

# Genes to reduce plant height without increasing diseases

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## Abstract

Recent research has highlighted the negative relationship between reduced plant height in wheat conferred by the 'Green Revolution' semi-dwarfing (Rht) genes and resistance to some diseases, in particular Fusarium head blight (FHB). This project used a number of parallel approaches to investigate the relationship between plant height and resistance to FHB and other important wheat diseases.

Near isogenic lines differing for plant height QTL on chromosomes 2A, 2D, 3A, 3B and 6A in two genetic backgrounds (Avalon and Cadenza) were assessed for FHB resistance. The 2D and 3B QTL had the greatest influence on plant height and on FHB resistance. In general, the allele conferring increased plant height was also associated with reduced FHB disease. The exceptions to this were the 6A QTL across both backgrounds in 2014 and the 2A QTL in the Cadenza background in both 2014 and 2015.

FHB assessment of lines derived from cultivar Paragon following EMS treatment again found that in general, the taller lines exhibited more resistance than the shorter ones. Such a finding suggests that the effect of plant height on susceptibility to FHB reflects a form of escape, possibly as a result of altered micro climate about the wheat head. However, it should be noted that many of the very short plants appeared to be affected for general fitness and produced fewer seed during the bulking process and fewer plants in field trial plots. Furthermore, while the association between plant height and susceptibility to FHB was moderate it is clear that a relatively large proportion of FHB resistance is independent of plant height.

Analysis of populations with both parents carrying the same semi-dwarfing allele but differing in FHB resistance revealed markedly different genetic basis for the differential resistance. The difference on FHB resistance between Barok and Bermude (both RhtD1-b) appears to be due to the effect of many genes of minor effect whereas that between Sokal and Oakley (both RhtB1-b) was determined to be due to the effect of three potent QTL on chromosomes 3A, 6D and 7B.

## 1. Introduction

Recent research has highlighted the negative relationship between reduced plant height in wheat conferred by the 'Green Revolution' semi-dwarfing (Rht) genes and resistance to some diseases, in particular Fusarium head blight (FHB).

This project used a number of parallel approaches to identify new alleles controlling plant height (PH) to counter the negative effect of height reducing alleles on major wheat diseases such as FHB.

- 1) Two novel height loci on chromosomes 2D and 3A were characterized for their influence on FHB and other important wheat diseases.

- 2) Height mutants identified within a mutagenized population of wheat cultivar Paragon were screened for disease resistance against FHB and other important diseases.

- 3) Populations with parents differing for FHB resistance but with the same Rht alleles (Rht1 or Rht2) were screened to identify quantitative trait loci (QTL) that enhance resistance to FHB in the presence of these height reducing alleles.

The goal of these studies was to identify alternative semi-dwarfing genes that can provide plant breeders with the desired plant height agronomic character while not compromising other important traits such as disease resistance, in particular FHB.

### Context and economic importance

Fusarium head blight (FHB) is important in many parts of the world. For example, in the USA from 1993-7 losses due to FHB exceeded \$1 billion. The incidence and severity of FHB has increased in Europe, linked to agronomic practices such as wheat-maize rotation and reduced or nil-tillage. In addition to reducing yield, this disease contaminates grain with fungal mycotoxins such as deoxynivalenol (DON) for which the EU has established legislative limits.

The introduction of the so-called Green Revolution Rht1 and Rht2 semidwarfing alleles (re-named Rht-B1b and Rht-D1b respectively) underpinned major advances in agricultural production across the world. However, it has become clear that the Rht1 and Rht2 alleles also have negative effects. In particular, susceptibility to FHB is greater in varieties carrying these alleles than in those that do not, the most severe effect being attributed to Rht2. In a recent study of 270 varieties either registered or cultivated in France in the past 20 years, 72% of them contained Rht2 and 22% contained Rht1, while only 6% had neither of them, most of which being EU varieties or Apache derivatives, and are tall.

Despite some recent advances in the breeding of varieties more tolerant to FHB, breeders still have to deal with the negative effects of these semi-dwarfing alleles which prevents major progress. This project aimed to identify alternative PH genes that provide plant breeders with the desired height without compromising resistance to FHB. In addition the goal was to identify genes that increase resistance to FHB, even in the presence of the conventional (Rht1 and Rht2) semi-dwarfing alleles.

### Scientific context

The introduction of Rht1 and Rht2 semi-dwarfing alleles were central to the increased yields achieved in wheat across the world in recent decades. These alleles permitted the application of nitrogen fertilizer to crops to achieve the desired yield and protein content essential for bread making without the risk of plants falling over, a serious problem in earlier tall varieties. Furthermore, varieties carrying these alleles apportion a larger percentage of biomass to

the grain than tall varieties. As a consequence of these important, agronomically beneficial traits conferred by Rht1 and Rht2, most modern varieties carry one of these alleles. Given the importance of these alleles to modern agriculture, it is highly disturbing that evidence has accumulated to show that they may also have negative effects for control of FHB.

Taller plant varieties generally show fewer FHB symptoms compared to shorter ones (Miedaner 1997; Steiner *et al.* 2004). In agreement with this, several QTL studies have found FHB resistance loci associated or coincident with PH QTL (Gervais *et al.* 2003; Steiner *et al.* 2004; Draeger *et al.* 2007; Klahr *et al.* 2007). However, not all height QTLs are coincident with FHB QTL indicating that the relationship between height and FHB is not simply a consequence of disease escape.

