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Type II Fusarium head blight susceptibility factor identified in wheat

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Short title

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Highlight

We have identified a Type II Fusarium head blight susceptibility factor on the short arm of wheat chromosome 4D and refined its position to a 31.7 Mbp interval.

Abstract

Fusarium head blight (FHB) causes significant grain yield and quality reductions in wheat and barley. Most wheat varieties are incapable of preventing FHB spread through the rachis, but disease is typically limited to individually infected spikelets in barley. We point inoculated wheat lines possessing barley chromosome introgressions to test whether FHB resistance could be observed in a wheat genetic background. The most striking differential was between 4H(4D) substitution and 4H addition lines. The 4H addition line was similarly susceptible to the wheat parent, but the 4H(4D) substitution line was highly resistant, which suggests that there is an FHB susceptibility factor on wheat chromosome 4D. Point inoculation of Chinese Spring 4D ditelosomic lines demonstrated that removing 4DS results in high FHB resistance. We genotyped four Chinese Spring 4DS terminal deletion lines to better characterise the deletions in each line. FHB phenotyping indicated that lines del4DS-2 and del4DS-4, containing smaller deletions, were susceptible and had retained the susceptibility factor. Lines del4DS-3 and del4DS-1 contain larger deletions and were both significantly more resistant, and hence had presumably lost the susceptibility factor. Combining the genotyping and phenotyping results allowed us to refine the susceptibility factor to a 31.7 Mbp interval on 4DS.

Key Words

Fusarium, scab, susceptibility, wheat, barley, aneuploid, deletion

Abbreviations

Days post inoculation (dpi)

Deoxynivalenol (DON)

DON-3-*O*-glucoside (D3G)

Ditelosomic (DT)

Fusarium head blight (FHB)

Fraction length (FL)

Quantitative trait locus (QTL)

UDP-glucosyltransferase (UGT)

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1 # Introduction

2 Fusarium head blight (FHB) is an economically important fungal disease of various
3 cereal crop species, in particular wheat (*Triticum aestivum*) and barley (*Hordeum*
4 *vulgare*). In wheat, the primary symptom is the premature bleaching of spikelets that
5 progressively spreads through the head. Infected spikelets produce shrivelled and
6 chalky grain, which can have a significant impact on yield. Furthermore, mycotoxins
7 accumulate in infected grain, which are harmful to humans and animal consumers.
8 The most important mycotoxin is deoxynivalenol (DON) which acts as a virulence
9 factor in wheat by promoting the spread of the fungus (Bai *et al*., 2002; Langevin *et*
10 *al*., 2004). *Fusarium graminearum* and *F. culmorum* are the most prevalent species
11 responsible for FHB. Both species are capable of producing large quantities of DON
12 (Scherm *et al*., 2013) and hence tend to be the most aggressive pathogens of wheat.

13 Resistance to initial infection (Type I) and to the spread of infection through the
14 rachis (Type II) were first proposed by Schroeder and Christensen (1963) and
15 remain the two most widely considered forms of resistance. Numerous small-effect
16 Type II and fewer Type I FHB quantitative trait loci (QTL) have been reported and
17 are reviewed by Buerstmayr *et al.* (2009) and more recently by Buerstmayr *et al.*
18 (2019). In addition to these two main types of FHB resistance, there is resistance to
19 kernel infection (Type III), host tolerance to FHB and/ or DON (Type IV) and
20 resistance to the accumulation of DON (Type V) (Boutigny *et al*., 2008; Gunupuru *et*
21 *al*., 2017). Single amino acid changes to the DON target, ribosomal protein L3
22 (RPL3), have been demonstrated to improve tolerance to DON in yeast and hence
23 this is a possible target to improve type IV resistance (Lucyshyn *et al*., 2007;
24 Mitterbauer *et al*., 2004). Type V resistance is commonly considered to be a
25 component of Type II resistance, as it typically limits disease spread (Gunupuru *et*
26 *al*., 2017), and can be subdivided into Class 1: processes that chemically modify
27 DON to a less toxic form, and Class 2: processes that prevent the accumulation of
28 DON and other trichothecene mycotoxins (Boutigny *et al*., 2008). The most widely
29 reported form of host detoxification of DON is by UDP-glucosyltransferase (UGT)
30 proteins, which glucosylate DON to the less toxic DON-3-*O*-glucoside (D3G)
31 (Poppenberger *et al*., 2003). More recent studies have identified other pathways
32 capable of detoxifying DON. For example, bacterial aldo-keto reductases were

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33 demonstrated to be involved in epimerising DON to 3-*epi*-DON (Hassan *et al.*, 2017;
34 He *et al.*, 2017).

35 Wheat and barley differ noticeably in Type II resistance. Wheat typically possesses
36 some degree of Type II susceptibility whilst, in contrast, barley is generally highly
37 resistant to fungal spread through the rachis (Langevin *et al.*, 2004). Furthermore,
38 whilst DON has been shown to function as a virulence factor in wheat (Langevin *et*
39 *al.*, 2004), DON does not appear to possess such a role during infection of barley
40 heads (Maier *et al.*, 2006).

41 The reasons for this marked difference in Type II susceptibility of wheat and barley
42 are not well understood. Defined genetic stocks of wheat containing all or part of
43 barley chromosomes offers an insight into which barley chromosomes contribute
44 most strongly to Type II FHB resistance and whether this resistance can be
45 expressed, and potentially utilised, in a wheat genetic background. Herein, we report
46 on a series of experiments to establish whether this difference in FHB susceptibility
47 is because barley carries genes conferring resistance, wheat carries genes
48 conferring susceptibility, or whether it is a combination of both factors. Following this,
49 we investigated the location of a major effect identified on wheat chromosome 4D
50 that appears to significantly compromise resistance to disease spread through the
51 rachis (Type II resistance).

52 To date, there have been few reports of FHB susceptibility factors. Garvin *et al.*
53 (2015) identified a spontaneous deletion of a portion of the long arm of 3D, which
54 appeared to be responsible for increased FHB resistance, suggesting that the
55 deleted region carries an FHB susceptibility factor in the cultivar Apogee. Ma *et al.*
56 (2006) point inoculated the existing ditelosomic lines of Chinese Spring that each
57 lack individual chromosome arms. They found that the loss of individual
58 chromosome arms can improve, as well as compromise, FHB resistance (Ma *et al.*,
59 2006). Their data suggested that some chromosome arms, especially 7AS, 3BL,
60 7BS and 4DS, are likely to contain FHB susceptibility factors (Ma *et al.*, 2006).
61 Although the gene(s) underlying *Fhb1*, the most widely deployed FHB resistance
62 QTL, remains controversial, there is evidence that *Fhb1* may be considered a
63 disrupted susceptibility factor (Su *et al.*, 2019; Su *et al.*, 2018). Plant hormones play
64 an important role in responding to disease. Host response to FHB infection is

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65 particularly sensitive to disrupting phytohormone production or perception. Plants
66 insensitive to ethylene and brassinosteroid signalling exhibits increased FHB
67 resistance, suggesting that the fungus is exploiting such physiological processes
68 (Chen *et al.*, 2009; Goddard *et al.*, 2014). There is significant potential in identifying
69 and characterising susceptibility factors, with the aim of eliminating them from elite
70 cultivars to enhance resistance to FHB and other economically important diseases.

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71 # Materials and Methods

72 ## Plant material

73 Wheat-barley addition, substitution and translocation lines were developed at the
74 Hungarian Academy of Sciences, Agricultural Institute, Centre for Agricultural
75 Research, Hungary (**Table 1**). An independent set of wheat-barley addition lines, of
76 the wheat variety Chinese Spring and the barley donor variety Betzes, were
77 generated by Islam *et al.* (1981) and obtained from the Genetic Resources Unit at
78 the John Innes Centre, Norwich, UK.

79 Chinese Spring and its 4D ditelosomic (DT) lines were acquired from the Germplasm
80 Resource Unit, John Innes Centre, Norwich, UK. The lines DT(4DL) and DT(4DS)
81 lack 4DS and 4DL, respectively. Four homozygous Chinese Spring terminal deletion
82 lines of 4DS, described by Endo and Gill (1996), were obtained from Kansas State
83 University, USA. The lines acquired were 4532 L1 (FL= 0.53), 4532 L2 (FL= 0.82),
84 4532 L3 (FL= 0.67) and 4532 L4 (FL= 0.77), henceforth referred to as del4DS-1,
85 del4DS-2, del4DS-3 and del4DS-4, respectively.

86 ## Marker development and genotyping

87 Homoeologue nonspecific markers were designed to simultaneously amplify
88 fragments of homoeologous genes on 4A, 4B and 4D. Sequence information of 4D
89 genes and corresponding homoeologous genes were obtained from Ensembl Plants
90 (http://plants.ensembl.org/Triticum_aestivum/Info/Index). Gene names and the
91 physical positions reported correspond to the IWGSC RefSeq v1.1 wheat genome
92 assembly (IWGSC, 2018). Sequence insertions and deletions (indels) between
93 homoeologous gene sequences were exploited to enable distinction of the three
94 resulting PCR products. Forward primers were M13-tailed to enable incorporation of
95 a fluorescent adaptor to PCR products, as described by Schuelke (2000). 37
96 markers designed as such were used to characterise the deletions in four Chinese
97 Spring 4DS terminal deletion lines (**Table 2**).

