FHB resistance and in all cases the less frequent allele conferred greater susceptibility to FHB.

Chromosome 5H was associated with contrasting effects in 2011 and 2012 with these being conferred by loci at each end of the chromosome. In 2011 at 16cM the less frequent allele (10%) was associated with increased FHB resistance, while in 2012 the other end of the chromosome at 140cM was associated with differential susceptibility to FHB but in this instance the more frequent allele was found to be contributing greater FHB resistance. The region at 107cM on 7H was associated with differential FHB resistance at JIC in 2011 but not in 2012 with the less frequent allele conferring greater susceptibility to FHB. Several of these regions had been identified at the NIAB trials in particular the region on 1H at 138cM, 2H at 122cM and on 7H at 107cM. Similarly several were in common with those identified using the combined scores (Table 1) including those on 1H at 90cM and 138cM; 3H at 123cM and 107cM on 7H.

3.6.5. Discussion

Several regions were consistently associated with differential FHB scores in one or more of the trials: 1H at 90cM and 138cM; 2H around 122cM; 3H between 123cM to 129cM; 5H around 130cM possibly extending to 140cM and 7H at 55cM and 107cM. The relative distribution of the alleles among the varieties indicates which should be selected for and which should be selected against in order to improve overall FHB resistance levels in barley. For the QTL on 1H and 3H the minor allele confers a reduction in disease incidence. In contrast those accessions with the minor allele on 5H and both

7H regions are associated with increased susceptibility. These findings suggest that FHB resistance of the majority of varieties may be improved by replacing the relevant regions of 1H and 3H with that from varieties carrying the less common allele. In both instances, over 75% of varieties would benefit from the replacement as the allele associated with greater resistance was present in less than 25% of varieties for both the 1H and 3H QTL. A similar allelic frequency was observed for the two QTL in 7H suggesting that most varieties already possess the more desirable alleles at these loci. However, the QTL on 5H was distributed equally among the varieties indicating that only 50% would benefit from changing the allele at the FHB associated locus. The contrasting effect on FHB resistance of the alleles in the 122cM region of 2H in different environments indicates that this locus may not be a suitable target for manipulation in order to provide stable improved FHB resistance.

3.7. Workpackage 4. Identification of the Optimal Integration of Host Resistance to Control Fusarium Ear Blight and Mycotoxin Accumulation.

3.7.1. Introduction

It is acknowledged that the control of FHB and the associated contamination of grain with DON and other mycotoxins poses a great challenge to growers. Current UK varieties generally exhibit weak to moderate resistance to FHB. It is probable that the absence of severe FHB disease pressure in the UK until the recent appearance of *F. graminearum* resulted in the development of varieties that had not been subjected to selection for resistance to FHB. This allowed the accumulation of alleles associated with susceptibility to this disease. Although some fungicides (e.g triazoles) have proven efficacy against *Fusarium* species they have limited capacity to reduce disease and mycotoxin levels under conditions of high disease pressure. As stated above, two broad forms of resistance to FHB are recognised in wheat: resistance to initial infection (Type 1) and resistance to spread within the head (Type 2). It is not known whether fungicide application can combine with enhanced host disease resistance to reduce disease and, more importantly, DON levels to below current legislative thresholds. The EU has established a limit on DON in unprocessed cereals other than durum wheat, oats and maize of 1250 µg/kg. Experiments were carried out to examine whether individual or combinations of FHB QTL of differing resistance (Type 1 and Type 2) could combine with selected fungicides to reduce FHB disease and suppress DON accumulation to below current EU limits even under high disease pressure.

3.7.2. Methodology

A series of trials were carried out between harvest year 2010 and 2013 to establish whether the application of fungicides would give additional benefit for control of FHB infection and mycotoxin production over and above that of varietal resistance alone. Trials were drilled to produce a plot size

of 1 m^2. Each winter wheat line was replicated four times, with a guard plot drilled between each replicate. In the growing seasons 2010/2011 and 2011/2012, six lines with differing fusarium head blight resistances were drilled (Table 4.1). In the 2012/2013 season, an additional six lines were drilled (Table 4.2). The different sources of resistance confer either Type 1 or Type 2 resistance. Type 1 resistance is associated with resistance to initial infection, however if overcome the symptoms will spread from the site of infection, whereas Type 2 resistance is associated with resistance to symptom spread but not to initial infection.

Table 4.1. Source and type of resistance for lines drilled 2010 to 2012.