Draeger *et al.* (2007) concluded that FHB susceptibility associated with Rht2 results from linkage or pleiotropy rather than an effect of height per se. This was supported by a study involving lines near isogenic for Rht2 and a DH population from a Spark x Rialto cross (Rialto carries the Rht2 allele) which revealed that Rht2 compromised Type 1 resistance either because of tight linkage to FHB susceptibility genes or due to a pleiotropic effect of the Rht2 allele itself (Srinivasachary *et al.* 2008). In a further study it was shown that, under high disease pressure, both Rht1 and Rht2 significantly decreased resistance to initial infection (Srinivasachary *et al.*, 2009). In addition, it has been concluded that the negative effects of these Rht alleles on FHB resistance are most probably due to the function of the genes themselves rather than because of linkage to nearby deleterious genes (Yan *et al.* 2011). A meta-QTL analysis confirmed the relationship between Rht2 and susceptibility to FHB and found that susceptibility at this locus had the smallest confidence interval of all those reported, further indicating that the increase in FHB susceptibility is due to the Rht allele itself rather than a nearby gene (Loffler *et al.*, 2009). Furthermore, a recent publication concluded that the negative influence of Rht2 on FHB resistance is so great that two major FHB resistance QTL are required to counter the adverse effect of Rht2 (Lu *et al.*, 2011). We have recently demonstrated, through the use of wheat and barley Rht near-isogenic and mutant lines that Rht also affects resistance to other diseases of cereals (Saville *et al.*, 2012). The product of the Rht gene is a transcription factor termed DELLA, because of the important regulatory motif in the protein (Peng *et al.* 1999). As well as controlling plant growth in response to GA, these proteins appear to control cell death and, as a response, affect resistance to pathogens. Saville *et al.* (2012) determined that the stabilised DELLA dwarf and semidwarf alleles (such as Rht1 and Rht2) show greater susceptibility towards biotrophic and hemi-biotrophic pathogens but show increased resistance to necrotrophic pathogens. This is a classical trade-off in resistance to pathogens with different lifestyles and illustrates the problems associated with this GA-insensitive class of plant growth genes.

Given the finding that GA-insensitive Rht alleles such as Rht1 and Rht2 have pleiotropic effects on disease resistance, it is important to determine whether alternative PH loci might be found that operate through different mechanisms and not impact on disease resistance. For example, in barley the semidwarf 'uzu' phenotype is conferred by mutation in the gene encoding the receptor (BRI1) of a second plant growth phytohormone, brassinosteroid (Chono *et al.*, 2003). Critically, the 'uzu' allele was not observed to influence resistance to FHB (Yoshida *et al.*, 2005).

Most significantly, a recent study has indicated that the reduced height conferred by Rht8 in wheat is also due to altered

sensitivity to brassinosteroid (Gasperini *et al.* 2012). Interestingly, it has been reported that Rht8 does not have any pleiotropic effect on FHB susceptibility, (although a locus conferring increased FHB susceptibility was present on chromosome 2D at a 10.5 cM distance) (Loffler *et al.*, 2009). Together, these findings indicate that PH alleles functioning through pathways other than GA biosynthesis or signalling may provide the agronomically desired plant stature without compromising disease resistance.

## 2. Materials and methods

### Disease assessment of the Avalon x Cadenza height QTL NILS

Several loci affecting plant height were identified in the UK reference mapping population Avalon x Cadenza. Five loci were identified with three (2A, 2D and 3A) derived from Cadenza that increased plant height and two (3B and 6A) derived from Avalon that increased plant height.

In 2013 near isogenic lines for the 2D and 3A plant height loci were analysed. Lines were selected at random and classed according to whether they carried the Avalon (A) allele or the Cadenza (C) allele at the respective height QTL locus. At JIC a total of 108 lines were tested in both Avalon and Cadenza backgrounds whereas at CETAC sites 38 lines were assessed in the Cadenza background only. Field trials were established at the JIC in UK and at CETAC sites in France: CS 28 (Reclainville), MH 28 (Allonnes), SC 78 (Maule) and SUR 60 (Estrees St Denis). Each experimental plot (2 rows of 1.5m) was replicated twice; 8 checks were added and the plots were randomized: Barok, Bermude, Sokal, Oakley, Rubisko, Renan, Oxebo, and Charger. Maize stubbles were spread after emergence and trials were mist irrigated 3 weeks before flowering to activate *Fusarium* inoculum and up to 1 month after flowering. Numerous traits were scored including height, heading date, lodging, leaf rust, yellow rust, *Septoria* and FHB.

In 2014 and 2015 additional NIL height QTL were available (2A, 3B and 6A). In addition lines were selected as pairs derived from parents heterozygous for the selected height QTL and this allowed pair-wise comparison for each height QTL across sites. These lines were tested both years at the JIC and only in 2014 at CS 28, MH 28 and SC 78 under the same experimental protocol.