98 DNA was extracted from freeze-dried leaf tissue as described by Pallotta *et al.*
99 (2003). PCR reactions were prepared using HotStarTaq Mastermix (Qiagen)
100 following the manufacturer’s instructions and amplified using the following steps: 95
101 °C 15 min; 35 cycles of: 95 °C 1 min, 58 °C 1 min, 72 °C 1 min; 72 °C 10 min. PCR
102 products were separated using an ABI 3730xl DNA analyser (Applied Biosystems)

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103 and resolved using Peak Scanner 2 software (Applied Biosystems). Up to five
104 markers were multiplexed following PCR to increase assay efficiency.

105 Primers were designed to specifically amplify within a 5H barley UGT-
106 glucosyltransferase (HORVU5Hr1G047150), whilst avoiding amplification of wheat
107 orthologues (primer sequences: GATGAGGTTTGAGATTTGCGGA,
108 CACGAGCACAACAGATGAATTCA). PCR reactions were prepared using Taq
109 Mastermix (Qiagen) and amplified using the following PCR settings: 94°C 3 min; 35
110 cycles of: 94 °C 30 sec, 58 °C 30 sec, 72 °C 1 min; 72 °C 10 min. PCR products
111 were separated on a 0.8 % w/v agarose gel.

112 **FHB evaluation and statistical analysis**

113 Highly virulent DON-producing isolates of *F. graminearum* or *F. culmorum* were used
114 in disease experiments. Production of inoculum was carried out as described
115 previously in Gosman *et al.* (2005). Wheat heads were inoculated at mid-anthesis.
116 The conidial suspension, adjusted to 1 $*10^6$ spores ml^{-1}, was injected in to a spikelet
117 approximately central on the wheat head. The spread of disease symptoms was
118 scored regularly after inoculation. Polytunnel experiments were organised in a
119 randomised complete block design with four replicates each containing four or five
120 plants per line. For the glasshouse experiment, at least 16 plants per lines were
121 randomised and individual inoculated heads were considered as replicates.

122 Disease data were analysed using a linear mixed model (REML) in Genstat software
123 (v18.1) to assess the variation attributable to line (fixed), inoculation date (fixed), the
124 interaction between line and inoculation date (fixed), and replicate (random), where
125 factors were significant in the model. Data from which residuals were not normally
126 distributed or where residuals did not appear independent of fitted values were log10
127 transformed, which was sufficient in correcting for these assumptions. Predicted
128 mean and standard error values were calculated for lines included in the REML.
129 Pairwise comparisons were made between the wild type wheat parent/ genetic
130 background and the other genotypes tested in each experiment using Fisher's
131 protected least significant difference. All predicted values generated from
132 transformed data were back transformed to the original scale for presentation.

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133 **DON evaluation and statistical analysis**

134 DON was purified to > 98 % at IFA-Tulln, as described by Altpeter and Posselt
135 (1994). DON application was carried out on wheat spikes at mid-anthesis, following a
136 protocol modified from Lemmens *et al.* (2005). Two adjacent spikelets opposite to
137 each other on the wheat head and approximately central on the head, were cut with
138 scissors approximately central on the spikelet. 1- 2 h after cutting, 10 µL of DON
139 solution (10 mg / mL amended with 0.01 % v/v Tween 20) was applied to the two
140 outer florets of each cut spikelet, between the palea and lemma. To increase the
141 humidity at the site of DON application, treated wheat heads were bagged. At 48 h
142 post-application, the DON application was repeated, and heads bagged again.
143 Hence, each treated wheat head received a total application of 0.8 mg DON. After a
144 further 48 h, crossing bags were removed from the DON treated heads. The severity
145 of bleaching for each treated wheat head was scored, out of ten, daily between five
146 and nine days post application (from the first application). A score of zero was given
147 when no evidence of DON damage was present and a score of ten was recorded
148 when the spike was completely bleached above the point of DON application. Scores
149 between one and nine were used to record the progressive yellowing and bleaching
150 of the DON treated wheat heads, which occurred relatively uniformly above the point
151 of DON application in the case of Chinese Spring (**Figure S1**). After the experiment,
152 DON-treated and untreated heads from each plant were harvested. From each plant
153 with a DON treated head, a comparable untreated head (with similar spikelet number
154 and head length) was selected for grain weight analysis. Grain number and grain
155 weight data were collected from DON treated and comparable untreated heads from
156 each plant, to observe any difference in the effect of DON on grain filling.

157 DON bleaching data and associated grain data were analysed using a REML. Both
158 DON bleaching data and grain data were log10 transformed to achieve normality of
159 residuals and to ensure residuals were independent of fitted values. For bleaching
160 data, line was included as a fixed term and replicate as a random term in the model.
161 For DON grain data, the REML model was constructed using line, treatment (DON
162 treated or untreated heads), and the interaction between line and treatment as fixed
163 terms, and replicate as a random term. Ratios between mean treated and untreated
164 values were calculated by subtracting the predicted mean of log10 DON treated
165 heads from the predicted mean of log10 untreated heads for each line. Standard

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166 errors of predicted means were calculated as the square root of the sum of the
167 squared standard errors of the predicted mean values. The calculated mean and
168 standard error values were back transformed, resulting in the presentation of DON
169 treated/ untreated mean grain weight ratios for each line.

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170 # Results

171 **Effect of barley chromosome additions, substitutions, translocations**
172 **and centric fusions on type II FHB susceptibility in the winter wheat**
173 **variety Martonvasari 9 (Mv9kr1)**

174 FHB point inoculation experiments of the wheat-barley material were conducted
175 twice and are described as experiment 1 (**Figure 1A**) and experiment 2 (**Figure 1B**)
176 henceforth. The experiments showed very similar results for most of the lines tested.
177 FHB symptoms were always restricted in both barley varieties, Igri and Betzes, and
178 did not spread from the inoculated spikelet. For this reason, Igri and Betzes were
179 only included as control lines in experiment 1 (**Figure 1A**). The primary wheat
180 parent, Mv9kr1, was susceptible to the spread of the fungus in both repeats of the
181 experiment.

182 The addition of barley chromosomes 2H (2H add) and 6HS (6HS add) appeared to
183 have no effect on FHB resistance in either experiment. Disease symptoms in these
184 lines were not statistically significantly different from that of Mv9kr1. The 6BS.6BL–
185 4HL translocation (6B-4H trans) was significantly more susceptible than Mv9kr1 ($p < 0.001$ in both experiments). Whilst the 3HS.3BL centric fusion line (3HS.3BL centric)
187 was more highly susceptible in experiment 1 ($p < 0.001$), the line showed similar
188 disease to Mv9kr1 in experiment 2 ($p = 0.566$). The addition of chromosomes 1HS
189 (1HS add) and 7H (7H add), in addition to the 5HS-7DS.7DL wheat-barley
190 translocation (5H-7D trans) and the 2DS.2DL-1HS translocation line (2D-1H trans)
191 all showed highly significant increases in FHB resistance compared to Mv9kr1 ($p < 0.001$ in both experiments for all lines). The 3H addition (3H add) was inconsistent
193 between the two experiments. In experiment 1, the 3H addition was significantly
194 more susceptible to FHB than Mv9kr1 ($p = 0.004$) whilst, in experiment 2, it was
195 significantly more resistant ($p < 0.001$).

196 A particularly strong resistant phenotype was seen with the 4H(4D) substitution, in
197 which disease was almost entirely restricted to the inoculated spikelet in both
198 experiments ($p < 0.001$ in both instances). In contrast to this, the addition of barley
199 4H (4H add) showed similar disease levels to Mv9kr1 in experiment 1 ($p = 0.841$,
200 **Figure 1A**) and exhibited only a small increase in resistance in experiment 2 ($p = 0.021$, **Figure 1B**).

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202 **Effect of barley chromosome additions, substitutions, translocations**
203 **and centric fusions on type II FHB susceptibility in the spring wheat**
204 **variety Chinese Spring**

205 An FHB point inoculation experiment was performed on wheat-barley addition lines
206 of the varieties Chinese Spring and Betzes, respectively (**Figure 2**). These lines
207 include addition lines of 5HS and 5HL, which were absent in the lines generated in
208 the Mv9kr1 wheat background. As previously observed, Betzes showed almost no
209 disease spread from the inoculation point. Chinese Spring, on the other hand,
210 showed evidence of disease spread. FHB symptoms in the majority of addition lines
211 were not significantly different from Chinese Spring. The addition lines carrying the
212 barley chromosome arms 2HL, 6HS, 7HL and 7HS all showed significantly increased
213 FHB susceptibility compared to Chinese Spring.

214 The 5HL addition line exhibited significantly increased FHB resistance when
215 compared with Chinese Spring ($p < 0.001$), although the line was still significantly
216 more susceptible than Betzes ($p = 0.042$). The 5HS addition line was also statistically
217 significantly more resistant compared to Chinese Spring ($p = 0.039$). A marker
218 targeting the barley UDP-glucosyltransferase gene, HORVU5Hr1G047150,
219 confirmed that this gene was present in Betzes and the 5HL addition line, but was
220 absent in the 5HS addition line (**Figure S2**). Consistent with the previous
221 experiments, the 4HL and 4HS addition lines both showed similar FHB susceptibility
222 to Chinese Spring.

223 **Type II FHB susceptibility and DON susceptibility in Chinese Spring 4D**
224 **ditelosomic lines**

225 The contrast in the effect of adding 4H or substituting 4D with 4H indicated that the
226 presence of 4D may be responsible for a significant proportion of the susceptibility of
227 both Mv9kr1 and Chinese Spring. To test this possibility, Chinese Spring and two
228 ditelosomic lines: DT(4DL) and DT(4DS), missing 4DS and 4DL, respectively, were
229 tested in three independent FHB point inoculation experiments. Data is presented
230 here from a 2013 experiment conducted in a glasshouse, but the results were
231 replicated in a 2013 experiment under controlled conditions and in a polytunnel
232 experiment conducted in 2016. Chinese Spring and DT(4DS), missing 4DL, showed
233 very similar disease symptoms to each other (**Figure 3**). In contrast to this, DT(4DL),

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234 missing 4DS, was highly resistant to the spread of infection when compared to wild
235 type Chinese Spring ($p < 0.001$).