Line	Type of FHB resistance
Hobbit (Sib)	FHB susceptible parent
HS WEK 1B	Type 2 on chromosome 1B
HS WEK 42	Type 1 on chromosome 5A and
	Type 2 on chromosomes 1B and 3B
HS WEK 5A	Type 1 on chromosome 5A
HS WEK 3B	Type 2 on chromosome 3B
DH 72	Type 1 on chromosome 4A

Table 4.2. Source and type of resistance for additional lines drilled in 2012.

Line	Source of resistance
QTL-20	Type 1 on chromosomes 4A and 5A
	Type2 on chromosomes 1B and 3B
1-4A3B	Type 1 on chromosome 4A
	Type 2 on chromosome 1B
8-4A1B	Type 1 on chromosome 4A
	Type 2 on chromosome 1B
13-5A4A	Type 1I on chromosome 5A and 4A
5-4A1B	Type 1 on chromosome 4A
	Type 2 on chromosome 1B
DH 81	Type 1 on chromosome 4A

Plots were inoculated during mid-flowering (GS65) two to three days following fungicide treatment with a conidial suspension made up of three DON producing isolates of *F. graminearum*. To encourage *F. graminearum* infection mist irrigation was initiated immediately after inoculation and continued for five days.

3.7.3. Fungicide application

In trials carried out in 2011 and 2013 the effect of Brutus® (37.5 g/l epoxiconazole + 27.5 g/l metconazole) applied at 2 l/ha was tested for efficacy against FHB infection and subsequent mycotoxin contamination. In 2012, the efficacy of two additional treatments, Brutus® applied at 1 l/ha and Swing Gold® (133 g/l dimoxystrobin and 50 g/l epoxiconazole) applied at 1.5 l/ha were also tested. The application of Swing Gold at 1.5 l/ha and Brutus at 2l/ha provided treatments with equivalent application rates of epoxiconazole. In each year, sprays were applied at the early stage of the flowering (GS63) in a 200 l ha^{-1} of water using flat fan nozzles (Tee Jet XR110VP Yellow).

3.7.4. Disease assessment and toxin analysis

A total of 60 ears for each treatment (15 per replicate plot) were assessed in each year. Disease severity was assessed at watery ripe (GS70) and early dough (GS80) growth stages. In each year plots were hand harvested and grain removed from ears using a HEGE Single Ear Thresher. The level of deoxynivalenol (DON) in cleaned grain samples was determined using RIDASCREEN DON *FAST* kits (R-Biopharm Rhône Limited) following the manufacturer's instructions.

3.7.5. Results

Similar results were obtained in each year and the data from 2011 provides an example that demonstrates the principal findings. Six closely related winter wheat lines (having a common background of Hobbit sib.) were drilled with differing sources of FHB resistance; these were Hobbit Sib. – FHB susceptible parent, HS WEK 1B – Type 2 resistance (associated with chromosome 1B), HS WEK 42 – combination of Type 1 resistance (associated with chromosome 5A) and Type 2 resistance (associated with chromosomes 1B and 3B), HS WEK 5A – Type 1 resistance (associated with chromosome 5A), HS WEK 3B – Type 2 resistance (associated with chromosome 3B) and DH 72 – Type 1 resistance (associated with chromosome 4A).

FHB symptoms

At the GS80 assessment the FHB symptoms had fully expressed and as such disease levels on lines with Type 2 resistance (resistance to symptom spread) should have increased less than those with Type 1 resistance only. Levels of FHB were significantly higher on Hobbit (Sib) and DH72 (Figure 4.4) compared to the other lines. Neither of these lines has resistance to spread of symptoms (Type 2 resistance). Line HS WEK 42 possesses both Type 1 and Type 2 resistances and exhibited significantly lower disease scores than the other lines. The level of disease on the remaining three lines (HS WEK 1B, 5A and 3B) did not differ significantly from each other.

The application of Brutus at 2 l/ha resulted in reduced disease levels across all lines with the reductions significant for all varieties other than HS WEK 42 (Figure 5). The greatest effect was seen where Brutus was applied to lines with Type 2 resistance only (HS WEK 1B and 3B) where 94% and

83% control was achieved, respectively. With these lines the fungicide appears to be contributing in a manner similar to the Type 1 resistance by reducing initial infection. Thereafter the Type 2 resistance present in both lines then acts to prevent the spread of the symptoms in the ear. The least effect was seen where the fungicide was applied to lines with Type 1 resistance (DH72 and HS WEK 5A), where the resistance and fungicide are working in a similar way – both reducing initial infection, with no mechanism present to prevent symptom spread once infection has occurred. For DH72 and HS WEK 5A, the disease control achieved was 42% and 36%, respectively, this is lower than the sensitive parent line (Hobbit (Sib)) where 69% disease control was achieved. A reduction in disease of 69% was seen for line HS WEK 42 (the line with both Type 1 and Type 2 resistance) following the application of fungicide. Even though this reduction was not significant it is evident that the application of fungicide was still of benefit. The level of disease recorded on the HS WEK 42 control (non-fungicide treated) plots was significantly lower than the disease recorded on plots of Hobbit (Sib), HS WEK 5A and DH 72 treated with fungicide.

