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Abstract and Summary

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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.