236 DON is widely believed to contribute towards Type II susceptibility by promoting the
237 spread of FHB. Hence, it is possible that the susceptibility factor may be responding
238 to DON and not the fungus itself. To confirm whether DON is involved, we applied
239 purified DON to wheat heads of Chinese Spring and two ditelosomic lines; DT(4DL)
240 and DT(4DS). Chinese Spring was moderately susceptible to DON, with an average
241 bleaching score of 3.39 (**Figure 4A**). DT(4DS), lacking 4DL, was not significantly
242 different from Chinese Spring (mean= 2.88; $p= 0.222$) (**Figure 4A**). On the other
243 hand, DT(4DL), lacking 4DS, was significantly more susceptible to DON induced
244 bleaching (mean= 7.64; $p< 0.001$) (**Figure 4A**).

245 Grain was harvested and dissected from DON treated and untreated heads to
246 assess any difference in grain weight. These data closely mirrored the bleaching
247 data. Chinese Spring and DT(4DS) showed similar reductions in grain weight when
248 comparing DON treated and untreated heads (mean ratios of 0.522 and 0.506,
249 respectively) (**Figure 4B**). In contrast, grain of DON treated DT(4DL) heads had a
250 proportionally much greater reduction in grain weight compared to untreated heads
251 (mean ratio= 0.290) (**Figure 4B**). The difference is evident when visually comparing
252 treated and untreated grain from the three lines; treated grain from DT(4DL) are
253 visibly smaller than those of Chinese Spring and DT(4DS) (**Figure 4C**).

254 These data suggest that DON is not implicated in the function of the susceptibility
255 factor. However, there does appear to be an independent DON resistance factor also
256 on 4DS.

257 Precise characterisation of deletion sizes in Chinese Spring 4DS
258 terminal deletion lines

259 Experiments using 4D ditelosomic lines strongly suggest that the FHB susceptibility
260 attributed to chromosome 4D is isolated to the short arm (4DS). Genotyping was
261 performed on four Chinese Spring lines with terminal deletions on 4DS to verify the
262 deletions present and more precisely position the deletion breakpoint in each line
263 relative to the physical map. Markers were designed that can reliably detect genes
264 on 4D and their homoeologues on 4A and 4B. The ability to detect and distinguish all
265 three homoeologues provides two internal positive controls for each marker when

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266 identifying deletions of any particular homoeologue. Up to five markers, tagged using
267 different fluorophores (NED, FAM, PET or VIC), were multiplexed into a single
268 sample for efficiency, using markers designed to produce PCR product sizes
269 sufficiently different for each gene target and its respective homoeologues when
270 resolved using capillary electrophoresis (**Figure 5**).

271 Genotyping was successful in identifying genes, and their respective physical
272 positions, flanking the deletion breakpoint in all four 4DS terminal deletion lines
273 (**Table 3**). A marker (BH0001) targeting the gene TraesCS4D02G001400 at the
274 extreme distal end of 4DS confirmed that all four lines were true terminal deletions.
275 The terminal deletion in del4DS-2 extends to between 50.6 and 51.6 Mbp. Line
276 del4DS-4 is deleted up to between 53.9 and 54.8 Mbp. The deletion in del4DS-3
277 ends between 83.3 and 85.6 Mbp. The deletion breakpoint in the largest terminal
278 deletion line, del4DS-1, ends between 111.1 and 140.9 Mbp.

279 **Chinese Spring 4DS terminal deletion lines and type II FHB susceptibility**

280 Euploid Chinese Spring and the four Chinese Spring 4DS terminal deletion lines
281 genotyped (del4DS-2, del4DS-4, del4DS-3 and del4DS-1, in ascending order of
282 terminal deletion size) were point inoculated in a polytunnel experiment in 2017
283 (**Figure 6**). Chinese Spring showed moderate levels of disease in this experiment,
284 with mean disease above the inoculation point of 1.84 bleached spikelets at 13 dpi.
285 Lines del4DS-2 ($p = 0.796$) and del4DS-4 ($p = 0.278$) showed similar disease levels to
286 that of euploid Chinese Spring (**Figure 6** and **Figure 7**). Lines del4DS-3 and del4DS-
287 1 both had significantly reduced disease with respect to euploid Chinese Spring ($p <$
288 0.001 for both lines) (**Figure 6** and **Figure 7**).

289 This information was used to infer that the susceptibility factor was present in the two
290 deletion lines carrying the smaller deletions (del4DS-2 and del4DS-4) but was lost in
291 the two lines containing the larger deletions (del4DS-3 and del4DS-1). Hence, the
292 FHB susceptibility factor appears to reside between the deletion breakpoints of
293 del4DS-4 and del4DS-3; a 31.73 Mbp interval (**Figure 8**).

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294 # Discussion

295 Previous studies have shown that barley is able to detoxify DON through
296 glucosylation by the UDP-glucosyltransferase UGT13248 (Schweiger *et al.*, 2010).
297 This gene has been transgenically expressed in Arabidopsis where it was
298 demonstrated to increase resistance to DON (Schweiger *et al.*, 2010). Furthermore,
299 expression of UGT13248 in wheat, under the maize ubiquitin promoter, increased
300 FHB resistance and transformants were demonstrated to more efficiently convert
301 DON to the less toxic DON-3-*O*-glucoside (Li *et al.*, 2015). However, Xing *et al.*
302 (2018) demonstrated that overexpression of a wheat UGT-glucosyltransferase also
303 increased FHB resistance and reduced the DON concentration in grain. How the
304 barley UDP-glucosyltransferase performs in wheat under its native barley promoter
305 has not yet been demonstrated and hence the increase in resistance attributed to the
306 barley UGT-glucosyltransferase in wheat may be due to overexpression. The barley
307 UDP-glucosyltransferase UGT13248 is encoded by gene HORVU5Hr1G047150
308 which is present near the centromere on chromosome 5H (Ensembl Plants). If the
309 breakpoints in the wheat - barley 5HS and 5HL ditelosomic addition lines are not
310 centromeric, this may explain the findings related to the high level of resistance
311 conferred by addition of both 5HS and 5HL. To confirm this, we designed primers
312 specific to the barley copy of the UDP-glucosyltransferase and will not amplify from
313 the orthologous wheat copies in the wheat-barley additions. This assay confirmed
314 that the UDP-glucosyltransferase was isolated to the 5HL addition line and was
315 absent in the 5HS addition line. Hence, it is likely that an independent source of FHB
316 resistance is present on 5HS.

317 In this study, we also found that addition of the barley chromosome 7H (7H add) or
318 the short arm of chromosome 1H (1HS add), as well as the translocation of 1H to 2D
319 (2D-1H trans), significantly increased Type II FHB resistance in the winter wheat
320 variety Mv9kr1. Despite the enhanced FHB resistance from the addition of 7H to
321 Mv9kr1, the addition of neither 7HS nor 7HL had an effect in the Asian spring wheat
322 cultivar Chinese Spring. No 1H addition lines were available in the Chinese Spring-
323 Betzes addition set, so this could not be compared between populations. These
324 findings suggest that barley contains genes conferring Type II resistance that are
325 lacking in one or both wheat varieties. The addition of barley chromosomes 5H and

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326 perhaps 1H and 7H are likely to offer the best opportunity of enhancing FHB
327 resistance, when considering the use of wheat-barley introgressions.

328 We confirmed the presence of a possible Type II susceptibility factor on the short
329 arm of 4D in three independent experiments. The loss of 4DS (line DT(4DL))
330 resulted in a high level of FHB resistance, whilst the loss of 4DL (line DT(4DS))
331 resulted in little change compared to euploid Chinese Spring. Ma *et al.* (2006)
332 phenotyped Chinese Spring ditelosomic lines for FHB susceptibility and they also
333 reported an increase in FHB resistance in the line missing 4DS. Together, these
334 studies strongly suggest the presence of a susceptibility factor in both winter
335 (Mv9kr1) and spring (Chinese Spring) wheat genetic backgrounds. We applied
336 purified DON to the 4D ditelosomic lines to test whether or not the susceptibility
337 factor is being influenced by DON. However, the loss of 4DS resulted in higher
338 susceptibility to DON, assessed both by scoring DON induced bleaching and by
339 comparing grain weights. This would indicate that there is an independent resistance
340 factor to DON present on 4DS and that the susceptibility factor is increasing
341 susceptibility to the fungus or another virulence factor.