### Fusarium head blight assessment of Paragon EMS-derived height mutant lines

A collection of lines derived from cv. Paragon treated with ethylmethanesulfonate (EMS) processed to M4 have been screened for altered plant height. A number of these lines were screened for resistance to FHB to establish whether any lines significantly shorter than the parent exhibited greater resistance to FHB. Trials were established at the John Innes Centre in 2014 and 2015 and at four CETAC sites (CS 28, MH 28, SC 78 and SUR 60) in 2015. The trials at JIC were subjected to spray inoculation with conidia of *F. culmorum* (1 x 10<sup>5</sup> conidia ml<sup>-1</sup>) at mid anthesis and three days later. Trials were mist irrigated for six days.

Limited seed was available at the start of the project for some lines and attempts to produce sufficient seed for field trials was not always successful. Thirty nine lines along with the Paragon parent were assessed at JIC in 2014. Fifty lines were assessed at JIC in 2015 while 34 lines were assessed at CETAC sites in 2015.

### Identifying FHB resistance quantitative trait loci (QTL) in RhtB1-b and RhtD1-b backgrounds

Previous studies have revealed that height reducing alleles at the plant height loci RhtB1 and RhtD1 on chromosomes 4B and

4D respectively significantly increase susceptibility to Fusarium head blight (FHB). Two populations were produced for the current study in which the parents carry the same semi-dwarfing allele (either RhtB1-b or RhtD1-b) but that have contrasting susceptibility to FHB. The Barok and Bermude parent lines both carry RhtD1b but differ markedly in FHB resistance with Barok being more resistant than Bermude. The Oakley and Sokal parent lines both carry RhtB1-b but also differ markedly in FHB resistance with Sokal being much more resistant than Oakley.

A population of 132 individuals was produced by single seed descent for the Barok x Bermude population. This was available at the start of the project and was screened for FHB resistance at 8 trial sites in France in 2014 (CS 28, MH 28 and SC 78), 2015 (CS 28, SC 78 and SUR 60) and 2016 (MH 59 and SC 78) under the same experimental design as described before.

A population of 81 genotypes was produced by doubled haploid method for the Oakley x Sokal population. This population was completed within the lifetime of the project and was screened for FHB resistance in 7 trial sites in France in 2015 (CS 28, MH 28, SC 78 and SUR 60) and 2016 (CS 28, MH 28 and SC 78).

DNA was extracted from individual lines of both populations and subjected to analysis to identify single nucleotide polymorphisms (SNPs) using the Affymetrix 'Breeder chip' array consisting of 35,000 SNP markers. DNA marker data of sufficient quality for use in the construction of genetic maps was obtained from 115 genotypes of the Barok x Bermude population and 81 genotypes of the Oakley x Sokal population.

### 3. Results

#### 1) Avalon x Cadenza height QTL :

- 2013

The Cadenza 2D allele conferred reduced susceptibility to FHB at all three sites where disease was recorded except for the second scoring at SC (FHB-SC). Similarly, the Cadenza 3A allele conferred reduced FHB susceptibility at two of the three sites (SC and CS). Note that at the SC site, there was a high level of infection of *Microdochium nivale* on the spikes together with a very high level of *Fusarium graminearum*.

The FHB inoculated trial at JIC clearly demonstrated that the 'Cadenza allele' for both 2D and 3A (which increase height) conferred reduced FHB susceptibility. The effect was greater for 3A than for 2D at the JIC site. (Table 1)

The combination of data from all sites strongly suggests that the 2D and 3A alleles that increase height (derived from Cadenza) confer reduced susceptibility to FHB.

The data is summarised for height and FHB in table 1.

The 2D QTL had no influence on heading date, leaf rust, lodging or Septoria disease. Surprisingly, the 2D QTL had an effect on resistance to yellow rust in several sites/score dates and for the average score, with the Cadenza allele being associated with reduced yellow rust disease levels.

- 2014

The 2D QTL had the most pronounced effect on plant height at JIC reducing height by an average of over 13cm across the two backgrounds. The 3B effect was smaller (almost 10cm) across the two backgrounds while the 2A, 3B and 6A effects were less than 5cm across the two backgrounds. Interestingly both the 2A and 3B effects were much more pronounced in the Cadenza background than in the Avalon background (See Table 1).

Disease in the Avalon background was much greater than that for NILs in the Cadenza background. In general there was a