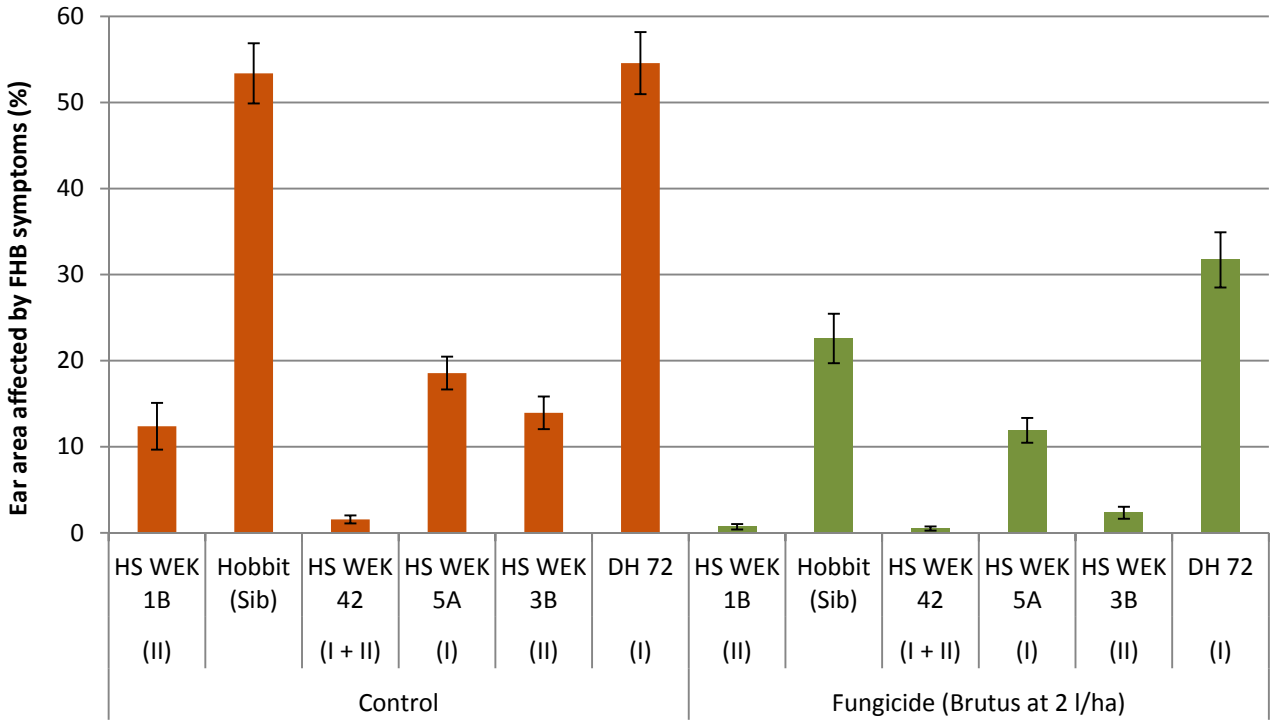

Figure 4.4. Effect of resistance and fungicide treatment on fusarium head blight (FHB) symptoms at GS80 (2011). (LSD 5% = 6.2). Transformed data presented, bars represent standard error of mean. Resistance type (I=Type 1, II=Type 2 or both) indicated in parenthesis below wheat line name.

Toxin analysis

There were significant differences in the concentration of DON detected in grain from the different lines (Figure 4.5). Grain from DH72 contained significantly more DON (27.4 ppm) than any other line, whereas grain from HS WEK 42 contained significantly less DON (1.6 ppm) than the other lines. In

general, grain from lines with Type 2 resistance had the lowest levels of DON, with the Type 2 resistance associated with chromosome 1B out-performing that found on chromosome 3B. The application of Brutus reduced the concentration of DON found in grain for all lines (Figure 6). The reduction was significant for all lines other than HS WEK 42. However, the reduction achieved for HS WEK 42 brought the DON concentration down from one that was above the legislative limit of 1.25 ppb (1.61 ppm) to one below it (0.97 ppm). As seen with the reduction of disease, the application of fungicide had the greatest effect when applied to the lines which contained Type 2 resistance only, with reductions of 72% and 69% seen for HS WEK 3B and 1B, respectively. The DON levels found in grain from the untreated HS WEK 42 plots was significantly lower than the toxin level in grain from the Hobbit (Sib), HS WEK 5A and DH 72 fungicide treated plots.