342 Endo and Gill (1996) developed a set of terminal deletion lines in Chinese Spring.
343 The lines have deletions from the ends of each chromosome arm, varying in size.
344 These stocks are a valuable resource for physically mapping genes to a defined
345 interval of a chromosome arm. The lines were characterised using C-banding and
346 the deletion size reported as a fraction length (FL) value; effectively the proportion of
347 the chromosome arm estimated to have been retained. C-banding is unlikely to be
348 capable of reliably detecting more complex deletions, such as interstitial deletions or
349 chromosome substitutions. Since their development, the Chinese Spring terminal
350 deletion stocks have not been more precisely characterised using more recent
351 advancements in genotyping. We have genotyped four lines containing terminal
352 deletions of 4DS, using a total of 37 novel homoeologue nonspecific markers
353 spanning the chromosome arm. These markers take advantage of the hexaploid
354 nature of wheat to create a robust genotyping assay for the detection of deletions on
355 4DS, and its homoeologous regions on 4BS and 4AL. A similar assay was used by
356 Chia *et al.* (2017) to verify deletions across homoeologous regions but this study
357 expands on this technique, using a much higher density of markers to characterise
358 deletion size. Homoeologous genes are simultaneously amplified with a single pair of

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359 primers but are distinguishable due to differences in the size of PCR products
360 corresponding to the A, B and D genome copies. The signal from the retained
361 homoeologues act as internal controls for a deletion in any homoeologue; in this
362 case, the 4D copy. This technique verified that all four Chinese Spring 4DS terminal
363 deletion lines were indeed true terminal deletions and the size of the deletions were
364 consistent with the FL values calculated by Endo and Gill (1996). For the lines
365 del4DS-2, del4DS-4 and del4DS-3, the physical position of the deletion endpoint has
366 been restricted to a small interval. For both del4DS-2 and del4DS-4, this interval is
367 smaller than 1 Mbp. The interval containing the deletion endpoint in del4DS-3 has
368 been refined to approximately 2.3 Mbp. The breakpoint in the largest deletion,
369 del4DS-1, was less precisely characterised and the deletion breakpoint was isolated
370 to a 29.8 Mbp interval. For the purposes of this study, it was not necessary to more
371 precisely characterise the deletion in del4DS-1, because the FHB susceptibility
372 factor appears to be situated between the deletion breakpoints in lines del4DS-4 and
373 del4DS-3.

374 We performed FHB disease experiments on the four Chinese Spring 4DS terminal
375 deletion lines that we genotyped. This clearly demonstrated that the lines with the
376 two smaller deletions, del4DS-2 and del4DS-4, retained the susceptibility factor and
377 showed a similar phenotype to euploid Chinese Spring. In contrast the lines del4DS-
378 3 and del4DS-1, containing the larger deletions, showed significantly improved FHB
379 resistance and hence the susceptibility factor has presumably been lost. As the
380 susceptibility factor was present in del4DS-4 but was lost in del4DS-3, it must be
381 situated between the deletion breakpoints of these two lines, restricting the
382 susceptibility factor to a 31.7 Mbp interval containing 274 high confidence genes
383 (IWGSC RefSeq v1.1). The positive effect of the deletion of the susceptibility factor
384 appears to be restricted to 4D and hence it is likely the gene responsible is 4D
385 specific and does not possess homoeologues. BLAST searches of each 4D gene in
386 the interval identified 20 genes that appear to lack homoeologues and hence are 4D-
387 specific. Alternatively, the 4D homoeologue may be preferentially expressed
388 compared to the 4A and 4B copies. It is also possible that the improved FHB
389 resistance is the consequence of altered dosage of the 4D susceptibility factor and
390 its homoeologues. The disrupted balance of a physiological process exploited by the
391 fungus is also likely to result in altered disease susceptibility.

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392 A population possessing smaller deletions is required to further refine the position of
393 the FHB susceptibility factor. We intend to utilise a gamma-irradiated population of
394 the UK spring wheat variety Paragon (Shaw *et al.*, 2013; Wheat Genetic
395 Improvement Network, 2019) to improve the resolution for the physical mapping of
396 the FHB susceptibility factor.

397 It may be considered surprising that an FHB susceptibility factor with such a powerful
398 effect has not been detected before now. However, we hypothesise that the FHB
399 susceptibility factor is highly conserved among wheat cultivars. The susceptibility
400 factor exists both in the Hungarian winter wheat cultivar Martonvasari 9 and in the
401 Asian spring wheat variety Chinese Spring. Preliminary experiments of gamma
402 irradiated Paragon lines, containing a deletion of the entire 31.7 Mbp FHB
403 susceptibility interval, indicated this line possesses potent resistance and hence
404 confirms that the susceptibility factor is also present in the UK spring cultivar
405 Paragon (data not shown). If there was sufficient allelic variation at the locus, the
406 effect of the susceptibility factor is likely to have been detected as an FHB QTL in
407 existing mapping populations. In the absence of such reports, we predict that the
408 FHB susceptibility factor is fixed in both spring and winter wheats.

409 Genetic resistance to fungal diseases is critical to the protection of food crops such
410 as wheat. The search and incorporation of resistance factors is common practice in
411 crop plant breeding. However, identifying novel sources of resistance to FHB is
412 challenging and time consuming. FHB resistance is quantitative, highly polygenic,
413 and often environmentally labile. Few large effect FHB QTL have been identified.
414 Attempts to clone the gene underlying the best known source of FHB resistance, the
415 *Fhb1* QTL, have been inconsistent and controversial (Ma *et al.*, 2017; Rawat *et al.*,
416 2017; Steiner *et al.*, 2017; Su *et al.*, 2017). Rawat *et al.* (2016) reported that they had
417 cloned a pore-forming toxin-like (*PFT*) gene underlying the *Fhb1* QTL. However, Jia
418 *et al.* (2018) disputed the findings of Rawat *et al.* (2016). Su *et al.* (2018) identified
419 that the presence of a deletion at the 5' end of a histidine-rich calcium-binding
420 protein within the *Fhb1* locus was sufficient in identifying varieties carrying *Fhb1*. Su
421 *et al.* (2019) have since reported that *Fhb1* possesses enhanced resistance due to
422 the loss-of-function of the histidine-rich calcium-binding protein and the wild type
423 allele is hence functioning as a susceptibility factor. Li *et al.* (2019) also identified
424 that mutation of the histidine-rich calcium-binding protein as the gene responsible for

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425 *Fhb1* resistance. However, in conflict with the findings of Su *et al.* (2019), their data
426 suggests that this is due to a gain-of-function resulting from an different start codon
427 positioned upstream to the original (Li *et al.*, 2019). Our data on the 3HS-3BL centric
428 fusion line does not suggest that 3BS contains a susceptibility factor, as the line was
429 either wild type-like or more highly susceptible to the spread of FHB. Furthermore,
430 Ma *et al.* (2006) reported that the Chinese Spring ditelosomic line missing 3BS
431 (DT(3BL)) was more susceptible to FHB, which is not compatible with the hypothesis
432 that FHB resistance from *Fhb1* being a loss-of-function susceptibility factor. It
433 remains possible that more than one gene is responsible for FHB resistance
434 conferred by *Fhb1*. Furthermore, it has proven difficult to utilise *Fhb1* in elite varieties
435 in high yielding European environments with few varieties released containing the
436 resistance. This suggests a linkage drag from the resistance or a pleiotropic effect
437 and demonstrates a need for novel methods of conferring resistance, such as
438 eliminating susceptibility factors.

439 Despite this, there has been relatively little research into susceptibility factors in
440 wheat and other cereals and how they may be used in plant breeding. The barley
441 *mildew resistance locus o* (*Mlo*) is one of the earliest and best characterised
442 examples of how disruption of a susceptibility factor could be exploited to improve
443 disease resistance; in this case, to powdery mildew caused by the biotrophic fungus
444 *Blumeria graminis* f. sp. *hordei* (Jorgensen, 1992). Induced and natural mutation of
445 the *Mlo* locus result is a recessive, race nonspecific and durable resistance which
446 has been widely deployed in European spring barley varieties (Jorgensen, 1992;
447 Lyngkjaer and Carver, 2000; McGrann *et al.*, 2014). *Mlo*-based resistance has since
448 been demonstrated in a number of other species affected by powdery mildew,
449 reviewed by Kusch and Panstruga (2017). The deployment of *mlo* in wheat is more
450 challenging due to its allohexaploid nature (Acevedo-Garcia *et al.*, 2017). However,
451 TALENs and CRISPR Cas9- derived gene knockouts (Wang *et al.*, 2014) and *Mlo*
452 TILLING mutants (Acevedo-Garcia *et al.*, 2017) have been used to demonstrate that
453 mutation of all wheat copies strongly enhances resistance to wheat powdery mildew.

454 R genes, usually nucleotide binding site- leucine rich repeat (NBS-LRR) genes, are
455 typically used by plants to detect and respond to attack by biotrophic fungi. However,
456 necrotrophic pathogens have evolved methods of exploiting such plant defences to
457 aid infection. *Parastagonospora nodorum* and *Pyrenophora tritici-repentis* are

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458 necrotrophic pathogens of wheat that utilise this strategy. Susceptibility to these
459 diseases operates in an inverse gene-for-gene interaction, in which a fungal
460 necrotrophic effector is detected by a corresponding host sensitivity gene product
461 (usually an NBS-LRR), triggering a hypersensitive response that results in necrosis
462 that benefits the fungus (Faris *et al.*, 2010). If either necrotrophic effector or host
463 sensitivity gene is absent, the interaction is impossible and host resistance is
464 maintained. There have been few reports of how NBS-LRRs are involved in
465 interactions with *Fusarium* spp. However, Zhang *et al.* (2019) found that the
466 expression of an LRR gene appeared to increase susceptibility to *F. graminearum* in
467 soybean (*Glycine max*).

468 *Fusarium graminearum* leads a hemibiotrophic lifestyle whereby the hyphal front
469 remains surrounded by living tissue but cell death is triggered soon after colonisation
470 (Brown *et al.*, 2010). Phytohormones play important roles in defence and there is
471 considerable evidence indicating that *F. graminearum* modifies phytohormone
472 expression for its own benefit. Disruption of ethylene signalling in wheat (Chen *et al.*,
473 2009) and brassinosteroid signalling in barley and *Brachypodium distachyon*
474 (Goddard *et al.*, 2014) results in enhanced resistance to FHB infection, suggesting
475 that the fungus is exploiting phytohormone signalling in order to aid infection.
476 Expression of 9-lipogenases are also manipulated by *F. graminearum* in both bread
477 wheat and *Arabidopsis thaliana* and are hence operating as susceptibility factors
478 (Nalam *et al.*, 2015).