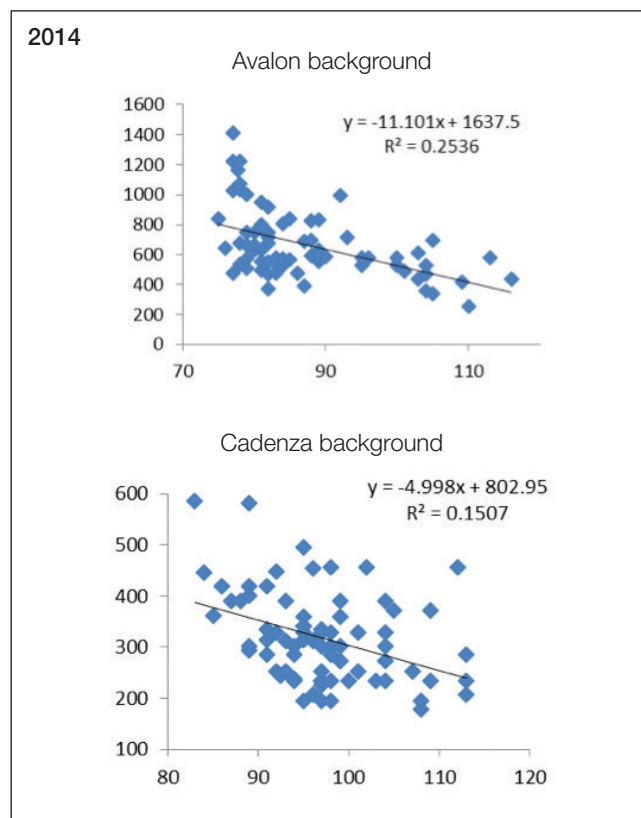
weak correlation between increased plant height and decreased FHB (See Figure 1). For most of the QTL the allele that increased plant height was associated with a decrease in FHB. The exception was 6A where the allele resulting in shorter height was associated with reduced FHB disease. Interestingly both the 2A and 3A QTL had different effects on FHB in the two backgrounds. The 2A allele conferring increased plant height also increased FHB disease in the Cadenza background while the 3A QTL had the same effect in the Cadenza background.

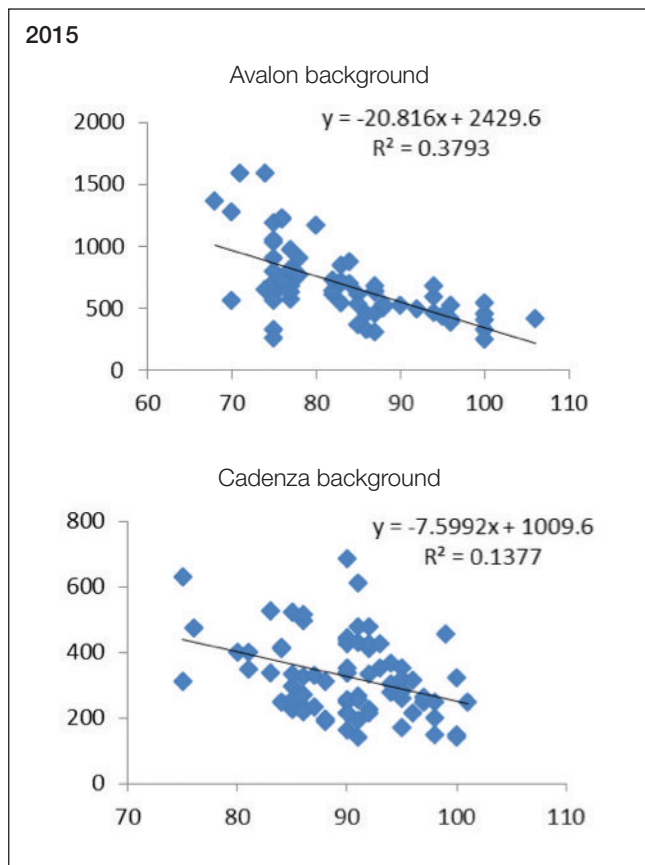
Few significant differences were observed for any of the traits measured at the CETAC sites in 2014. The 2D QTL significantly influenced plant height at all three of the sites (CS 28, MH 28 and SC 78) while the 3B and 6A QTL only significantly influenced height at MH 28. The relative effect of the QTL on plant height was broadly similar at the French and JIC sites. The 2D QTL had the most pronounced effect (average of 12.6 cm) while the 3B effect was again much more pronounced in the Cadenza background (See Table 1). The 2D QTL also influenced the second FHB score at both CS 28 and MH 28 as well as Septoria tritici disease at SC 78.

- 2015

The effects of the QTL on plant height were broadly similar at JIC to those observed in 2014. The 2D QTL had the greatest effect (12.7 cm) with the other QTL being much less potent (3-6 cm). Again, the effect of the 3B QTL was much greater in the Cadenza background than in the Avalon background. Disease in the Avalon background was much greater than that for NILs in the Cadenza background. In general there was a weak correlation between increased plant height and decreased FHB (See Figure 1). Across both backgrounds the allele conferring increased plant height was associated with a decrease in FHB with the exception of the 2A QTL in the Cadenza background (See Table 1).

**Figure 1 :** Regression of FHB disease AUDPC against plant height for Avalon x Cadenza near isogenic lines differing for QTL 2A, 2D, 3A, 3B and 6A.





Background and year	QTL	A versus C allele differential (cm)
Cadenza 2013	2D	7.3
	3A	4.5
Avalon 2014	2A	2
	2D	12.1
	3A	3.2
	3B	-5
Cadenza 2014	6A	-2.9
	2A	4.5
	2D	13.2
	3A	1.6
Cadenza 2014	3B	-16.2
	6A	-6.2
	3B	-16.2

**Table 1** : Effect of five height QTL on plant height and Fusarium head blight disease at sites in France and the UK across 2013, 2014 and 2015.

The 'C' allele for 2D clearly has a major effect in increasing height in both backgrounds while the 'C' allele for 3B has a major effect in reducing height primarily in the Cadenza background.

Average height and Fusarium head blight disease (AUDPC) differential for the Avalon x Cadenza NILs across two years (2014, 2015) at the JIC site.