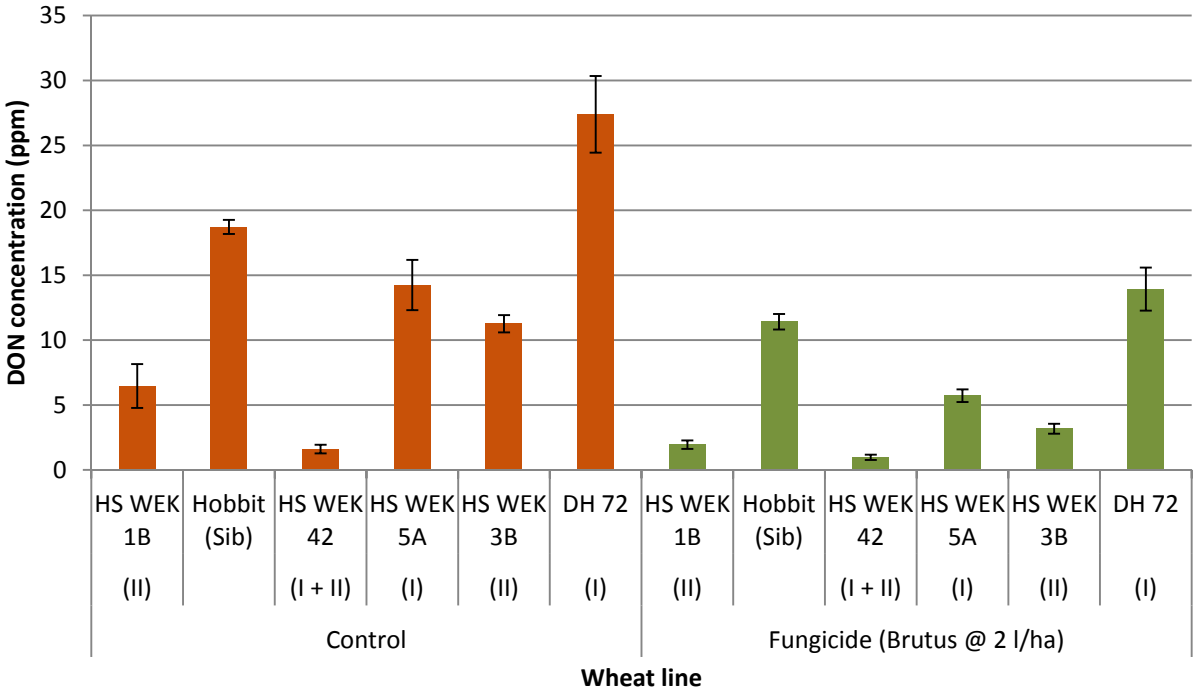

Figure 4.5. Effect of resistance and fungicide programme on deoxynivalenol (DON) contamination in grain (2011). (LSD 5% = 3.85, bars represent standard error of mean). Resistance type (I=Type 1, II=Type 2 or both) indicated in parenthesis below wheat line name.

3.7.6. Discussion

Differences in resistance to fusarium head blight were evident among the lines with those carrying Type 2 resistance generally outperforming those with Type 1 resistance. The Type 1 resistance derived from *Triticum macha* and the subject of study in Work package 5 had only very limited effect against *F. graminearum* and did not control accumulation of DON mycotoxin in grain. The presence of Type 2 resistance, however, not only reduced symptom development but also reduced DON

mycotoxin levels. The application of fungicide reduced the level of disease and mycotoxin. Across the different seasons and wheat lines, the application of fungicide reduced DON levels by approximately 50%. It was noted that the benefit of the fungicide appeared to be greater following treatment of lines with Type 2 resistance. It appears that fungicide is reducing the ability of the fungus to infect the host thereby mimicking a Type 1 resistance. This reduced incidence of infection is complemented by the host Type 2 resistance where, following infection, colonisation of the host and accumulation of mycotoxin is reduced.

Most significantly, throughout the series of experiments, in many instances, the combination of wheat lines with high levels of FHB resistance plus application of fungicide could reduce DON levels in grain to below the EU threshold. Therefore the data support the view that an integrated approach, combining improved host resistance with application of fungicide when conditions are favourable to the development of FHB is appropriate for the limitation of threat posed by this disease under UK conditions.