479 In this study, we provide compelling evidence for the presence of an FHB
480 susceptibility factor on the short arm of chromosome 4D. We have demonstrated that
481 the removal of the susceptibility factor is sufficient to significantly improve Type II
482 FHB resistance and have refined its position to a 31.7 Mbp interval containing 274
483 high confidence genes. We have designed markers that can reliably detect deletions
484 on 4DS. A subset of these markers covering the susceptibility interval will be utilised
485 in further studies to identify lines containing relatively smaller deletions across the
486 FHB susceptibility interval in a gamma irradiated Paragon population. This will
487 reduce the number of gene candidates for the FHB susceptibility and may lead to the
488 identification of the causal gene.

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Tables

Table 1 Wheat-barley addition, substitution, translocation and centric fusion lines used in FHB experiments.
The primary wheat parent was Martonvasari 9 kr1 (Mv9kr1) for all lines and the barley donor parents were Igri or Betzes. Associated references contain detailed descriptions of line generation and composition.

Line abbreviation	**Description**	**Reference**
Mv9kr1	Martonvasari9 *kr1*	MolnarLang *et al.* (1996)
1HS add	Mv9kr1–Igri 1HS disomic addition	Szakacs and Molnar-Lang (2007)
2H add	Mv9kr1–Igri 2H disomic addition	Szakacs and Molnar-Lang (2007)
3H add	Mv9kr1–Igri 3H disomic addition	Szakacs and Molnar-Lang (2007)
4H add	Mv9kr1–Igri 4H disomic addition	Szakacs and Molnar-Lang (2007)
6HS add	Mv9kr1–Igri 6HS disomic addition	Szakacs and Molnar-Lang (2010)
7H add	Mv9kr1–Igri 7H disomic addition	Szakacs and Molnar-Lang (2010)
2D-1H trans	2DS.2DL-1HS translocation	Nagy *et al.* (2002)
3HS.3BL centric	3HS.3BL centric fusion	Nagy *et al.* (2002)
4H(4D) sub	4H(4D) wheat-barley substitution	Molnar *et al.* (2007)
6B-4H trans	6BS.6BL–4HL translocation	Nagy *et al.* (2002)
7D-5H trans	5HS-7DS.7DL wheat-barley translocation	Kruppa *et al.* (2013)

Table 2 Homoeologue nonspecific markers used to genotype four Chinese Spring 4DS terminal deletion lines. Primer sequences, fragment sizes (corresponding to the 4A, 4B and 4D homoeologous gene targets) and the 4D gene target of markers used to characterise the deletion sizes present in four Chinese Spring 4DS terminal deletion lines. The lowercase sequence in the forward primer indicates the M13 tail. All markers amplified at 58 °C annealing temperature.

Marker	Forward primer	Reverse primer	Fragment A; B; D (bp)	4D gene target
BH0001	tgtaaaacgacggccagtTCCTCCAATAAGAAGGTATGTC	TGGCACTGCCCTTATAGCAA	356; 330; 228	TraesCS4D02G001400
BH0002	tgtaaaacgacggccagtTGTCGTTGTTCCAGTTAAAG	TCAGGCGCATCAGACATTTG	205; 172; 163	TraesCS4D02G009200
BH0013	tgtaaaacgacggccagtGGGGAATTGTCCAAAGCGT	TGCAAGAGATGTTGGGATTTT	211; 155; 207	TraesCS4D02G014500
BH0003	tgtaaaacgacggccagtCTCCACTTTATCATTTGAAGACA	ACAAAACCTTTCACATGGCC	452; 264; 491	TraesCS4D02G017300
BH0004.2	tgtaaaacgacggccagtGTGTTCCCATTGTCGCCG	TAGTCCGCCTCCTTGCTCCT	168; 152; 194	TraesCS4D02G035700
BH0025.2	tgtaaaacgacggccagtACAATCCCGAGGTTGCCAGA	CGAAGAGGAGGGCATACATA	275; 359; 378	TraesCS4D02G039400
BH0005.2	tgtaaaacgacggccagtTGGTGCTTCATTATCCTTCTGAT	TGGTGTCCAGAGTAAACTCGATA	443; 448; 319	TraesCS4D02G040700
BH0020	tgtaaaacgacggccagtCGACCTCCTCTCAGCTTTTAG	ATGAGGATACACGGTGCTGC	304; 193; 220	TraesCS4D02G045500
BH0029	tgtaaaacgacggccagtGAGCAGATCTTCAACGTACG	ATCACAAAGGGATGGACCTG	183; 196; 159	TraesCS4D02G050300
BH0024	tgtaaaacgacggccagtAAAGTAAAATCCTCTTCCCTGAG	GCTAAACTTGCTGTCAGACAAG	274; 298; 389	TraesCS4D02G051400
BH0006.2	tgtaaaacgacggccagtGGCCAAGGTGCGTAATCCA	CGCGAGCTGAACACAAGC	265; 121; 313	TraesCS4D02G052300
BH0022	tgtaaaacgacggccagtAGTATTAGGCAATGTGTTCCACT	TGAGAAGGTTCCAAGAACCAAC	288; 459; 260	TraesCS4D02G057100
BH0021	tgtaaaacgacggccagtTCATTCAACATGCAGATCTAGGC	GACAAACTTCAATGGCATAAGC	123; 155; 130	TraesCS4D02G065300
BH0014	tgtaaaacgacggccagtCCATTGCATTCCTTCACTTGT	CGTCGTCCCATACTTCACAAA	110; 113; 107	TraesCS4D02G066900
BH0026	tgtaaaacgacggccagtCGATACACCAGTTAATTGAAATATG	CTAGGAGTTCCTTCATGGACATT	289; 471; 318	TraesCS4D02G073200
BH0015.2	tgtaaaacgacggccagtCACAACTTGTGCAGGTATAACC	GGAAAGTCAAGACAGGCACAA	198; 346; 426	TraesCS4D02G074200
BH0008	tgtaaaacgacggccagtGTATCGACGAAGCCGCAGTT	TTCCGGAGCGTCCTACGACAA	309; 190; 199	TraesCS4D02G074500
BH0040	tgtaaaacgacggccagtGCGCAGTGAGACAAAACTC	AAGTAGAAGAGCAGCGCCAT	442; 448; 451	TraesCS4D02G075300
BH0041	tgtaaaacgacggccagtAACAAATCCATGTGACCCC	CTACAAGGACGCGTGGTTAT	299; 338; 302	TraesCS4D02G076000
BH0042	tgtaaaacgacggccagtCGGACAACATTTCAGGATTTC	ACCGGAACAAGGCTGCAC	379; 135; 125	TraesCS4D02G077600
BH0027.3	tgtaaaacgacggccagtGGTAACATTCCTTTGGTATACTCGG	TGTGCTAAGATCTACAACATC	303; 350; 266	TraesCS4D02G078900
BH0032	tgtaaaacgacggccagtTTGTGGCCTGCTTACATTGC	TGATCTGCAGGTGTTGGC	317; 305; 300	TraesCS4D02G079900
BH0033	tgtaaaacgacggccagtTGCCCGTGTTTTATGCACTG	GGTAAGTAAAATGGGAAGAAAGC	201; 167; 185	TraesCS4D02G081000
BH0034	tgtaaaacgacggccagtCTGCCGTATCTCCAACTC	ATGAGCGCCATCAGGAAC	209; 297; 217	TraesCS4D02G082500
BH0035	tgtaaaacgacggccagtACGCGGACCCGAATTCAAA	TCCTTGGGCATAGAGGAAG	190; 167; 162	TraesCS4D02G083100
BH0036	tgtaaaacgacggccagtATGTTAGCCGTCCTTTGTTTC	TGGCTGACAGCTATACTTCTAGT	246; 255; 223	TraesCS4D02G084000
BH0037	tgtaaaacgacggccagtGACGGACAATTCTTATGATTGTG	TATGTCCTGCCCCTTCTCCAT	191; 187; 166	TraesCS4D02G085100

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Table 2 (continued) Homoeologue nonspecific markers used to genotype four Chinese Spring 4DS terminal deletion lines. Primer sequences, fragment sizes (corresponding to the 4A, 4B and 4D homoeologous gene targets) and the 4D gene target of markers used to characterise the deletion sizes present in four Chinese Spring 4DS terminal deletion lines. The lowercase sequence in the forward primer indicates the M13 tail. All markers amplified at 58 °C annealing temperature.