Both backgrounds 2014			
QTL	Height differential	AUDPC differential	AUDPC per cm
2A	-3.17	170.05	-53.5
2D	-13.19	109.23	-8.279
3A	-4.62	121.5	-26.27
3B	9.75	-173.87	-17.83
6A	4.75	51.718	10.88

Avalon background 2014			
QTL	Height differential	AUDPC differential	AUDPC per cm
2A	-0.25	86.75	-347
2D	6	-87.5	-14.58
3A	-3.83	-80.16	-20.91
3B	-5.5	195.75	-35.59
6A	-5.37	-43.56	8.10

Cadenza background 2014			
QTL	Height differential	AUDPC differential	AUDPC per cm
2A	4.25	44.04	10.36
2D	12.08	-98.08	-8.11
3A	5.1	-93.9	-18.41
3B	-14	152	-10.85
6A	-6.4	-10.1	1.58

Both backgrounds 2015			
QTL	Height differential	AUDPC differential	AUDPC per cm
2A	-3.0	5.5	-1.81
2D	-12.7	216.2	-17.0
3A	-5.2	223.3	-43.1
3B	6.0	-207.8	-34.6
6A	3.6	-35.6	-10.0

Avalon background 2015			
QTL	Height differential	AUDPC differential	AUDPC per cm
2A	0.75	-20.6	-27.46
2D	13.85	-277.78	-20.04
3A	4.5	-312.3	-69.4
3B	-1.25	271.2	-216.96
6A	-5.5	39.625	-7.20

Cadenza background 2015			
QTL	Height differential	AUDPC differential	AUDPC per cm
2A	5.25	9.65	1.83
2D	12.83	-132.11	-10.29
3A	5.8	-169.88	-29.28
3B	-10.75	144.4	-13.43
6A	-0.875	31.6	-36.11

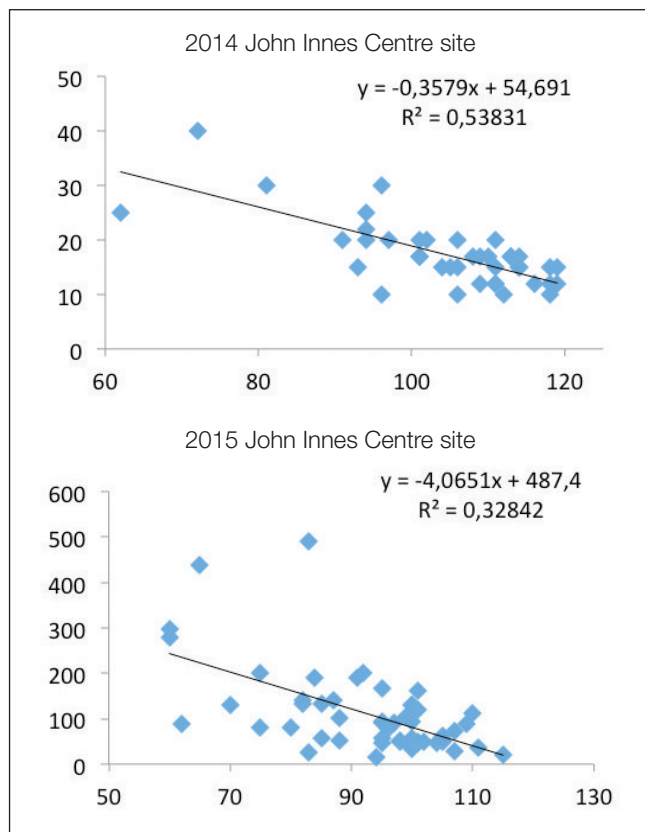
## 2) Fusarium head blight assessment of Paragon EMS-treated lines

In 2014 disease levels were high and FHB developed moderately rapidly. Paragon height was 101cm and the height of the mutant lines ranged from 62 cm (line 2989b) to 119 cm (line 1342a). Taller lines tended to be more resistant than the Paragon parent while shorter lines tended to be more susceptible. The correlation between height and FHB disease was 0.54 (see Figure 2). The line that was most resistant for its height was 1461b which had a FHB score of 10% while the line most susceptible for its height was 2132B with a score of 30%. Both lines were 96cm tall.

In 2015 FHB disease developed very slowly at the JIC site because of very cold weather at anthesis. Paragon height was 97 cm and the height of the mutant lines ranged from 60 cm (line 1011a) to 115 cm (line 192a). As observed in 2014, the taller lines tended to be more resistant than the Paragon parent while shorter lines tended to be more susceptible. The correlation between height and FHB disease was less pronounced than in 2014 ( $R^2=0.33$ ) (see Figure 2). The line that was most resistant for its height was 2603a which had a cumulative AUDPC FHB score of 24.5 while the line most susceptible for its height was 1461b with a score of 490. Both lines were 83 cm tall.

In 2015 FHB was assessed at three CETAC sites (CS, MH and Sec). At the MH site, the range of height differed by 50 cm between the shortest and tallest lines. The Paragon height was 105cm and the height of the mutant lines ranged from 70 cm (line 2914a) to 120 cm (line 2184b). At the Sec site the range of heights was much less (33 cm). Paragon height was 100cm and the height of the mutant lines ranged from 80 cm (line 3442a) to 113 cm (line 1419b). Disease developed at the two sites but the limited number of categories in the scale obscured any relationship between height and disease.