3.8. Workpackage 5. Fine mapping of the Type 1 FHB resistance on chromosome 4AS of *Triticum macha*.

3.8.1. Introduction

Inheritance of resistance to fusarium in wheat is quantitative with a large volume of literature identifying more than 100 quantitative trait loci (QTL) for resistance (Buerstmayr et al., 2009). Several forms of resistance have been postulated but resistance is generally differentiated into two types: Type 1 (resistance to initial infection) and Type 2 (resistance to spread within the head) (Schroeder and Christensen, 1963). The majority of resistance QTL identified confer Type 2 resistance (Buerstmayr et al., 2009). This includes the potent 3BS QTL derived from Sumai-3, *Qfhs.ndsu-3BS* (Anderson et al., 2001), which was subsequently mapped as a single Mendelian gene termed *Fhb1* (Cuthbert et al., 2006), and a QTL identified on chromosome 1B that is thought to be located at or closely linked to the 1BL-1RS wheat-rye translocation (Ittu et al., 2000; Schmolke et al., 2005; Shen et al., 2003). Type 1 resistance is considered to be advantageous, because it confers resistance to colonisation both by toxin producing *Fusarium* species and non-toxin producing *Microdochium* species. However, it is difficult to identify and select for Type 1 resistance as it must be inferred following assessment by both single spikelet (point) inoculation to assess Type 2 resistance and spray inoculation to assess both Type 1 and Type 2 resistance (Mesterházy et al., 2008). A Type 1 resistance QTL was identified on chromosome 4A of *T. macha*, a hexaploid wheat endemic in the Caucasus region (Steed et al., 2005). Importantly this resistance both reduced visual disease symptoms and DON levels, suggesting that it may be useful for deployment in elite varieties to provide protection against FHB. This resistance was mapped as a single gene to the short arm of chromosome 4A (4AS) using a double haploid (DH) population, where it co-segregated with the SSR

marker Gwm165 and was named *QFhs.jic-4AS*. However, the limited number of recombinants (43 lines), combined with a lack of polymorphic distal flanking markers prevented accurate localisation of the QTL (Steed et al. 2005). In the present project we used a 288 line F_4 population developed from the susceptible parent Hobbit 'sib' and the resistant line DH81, previously developed by Steed et al. (2005), to refine the localisation of the 4AS *T. macha* Type 1 resistance and to identify SNP markers to aid selection and pyramiding with other FHB resistance QTL by plant breeders.

3.8.2. Development of plant materials and map construction:

Seed was obtained of the single chromosome recombinant double haploid line (DH81) previously developed from the cross between HS/Tm4A x Hobbit 'sib' and shown to possess the FHB QTL (Steed et al., 2005). DH81 was backcrossed to HS and a population of 288 F_4 plants was generated. Seedlings were screened, using the markers described below, to detect recombination in the region of the 4A chromosome believed to harbour the FHB resistance. Homozygous recombinant F_5 lines were bulked for use in phenotypic evaluations of FHB resistance.

To identify SSR markers for mapping the *T. macha* 4A resistance, HS and DH81 were screened with 39 publically available SSR markers that were reported to be located on chromosome 4A, to identify polymorphic and co-dominant markers. Polymorphic SSR markers were applied to the HS x DH81 F_4 population and the resulting F_5 recombinant lines.

The parent lines of the population (HS and DH81) and the single chromosome substitution line HS/Tm4A were screened with a wheat SNP panel and polymorphic markers were identified to provide an even coverage of chromosome 4AS and primer sets obtained to apply to the HS x DH81 F_4 population and the resulting F_5 recombinant lines. Additional SNP polymorphisms between the parent lines were identified using the iSelect 90k wheat SNP chip (Wang et al., 2014, in press) at the University of Bristol Genomics Facility (http://www.bristol.ac.uk/biology/research/transcriptomics/). Sequences for the polymorphic SNPs were aligned to the *Brachypodium*, rice and Sorghum genomes using Phytozome v9.1 (www.phytozome.net) to identify the orthologous loci in these species, where present.

3.8.3. FHB resistance phenotyping of the HS x DH81 population:

In total, 78 recombinant lines were selected from the HS x DH81 F_4 population for use in the current study. 39 stable recombinant F_5 lines were assessed for FHB resistance in a field trial at JIC in 2012. The FHB resistance of all 78 recombinant F_5 lines was assessed during the summer of 2013 in two independent field trials; one at Church Farm (CF) near Norwich and one at JIC each with three replicate plots per line. All trials were inoculated with a highly virulent DON-producing *F. culmorum* isolate (Fu42) and conducted as described above. Disease was assessed as percentage of infection within each plot at 16, 22, 25 and 30 days post infection (dpi). The area under the disease progress

curve (AUDPC) was again calculated to provide an integrated measure of disease and percentage infection at 30 dpi was used as a measure of disease severity (%FHB).