Marker	Forward primer	Reverse primer	Fragment A; B; D (bp)	4D gene target
BH0038	tgtaaaacgacggccagtATCTGCGTCCAGGTGAGC	TCAGCTAAGACAACTGGCAC	359; 341; 318	TraesCS4D02G085900
BH0009.3	tgtaaaacgacggccagtTAGAGGGAGCAGGGATGACAT	TCTCCGTCTGGTTCATTCGT	106; 103; 111	TraesCS4D02G087200
BH0010.2	tgtaaaacgacggccagtACGTGGTCTTCAAATCTGGC	CTGCAATATAAGGTGGCAAATC	189; 155; 159	TraesCS4D02G098400
BH0017	tgtaaaacgacggccagtCAGATTGTACGAACATCTTCTGC	AGCAGAACAAAATCTCATGG	252; 246; 263	TraesCS4D02G105100
BH0018	tgtaaaacgacggccagtGTGAGCAGAGCACCCTCC	CTGCACCACCACAGAAAAGA	226; 195; 214	TraesCS4D02G107300
BH0011	tgtaaaacgacggccagtATGCTCGTCTTCATCGAGGTAA	ATGCATTGCAGACACATCAAG	128; 160; 135	TraesCS4D02G114700
BH0012.2	tgtaaaacgacggccagtGGTCCTTCATGAAGCTTGTTC	GGCAAATAAGAGAGTTGCATAGG	275; 289; 280	TraesCS4D02G117800
BH0030	tgtaaaacgacggccagtGGCAATGTGATCCTGCAGTTC	GCCCAAAGAAATAGCAAGGGAAA	145; 174; 189	TraesCS4D02G126600
BH0057	tgtaaaacgacggccagtGCACATCCTGCTGTACCA	CTCCTTGGGAATCTTAATGCA	464; 356; 322	TraesCS4D02G147800
BH0058	tgtaaaacgacggccagtCCATTTAGATTCATGGCGAT	AGGCATATTGCAAACCCAAC	190; 315; 179	TraesCS4D02G149800

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Table 3 Flanking genes and markers of deletion breakpoints in four Chinese Spring 4DS terminal deletion lines.
The breakpoint interval is the size of the interval between two adjacent markers where the marker signal was retrieved, indicating the end of the deletion.

Line	Left flank gene	Left flank marker	Right flank gene	Right flank marker	Breakpoint interval (Kb)
del4DS-2	TraesCS4D02G076000	BH0041	TraesCS4D02G077600	BH0042	976
del4DS-4	TraesCS4D02G079900	BH0032	TraesCS4D02G081000	BH0033	949
del4DS-3	TraesCS4D02G105100	BH0017	TraesCS4D02G107300	BH0018	2313
del4DS-1	TraesCS4D02G126600	BH0030	TraesCS4D02G147800	BH0057	29776

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Figure legends

Figure 1 FHB disease above inoculation point in wheat-barley addition, substitution, translocation and centric fusion lines from a) polytunnel experiment 1, including barley parents Igri and Betzes as controls, and b) polytunnel experiment 2. Predicted means were generated using a linear mixed model. Error bars are ± standard error. * p< 0.05; ** p< 0.01; *** p< 0.001 compared to Mv9kr1.

Figure 2 FHB disease, as a percentage of total number of bleached spikelets, from data combined from 13 dpi and 14 dpi. Predicted means were generated using a linear mixed model. Error bars are ± standard error. * p= 0.05-0.01 compared to Chinese Spring; *** p< 0.001 compared to Chinese Spring.

Figure 3 FHB disease at 17 dpi in euploid Chinese Spring and 4D ditelosomic lines DT(4DL) and DT(4DS), missing 4DS and 4DL, respectively. Diagrams of 4D are included above ditelosomic lines to illustrate their genetic state. Error bars are ± standard error. *** p< 0.001 compared to Chinese Spring.

Figure 4 DON application experiment to heads of Chinese Spring and ditelosomic lines DT(4DL) and DT(4DS), lacking 4DS and 4DL, respectively. a) average DON bleaching scores at 7 days post application. Predicted means were generated using a linear mixed model. Error bars are ± standard error. p< 0.001 compared with Chinese Spring. b) ratio of DON treated/ untreated mean grain weight above the DON application point, or comparable point in untreated heads, dissected after the experiment. Ratios were calculated by subtracting the log10 mean grain weight of DON treated heads from untreated heads for each line, followed by back transformation to obtain a treated/untreated ratio for each line. Predicted means were generated using a linear mixed model. Error bars are ± standard error. c) photograph showing three representative examples of untreated and DON treated grain taken from above the DON application point, or comparable point in untreated heads for each line.

Figure 5 Example outputs of five multiplexed markers BH0014 (left black), BH0030 (blue), BH0018 (red), BH0017 (green) and BH0026 (right black) in a) Chinese Spring; b) del4DS-2; c) del4DS-4; d) del4DS-1. The line del4DS-3 showed the same deletion pattern for the markers visible in the selected multiplex and was hence omitted. X axis is fragment size (bp) and Y axis is the strength of fluorescence (relative fluorescence units). Images were extracted as screenshots from Peak Scanner 2 software (Applied Biosystems).

Figure 6 FHB disease above the inoculation point at 13 dpi, following point inoculation of euploid Chinese Spring and four terminal deletion bins; del4DS-2, del4DS-4, del4DS-3 and del4DS-1. Error bars are ± standard error. *** p< 0.001 compared to Chinese Spring.

Figure 7 Representative FHB disease symptoms in the Chinese Spring terminal deletion lines del4DS-4 and del4DS-3 at 16 dpi.

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Figure 8 Diagrams of 4DS in euploid Chinese Spring and four 4DS terminal deletion lines, as characterised by genotyping with 35 markers spanning 4DS. The spotted interval indicates the breakpoint interval; the distance between two markers where the 4D signal was retrieved. The bottom diagram indicates the interval on 4DS inferred to contain an FHB susceptibility factor (diagonal stripes), following point inoculation of the Chinese Spring terminal deletion lines. Values in bold indicate the physical position in Mbp.

a)

Infected spikelets above

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

*** ** *** *** *** *** *** *** *** ***

Betzes
1HS add
2H add
3H add
4H add
6HS add
7H add
Mv9kr1
4H(4D) sub
3HS.3BL centric
2D-1H trans
7D-5H trans
6B-4H trans
Igri

Line

b)

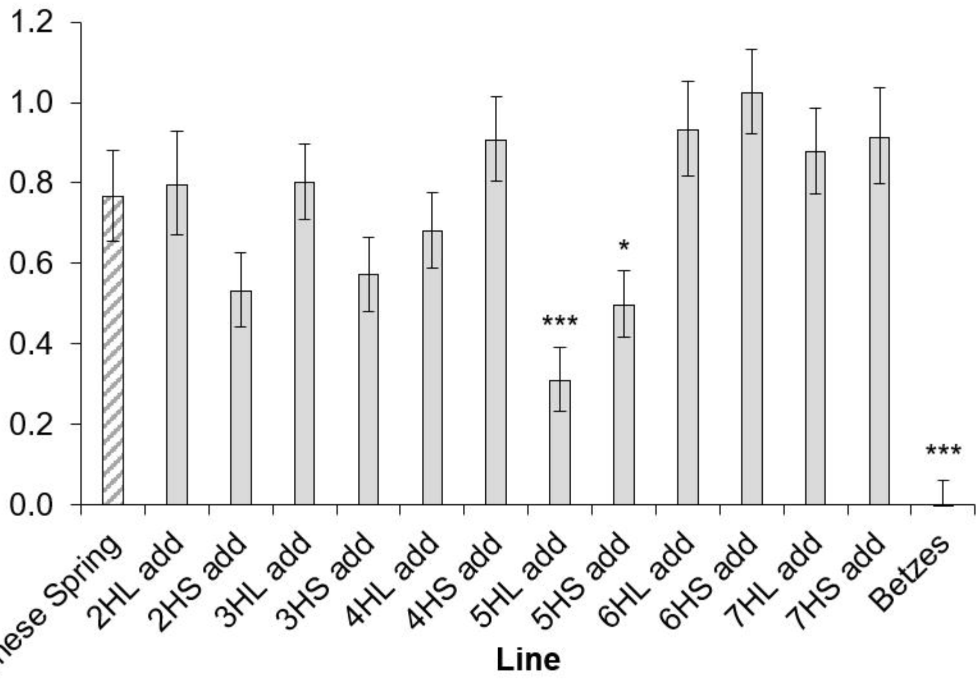
Infected spikelets above

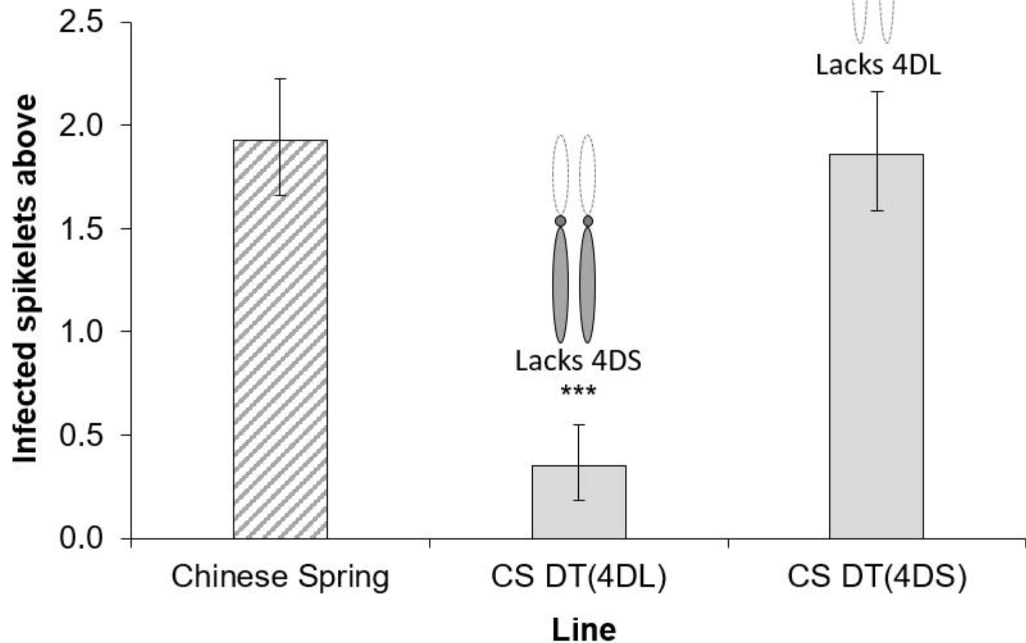
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