**Figure 2 :** Regression of *Fusarium* scores against plant height for *cv. Paragon EMS mutant lines*.



### 3) Identifying FHB resistance quantitative trait loci (QTL) in *RhtB1-b* and *RhtD1-b* backgrounds.

#### Barok x Bermude population

The genetic map of the Barok x Bermude population consisted of 2483 markers distributed across 47 linkage groups with a total length of 4272 cM.

The population was fairly homogeneous for height (ranging from 73 cm to 89 cm) and flowering date (ranging from 145 days to 150 days). 2014 was a favourable year for *Fusarium* although the disease pressure was medium at MH 28 site. There was already a mixture of *Fusarium graminearum* and *Microdochium* spp. at SC 78 site (fungal analyses, data not shown). The dry conditions in 2015 did not help the expression of FHB in both sites. In 2016, the relatively low temperatures and exceptionally wet conditions at flowering time favoured the development of *Microdochium* spp. to a level where it was often predominant over *Fusarium graminearum*. The Spearman correlation matrix on *Fusarium* scores (data not shown) demonstrated that the scores at SC 78 over the 3 years correlated poorly with the other locations; this is certainly due to the high infection of *Microdochium* spp. in this location.

Year	Trial	FHB score	Effectives		Values			ANOVA	
			Nb. lines	Nb. Rep.	Min	Max	Avg	F value	PR(>F)
2014	CS 28	1	139	2	1	9	4.4	4.21	3.20E-16
2014	MH 28	1	139	2	1	6	3.3	2.22	1.85E-06
2014	MH 28	2	139	2	2	6	4.0	3.21	1.31E-11
2014	SC 78	1	139	2	1	6	3.0	1.32	5.02E-02
2014	SC 78	2	139	2	4	8	6.0	1.64	1.83E-03
2015	CS 28	1	118	1	1	7	3.0		
2015	SC 78	1	139	2	1	5	1.9	2.15	4.39E-06
2015	SC 78	2	139	2	2	7	3.6	1.64	1.84E-03
2016	MH 59	1	133	1	1	4	1.8		
2016	MH 59	2	133	1	1	6	3.0		
2016	SC 78	1	139	2	2	7	5.2	2.33	5.16E-07
2016	SC 78	2	139	2	4	9	6.7	2.67	7.72E-09

**Table 2 :** Descriptive statistics of the Barok x Bermude population FHB trials

Numerous QTL for FHB resistance were identified across the different trials (1B, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 5D, 6A, 6D, 7A, 7B, 5B-7B and 7D). In most instances these QTL were of minor effect (explaining less than 10% of the phenotypic variance). The FHB resistance conferring alleles were contributed by both parents although the majority were contributed by the resistant parent (Barok) (See Table 3).

FHB resistance QTL derived from Barok were observed on 12 linkage groups with more than one QTL being present on 2B, 2D, 5A and 7A. However most QTL were observed only at single trial sites or at single score dates. One of the 2B QTL (219-306 cM) was observed at three sites and accounted for up to 16% of the phenotypic variance. The second 2B QTL (0-16 cM) was also observed more than once. It is unclear whether the effect conferred is due to the presence of two independent QTL or reflects inherent imprecision in locating the peak markers associated with the trait.

The FHB QTL of greatest magnitude were detected on chromosome 2D. In 2014 at the MH site the QTL positioned at 10cM and accounted for 23% of the variance while in 2016 a QTL on “D” was located at 110 cM and accounted for 15% of the variance. The FHB resistance was derived from Barok in both cases and was observed at the first score date suggesting that it may be considered to be a ‘type 1’ resistance.

FHB resistance QTL derived from Bermude were observed on 10 linkage groups with more than one QTL being present on chromosomes 1B and 4A. FHB QTL were generally observed only at single locations or at single score dates but the Barok-derived FHB resistance on 1B was observed at all three score dates at the MH site in 2014 as well as at the first score date at the SC site in 2016. In some instances the ‘phase’ of the QTL was different for different sites or scores. For example, the QTL on 2B between 132 and 148 cM and the QTL on 4A at 41-47 cM both had the resistance associated with both parents (depending upon the trial). It was noted that in such instances the outlying origin was observed using the data from the second replicate of the 2015 trial at the SC site.

A single height QTL was detected on chromosome 3A in this population with Barok contributing the allele for greater height. Curiously, this allele was also associated with greater FHB susceptibility for one set of data (2015 SC site FHB first score). No QTL for flowering time were detected within this population.

QTL of major effect for foliar diseases were detected in this population. A QTL for yellow rust resistance derived from Bermude accounted for up to 59% of the variance and was observed at three sites across different years. QTL of minor effect for yellow rust resistance were also observed on chromosomes 6D and 7B with Bermude and Barok, respectively contributing the resistance.

Barok contributed a QTL on 7B of major effect (21% variance accounted for) for brown rust resistance at the SC site in 2016. Barok also contributed a QTL on 2B of major effect (18% variance) for *Septoria tritici* resistance while Bermude contributed two QTL of minor effect on 5A and 6A.