The 39 stable recombinant HS x DH81 F_5 lines initially identified and generated from the F_4 population were assessed for FHB resistance in 2013 at JIC in an unheated polytunnel with capillary matting irrigation. 15 plants per line were arranged in a randomised complete block design with 4 blocks (3-4 plants per line within each block). Inoculations were conducted and plants were scored as described for the above polytunnel trial.

3.8.4. QTL analysis:

A genetic linkage map of chromosome 4A was constructed using 16 KASP wheat SNPs, 3 SSRs and 2 EST-SSRs applied to the DNA from 288 F_4 lines. The linkage analysis was performed in Joinmap (version 3.0) (Van Ooijen and Voorips, 2001), using 0.4 as the maximum recombination fraction and 5.0 as the logarithm of the odds ratio (LOD) and the linkage map was drawn using MapChart (Voorips, 2002).

Predicted mean FHB% and AUDPC scores from the field trials and polytunnel experiment of F_5 recombinant lines were used alongside marker data from the same lines in a single marker regression analysis to identify QTL locations for each trait within each experiment. A single marker regression analysis was utilised as there were relatively few markers (21) densely spaced on a single linkage group. Markers were only determined to be associated with the phenotype where $p<0.01$ to reduce the likelihood of false positives. To provide a more accurate estimation of QTL location, composite interval mapping (CIM) was also conducted. All QTL analyses were conducted in Genstat v.15.2.

3.8.5. HS x DH81 marker analysis, genotyping and map construction:

The genetic map in the original publication describing the identification of the 4A FHB QTL (*QFhs.jic.4A*) was extremely sparse, reflecting the paucity of wheat molecular markers available at that time (Fig 4.6). With the advent of the new DNA sequencing technologies and the increase in available SNP markers for wheat it is possible to produce far superior genetic maps as illustrated by that produced in the present study (Fig 4.6). Using the new set of recombinants combined with the vastly increased number of genetic markers it was possible to produce a genetic map of the relevant region of 4A with good density of makers and, most importantly to extend the genetic map towards the telomere.