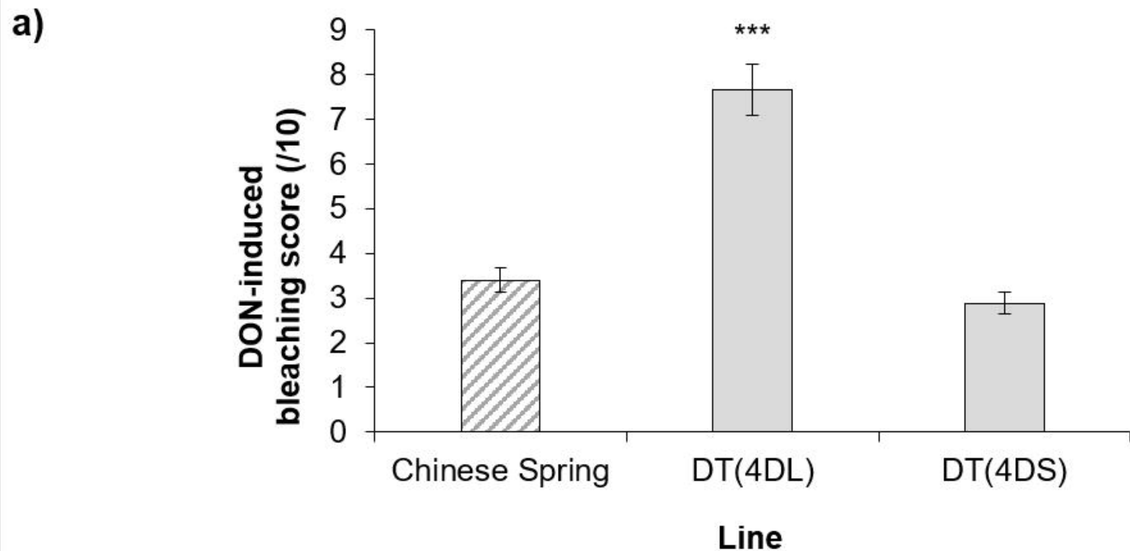
*** *** * *** *** *** *** ***

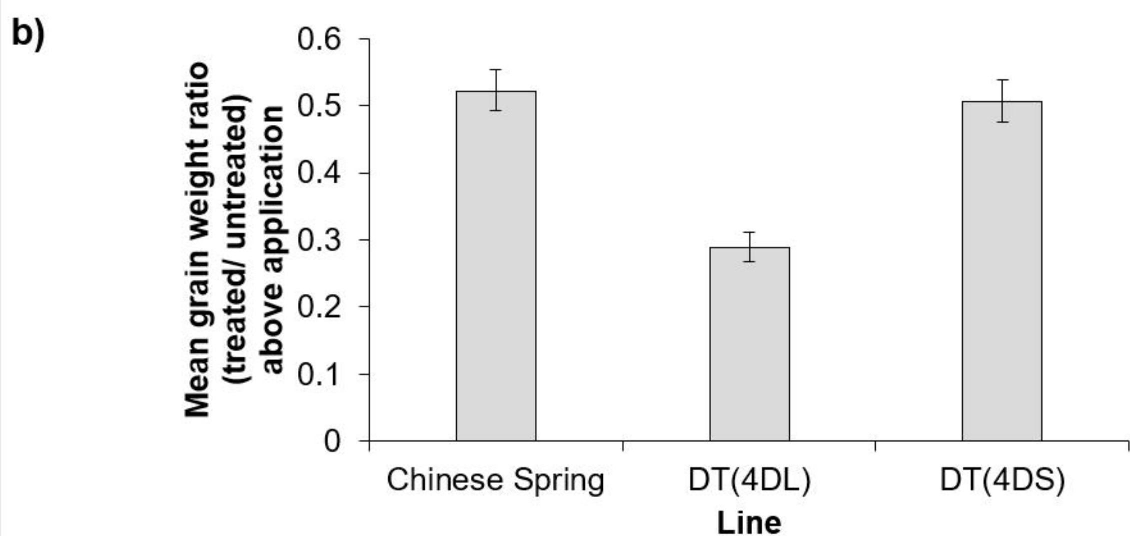
1HS add
2H add
3H add
4H add
6HS add
7H add
Mv9kr1
4H(4D) sub
3HS.3BL centric
2D-1H trans
7D-5H trans
6B-4H trans

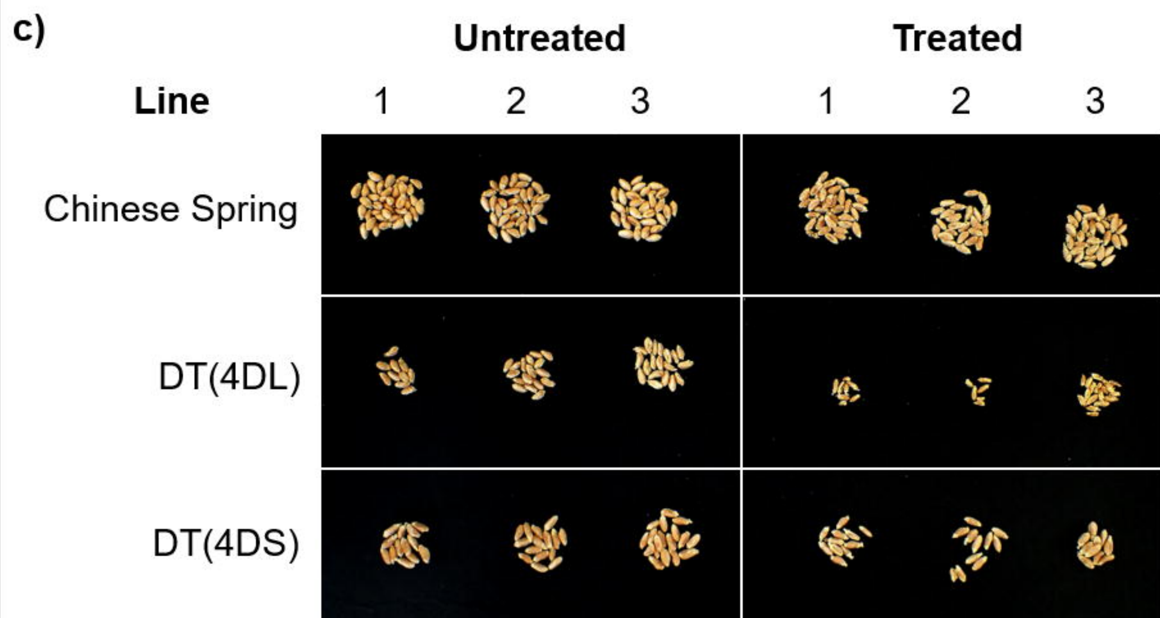
Line


Infected spikelets above
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
*
***
Chinese Spring
2HL add
2HS add
3HL add
3HS add
4HL add
4HS add
5HL add
5HS add
6HL add
6HS add
7HL add
7HS add
Betzes
Line

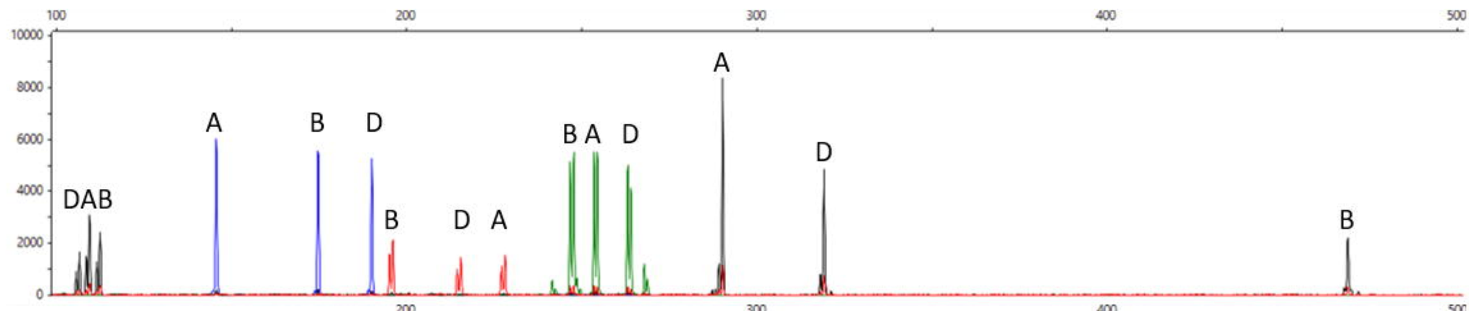

Infected spikelets above
2.5
2.0
1.5
1.0
0.5
0.0
Lacks 4DL
Lacks 4DS
***
Chinese Spring
CS DT(4DL)
CS DT(4DS)
Line


a)
DON-induced bleaching score (/10)
9
8
7
6
5
4
3
2
1
0
***
Chinese Spring
DT(4DL)
DT(4DS)
Line