Marker	Chrom. Position (cM)	Log(10)	Max. % Expl.	High Allele
AX-94758722_1BL	1B (3.2 cM)	4.19	11.75	Barok
AX-95160899_2BS	2B (278 cM)	6.07	16.99	Bermude
AX-95024486_2DL	2B (16 cM)	3.25	14.50	Bermude
AX-94574405_5AL	5A (129 cM)	4.6	10.33	Bermude
AX-95008466_7AL	7A (66 cM)	5.58	13.11	Bermude

**Table 3 :** Main QTL identified in the Barok x Bermude population for *Fusarium head blight* (FHB).

### Oakley x Sokal population

The genetic map of the Oakley x Sokal population consisted of 927 markers distributed across 57 linkage groups with a total length of 3784 cM.

The range of height (from 68 cm to 90 cm) in this population was wider than the previous one. Flowering date range was very large and varied from 144 days to 158 days, which may be a problem to assess FHB properly. Weather conditions and *Fusarium* and *Microdochium* spp. occurring on the heads were the same as described above. Due to a very low infection, the ANOVA showed no varietal effect at the CS 28 site in 2015. The Spearman correlation matrix on *Fusarium* scores (data not shown) demonstrated that the 2015 SC 78 score and MH 28 score 2 correlated poorly with the locations in 2016 where *Microdochium* spp. was predominant.

Year	Trial	FHB score	Effectives		Values			ANOVA	
			Nb lines	Nb. Rep.	Min	Max	Avge	F value	PR(>F)
2015	CS 28	1	103	2	1	6	2.2	1.13	2.67E-01
2015	MH 28	1	100	2	2	7	3.2	3.90	3.20E-11
2015	MH 28	2	100	2	2	7	4.5	2.81	2.37E-07
2015	SC 78	1	96	2	1	9	3.7	5.55	1.08E-15
2016	CS 28	1	93	1	1	9	5.8		
2016	MH 28	1	89	2	2	6	2.9	2.48	1.40E-05
2016	MH 28	2	88	2	2	9	5.0	9.62	2.20E-16
2016	SC 78	1	92	2	2	7	4.4	4.58	1.2E-12
2016	SC 78	2	92	2	4	9	6.5	6.63	2.2E-16

**Table 4 :** Descriptive statistics of the Barok x Bermude population FHB trials

Fewer FHB QTL were detected in the Oakley x Sokal population than in the Barok x Bermude population with QTL detected on chromosomes 2B, 3A, 3B, 6D 7A, and 7B-5B. Almost all the FHB resistance derived from Sokal. A QTL on 2B of major effect was observed at the MH site for the first score date of 2016. This QTL was not observed at any other occasion. Chromosome 3A conferred FHB resistance QTL at 113 cM that was derived from Sokal and accounted for up to 42% of the variance. This QTL was detected at numerous sites and in different years. It is highly unusual to detect a QTL for FHB resistance of such potency in European winter wheat varieties. 3A also contributed a QTL from Sokal of lesser magnitude in different trials or at different score dates being positioned at 162-170 cM. These may represent a single QTL or represent the effects of independent QTL acting at different times or under different environmental conditions.

A QTL derived from Sokal on chromosome 7A was observed at the SC site in 2016 being more pronounced in effect at the second score date (27% variance accounted for). The linkage

group identified as 7B-5B also carried a FHB QTL with resistance derived from Sokal. The position of the peak marker for this QTL was between 137 and 139 cM and the QTL accounted for up to 23% of the observed variance.

QTL for resistance to foliar diseases were detected in various years and at different trial sites. Chromosome 1B appeared to carry two QTL of great potency against yellow rust with the resistant allele derived from Sokal in both cases. The QTL located at approximately 36-39 cM was detected in three trials (MH in 2015, SUR in 2015 and MH in 2016). This QTL accounted for up to 56% of the observed variance and probably reflects the presence of a so-called R gene for yellow rust resistance in Sokal. A second QTL of similar magnitude was detected at 115-119 cM at both score dates in the SUR trial in 2015. The detection of the two potent QTL on 1B in the same trial indicates that they may represent distinct loci. QTL for yellow rust resistance were also detected on chromosome 4B, 6A and 6D with resistance conferred by both parents (Oakley - 4B and Sokal 6A and 6D). A single QTL of major effect on brown rust resistance was detected on chromosome 4A at 33 cM accounting for 72% of the observed variance suggesting the effect of a major gene, probably a conventional so-called R-gene.

QTL for resistance to *Septoria tritici* were also detected in this population. Four QTL derived from Sokal were present on 1A, 1B (two QTL) and 6D. While the 1A and 6D QTL were of minor effect, both the 1B QTL were of major effect accounted for up to 34% of the observed variance. A QTL for *Septoria tritici* resistance was detected on 3A with resistance derived from Oakley. This QTL was coincident with one for heading date where Oakley contributed the allele for later heading and resistance to *S. tritici*. The same locus also contributed to FHB resistance for the first score date at the MH site in 2016.