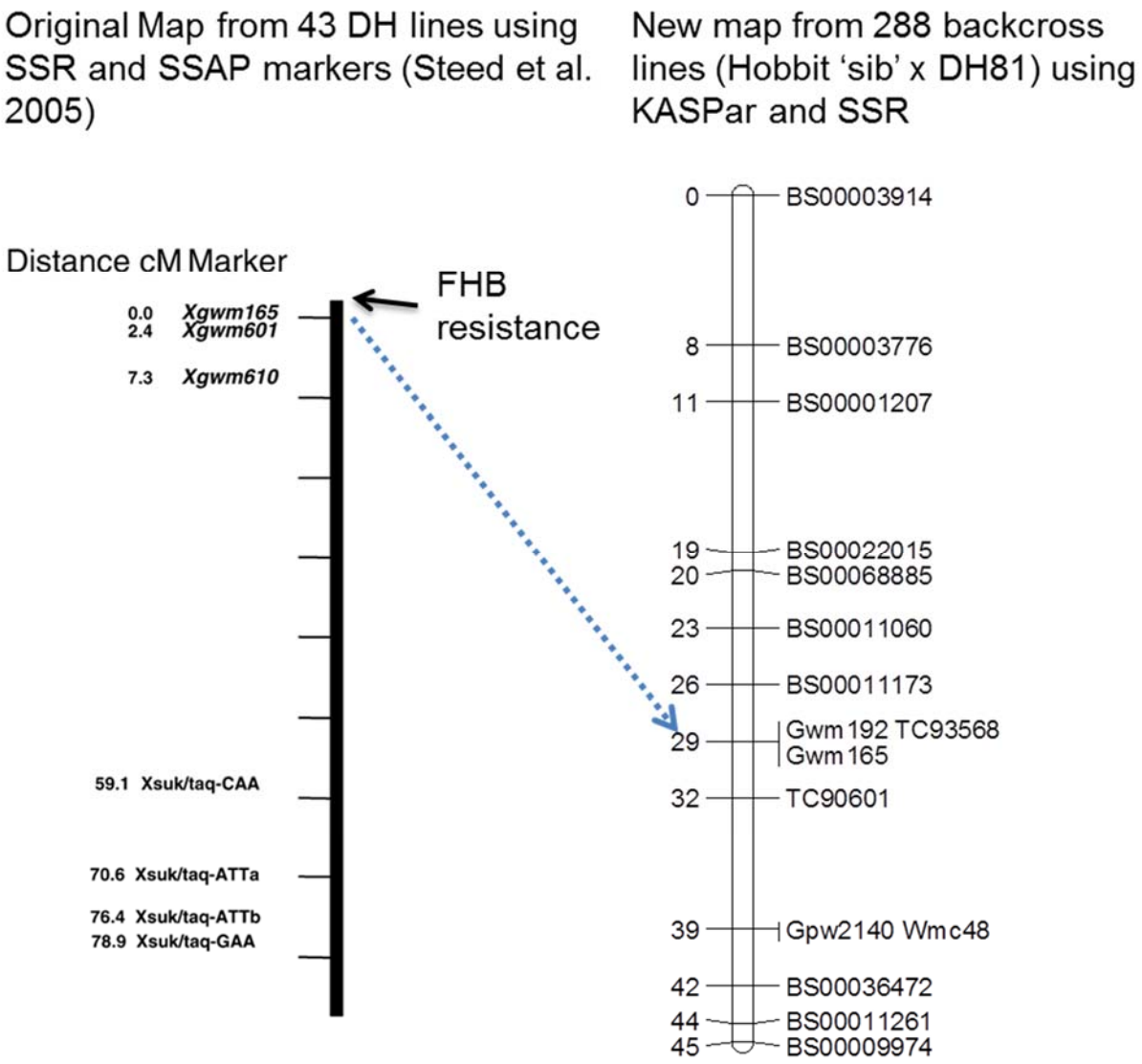

Figure 4.6. Linkage map of chromosome 4AS from the original paper of Steed et al (2005) compared with that produced in the current study of the Hobbit 'sib' (HS) x DH81 population indicating the position of the marker explaining most of the phenotypic variance in each case. The top of the linkage group relates to the region closest to the telomere and the bottom of the linkage group relates to the region closest to the centromere of the chromosome.

3.8.6. HS x DH81 QTL analysis:

Despite the large number of replicated trials and the number of recombinant lines it was not possible to define the precise location of the FHB resistance on 4A as a single gene. The resistance always behaved as a quantitative trait and the position of the resistance was ascribed to a region covering several markers rather than within an interval between two markers. Due to the quantitative nature of the resistance the position shifted slightly between trials.

Similar regions were identified when assessed using AUDPC scores as for %FHB scores in all four trials using both single marker regression and CIM. However, the significance of the QTL detected

was generally higher for %FHB than for AUDPC scores. Both the CIM and single marker regression analyses from the JIC trial in 2012 identified Gwm165 as the marker with the most significant relationship with AUDPC and % FHB. This lies within the region predicted by the polytunnel and but lies outside of the region identified by the single marker regression analysis of the JIC field trial in 2013. The presence of a DH81 allele in a 4.7 cM region from markers BS00182960 to TC93568 was associated with a reduction in the AUDPC ($p<0.01$) in the CF field trial in 2013. An overlapping but smaller 2.1 cM region was identified from the polytunnel trial with DH81 alleles at markers from BS0011396 to Gwm192 associated with a significant reduction in AUDPC ($p<0.01$). The CIM results confirmed this, locating QTL peaks within these regions on BS00011173 in the CF field trial and at TC93568 in the polytunnel experiment.

In both the polytunnel trial and CF field trial the presence of a DH81 allele at markers in a 5.3 cM region from BS00182960 to Gwm192 was associated with a reduction in % FHB. A similar region was identified in the JIC field trial in 2013 with the presence of a DH81 allele associated with a reduction in %FHB in a 5.9 cM region between markers BS00011060 to TC93568.

The polytunnel trial found the EST-SSR marker TC93568 to account for the highest proportion of variation for both AUDPC and % FHB traits, using both single marker regression and CIM. The greater amount of variation accounted for by markers within the polytunnel trial, compared to the field trials, may be due to a more homogenous environment and/or the more detailed scoring of individual spikelets in this procedure. Steed et al. (2005) conducted all phenotyping in a polytunnel, and this may have assisted the detection of the resistance as a single gene with Mendelian inheritance. However, the QTL with the highest location confidence was detected in the JIC 2013 field trial for %FHB centring about marker BS00011173.

3.8.7. Discussion

Prior to this study the map location of *QFhs.jic-4AS* was imprecise. Previous efforts to map this resistance have been thwarted because of a lack of polymorphic markers. Steed et al. (2005) utilised existing SSR and developed novel sequence-specific amplified polymorphism (SSAP) markers, but were not able to identify any distal markers to flank the resistance to facilitate marker assisted selection of the resistance by plant breeders. Developments in SNP technology and the availability of wheat SNPs both through the KASP assays (Allen et al., 2013) and the wheat 90K iSelect genotyping assay (Wang et al., 2014) enabled saturation of the region surrounding the 4AS QTL. It was therefore possible to identify breeder-friendly KASP markers underlying the QTL region such as BS00011173 and BS00113963. It was also possible to identify distal flanking KASP assay markers such as BS0006885 and BS00022015 and proximal flanking markers such as the iSelect derived KASP BS00164805 and the KASP assay BS00036472 that would be suitable for selection of the region containing *QFhs.jic-4AS*.