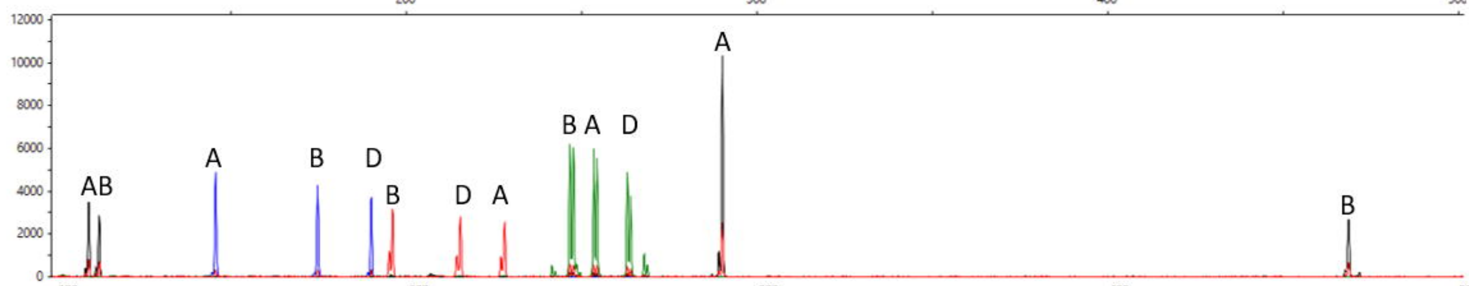

b)
Mean grain weight ratio (treated/ untreated) above application
0.6
0.5
0.4
0.3
0.2
0.1
0
Chinese Spring
DT(4DL)
DT(4DS)
Line


c)
Untreated
Treated
Line
1
2
3
1
2
3
Chinese Spring
DT(4DL)
DT(4DS)

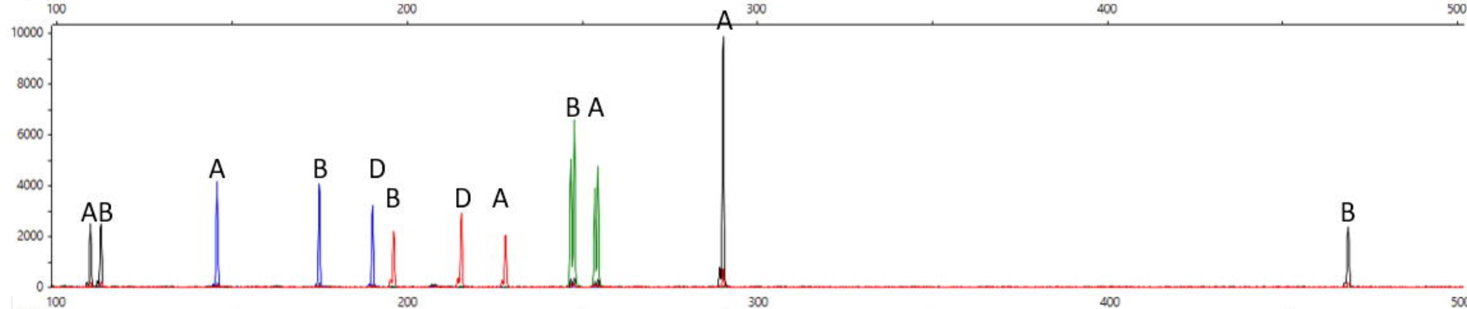
a) Chinese Spring



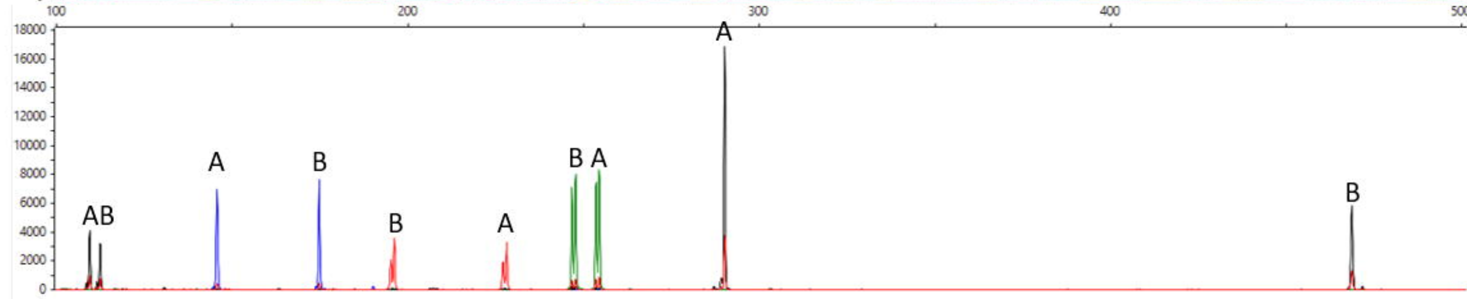
b) del4DS-2

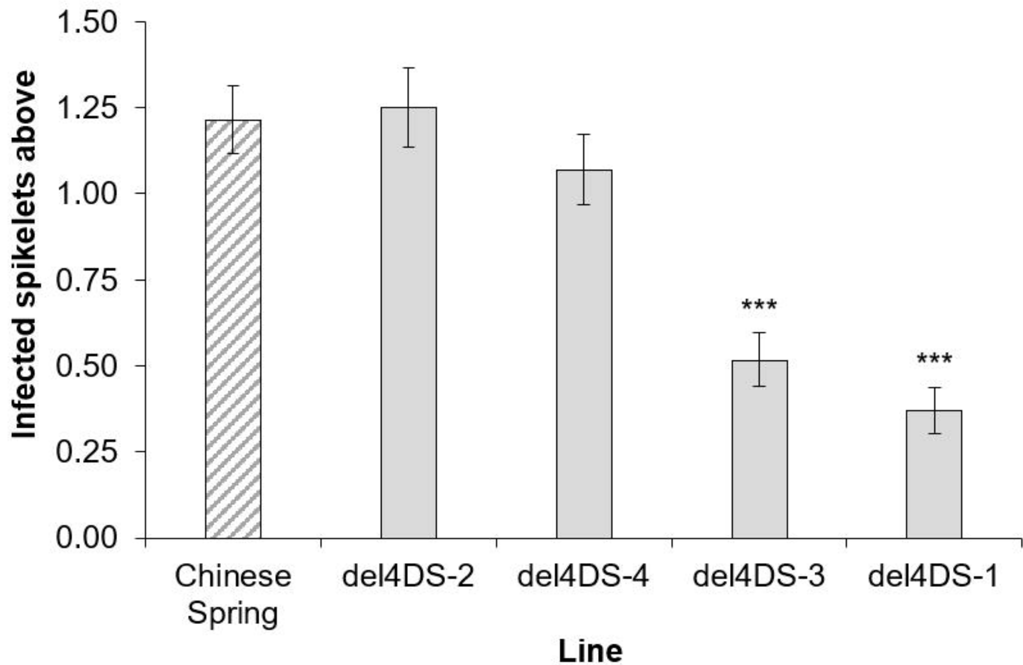


c) del4DS-4



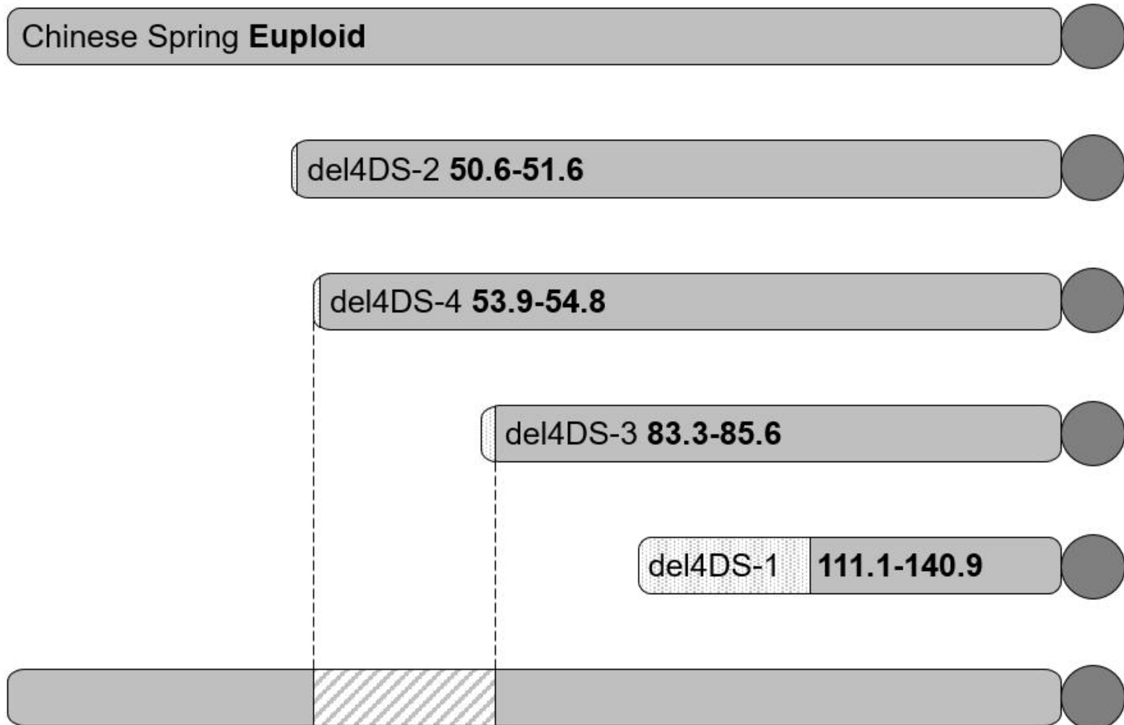
d) del4DS-1




Infected spikelets above
1.50
1.25
1.00
0.75
0.50
0.25
0.00
***
***
Chinese Spring
del4DS-2
del4DS-4
del4DS-3
del4DS-1
Line

a)
del4DS-4

b)
del4DS-3


20 Mbp
Centromere
Chinese Spring Euploid
del4DS-2 50.6-51.6
del4DS-4 53.9-54.8
del4DS-3 83.3-85.6
del4DS-1
111.1-140.9
Susceptibility interval
53.9-85.6