QTL for plant height were also detected in this population. Chromosome 3A had a QTL at 129-133 cM with the allele for greater height contributed by Sokal. This location is only 10 cM from the major FHB QTL. However the effect on plant height was only observed at two sites whereas that for FHB was observed at 5 sites suggesting that the FHB resistance is not pleiotropic to the plant height allele. Oakley contributed three plant height QTL (3B, 7A and 7B-5B). While the 3B and 7A effects were minor, the QTL on 7B-5B accounted for up to 43% of the observed variance. The peak-marker for this QTL was approximately 40-70 cM distant from that for FHB resistance suggesting that the two are independent. Furthermore the allele for greater plant height and increased susceptibility to FHB both derived from Oakley indicating that the effect on FHB is not due to some form of escape.

QTL for heading and flowering time were also detected. The major effect for heading and flowering time was observed on 5A at approximately 85 cM. Curiously two additional QTL for flowering time were detected on this chromosome with one positioned at 4 cM and one at 28 cM with the allele for later flowering contributed for the former by Sokal and for the latter by Oakley. Additional QTL for heading date were detected on 5B at 53 cM and 109 cM, again with the allele for later flowering contributed for the former by Sokal and for the latter by Oakley.

Marker	Chrom. Position (cM)	Log(10)	Max. % Expl.	High Allele
AX-94433044_3AS	3A (113cM)	8.66	41.91	Oakley
AX-94540502_3DL	3A (117 cM)	4.75	20.69	Oakley
AX-94571885_1DL	6D (20 cM)	6.03	21.36	Oakley
AX-94465240_7BL	7B/5B (139 cM)	5.12	23.69	Oakley

**Table 5 :** Main QTL identified in the Oakley x Sokal population for *Fusarium head blight* (FHB).

## 4. Discussion

### Effect of plant height QTL on FHB and other diseases

Across the different years and trial sites the 2D and 3B QTL had the most significant effect on plant height (See Table 1). The 2D QTL had the greatest influence on plant height and appeared to be equally potent in both the Avalon and Cadenza backgrounds. Curiously, the effect of the 3B QTL was much greater in the Cadenza background than in the Avalon background (See Table 1). The 2A, 3B and 6A had much less effect on plant height and the effect of the 2A QTL was greater in the Cadenza than in the Avalon background.

In general, the allele conferring increased plant height was also associated with reduced FHB disease. The main exceptions to this were the 6A QTL across both backgrounds in 2014 and the 2A QTL in the Cadenza background in both 2014 and 2015.

### Effect of EMS-induced alteration of plant height on FHB resistance

No EMS line was observed to express very high levels of resistance to FHB in any trial. In general, the taller lines exhibited more resistance than the shorter ones. If it is assumed that lines altered in plant height may be altered in other genes in a random manner then it might be concluded that the relationship between plant height and disease resistance to FHB is at least in part due to some passive form of disease escape. This view is supported by the moderate correlation between plant height and FHB disease observed at the JIC site in both 2014 and 2015. However, many of the shorter plants appear to be affected for general fitness. Many of these lines produced fewer seed during the bulking process and fewer plants in field trial plots. Overall, the results from this investigation indicate that, while taller plants tend to be more resistant to FHB than shorter plants a relatively large proportion of FHB resistance may still be independent of plant height.

### QTL analysis of FHB resistance of populations with parents differing in FHB susceptibility but fixed for RhtB1-b or RhtD1-b

The two populations differed markedly in the apparent genetic basis for the differential FHB resistance of the parents.

The larger, single seed decent-derived, population of Barok x Bermude (both RhtD1-b) was subjected to more field trials than

the smaller doubled-haploid derived Sokal x Oakley (both RhtB1-b) population. This potentially provided more opportunity to identify QTL associated with the differential resistance of the parents. The FHB QTL identified in the Barok x Bermude population tended to be of small effect, observed only at single or very few locations and were often ill defined for position on the linkage group. Furthermore, both parents appeared to contribute alleles for greater FHB resistance. These results suggest that the differential FHB resistance of these two varieties is due to the action of a relatively large number of genes of small effect. In contrast to the resistance to FHB, the genetic basis of differential resistance against yellow rust, brown rust and *Septoria tritici* appeared to be due to the action of relatively few genes of major effect.

The differential resistance of Sokal and Oakley to FHB appeared to be relatively simply inherited with QTL of major effect being present on linkage groups ascribed to chromosomes 3A, 6D and 7B-5B. The alleles for greater FHB resistance all originated from Sokal and they were relatively robust, being observed across years and trial sites. Again, QTL of major effect against yellow rust, brown rust and *Septoria tritici* foliar diseases were also observed in this population.

The QTL analysis reveals loci where the two parents differ in the alleles that they carry against FHB. It is not possible to determine whether the Sokal parent carries particularly potent alleles or whether the Oakley parent carries particularly ineffective alleles relative to other varieties. However, comparison of the markers associated with FHB resistance in Sokal with those of other varieties may indicate whether Sokal carries potentially interesting alleles that might be used on breeding programmes through use of marker-assisted selection.

The QTL for foliar diseases often accounted for a very large proportion of the variance indicating that they are so-called R-genes. Two QTL for yellow rust resistance on 1B of Sokal were of particular significance because they co-localised with those for resistance to *Septoria tritici*. Additional research will be required to establish whether the same genes are functioning against the two pathogens. It is conceivable that the effect observed at the SUR site reflects confusion between symptoms for yellow rust and *S. tritici* blotch where those identified for the latter might actually be yellow rust lesions in which the spores have been lost.

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