Previously, Steed et al. (2005) located the *QFhs.jic-4AS* as a single gene using visual disease symptoms observed in a polytunnel using a population 43 DH lines. The genetic effect of the region as a whole appears to be relatively large, providing heritability estimates of 0.70 – 0.73 for the FHB resistance traits recorded. In contrast, in the present study using 78 F_5 lines from a recombinant population, we were unable to resolve the resistance as a single gene in any experiment, but were able to locate it quantitatively using single marker regression and CIM. It is possible that the *T. macha* 4A resistance is conferred by multiple genes of small effect distributed over the approximately 12.2 cM region between markers BS00011060 and BS00164805. The additional recombinants within the 288 F_4 lines and the high marker density in the present study, compared to the limited recombinants within the 43 DH lines studied by Steed et al. (2005), may have fractionated *QFhs.jic-4AS* into multiple QTL within a small region. However, failure to resolve the QTL as a single gene may reflect the difficulty of accurately phenotyping FHB using only one score (%FHB) at a particular day after inoculation. However, from the current data, it is not possible to determine whether the effect is due to the presence of are multiple QTL or as a consequence of unexplained variation in the experiments. Other factors may have hindered resolution of *QFsh.jic-4AS* as a single gene. Accurate phenotyping of Type 1 resistance is recognised to be challenging because of confounding effects of Type 2 susceptibility in lines such as Hobbit sib. used in the present study. Furthermore, disease pressure was extremely high in all trials and this may have resulted in the fungus overcoming the resistance conferred by *QFsh.jic-4AS*. Additional, detailed phenotyping research using reduced disease pressure will be required to resolve this issue.

The *QFhs.jic-4AS* QTL was detected in approximately the same region, using both disease development (represented by AUDPC) and disease severity (represented by %FHB) measurements, in four independent phenotyping experiments. This suggests that the resistance can be considered to be stable, as it was expressed across different environments. Significantly, the line carrying *QFhs.jic-4AS* used in the fungicide experiments described above was not found to be effective against *F. graminearum* in any trial. Further work is required to establish the basis of these discrepancies. From the present work, however, we have identified a number of markers suitable for high throughput genotyping to allow plant breeders to integrate and follow this resistance within their breeding programmes.

4. Conclusions

The INSPYR project was intended to benefit the industry, through assisting breeders to more effectively develop varieties with resistance to all FHB species by expanding knowledge of the genetics of resistance.

The project was successful in unravelling the FHB susceptibility associated with the *Rht2* (*RhtD1b*) gene. Molecular markers were developed to allow breeders to select for the *Rht2* allele while selecting against the presence of a nearby FHB susceptibility factor. The project also demonstrated that Type 1 and Type 2 resistances can be combined within UK semi-dwarf varieties to greatly

increase overall FHB resistance. These findings should assist breeders in developing new highly resistant varieties with desirable agronomic characteristics (e.g. semi-dwarf)

The INSPYR project set out to identify new sources of Type 1 resistance as this form is relevant to both wheat and barley, to all FHB pathogens and to attempts to limit the accumulation of DON, T-2 and HT2 mycotoxins in grain. New molecular markers were developed to assist breeders to select for the Type 1 FHB QTL (*Qfhs.jic-4A*). The presence of awns was demonstrated to increase type 1 resistance in three segregating populations. It is suggested that breeders could improve FHB resistance by selecting awned lines and that this would increase resistance to both toxin and non-toxin producing FHB pathogens.

The project clearly demonstrated the benefits of an integrated approach to the control of FHB by combining the growing of more resistant varieties with appropriate fungicide application. Additional work is required to identify the optimal combination of FHB QTL and fungicides for maximising control of DON accumulation in grain. This approach is important as it provides a means to maintain low DON levels in harvested grain even under high disease pressure.

Increasing FHB resistance in barley is particularly challenging because of significant environment x genotype interactions, meaning that the same allele does not have the same effect on resistance in different environments (years or locations). No robust, large effect QTL were identified among the elite two-row varieties investigated. A QTL of relatively large effect was, however identified in the heritage variety ‘Chevallier’. Further work is required to refine the position of this QTL to make it useful to barley breeders.

Overall, INSPYR has provided new insights into the control of FHB in wheat and barley and provided breeders and growers with new tools and approaches. INSPYR has also identified areas for potential future research.

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