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1 Reduced height alleles (*Rht*) and Hagberg falling number of wheat

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12 *Keywords: Rht, wheat, Hagberg falling number, Ppd*

13

14 *Abbreviations: DF, degrees of freedom; DH, doubled haploid; GA, gibberellic acid; HFN, Hagberg falling*

15 *number; I, intensive farming system; LMA, late-maturity alpha-amylase; NIL, near isogenic line; O, organic*

16 *farming system; PHS, pre-harvest sprouting; Ppd, photoperiod response allele; QTL, quantitative trait loci;*

17 *REML, residual maximum likelihood; Rht, reduced height allele; SED, standard error of difference*

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22 ABSTRACT

23 Near isogenic lines varying for alleles for reduced height (*Rht*) and photoperiod insensitivity (*Ppd-D1*) in cv.
24 Mercia (2005/6 to 2010/11; *rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht8c+Ppd-D1a*, *Rht-D1c*, *Rht12*) and cvs
25 Maris Huntsman and Maris Widgeon (2007/8 to 2010/11; *rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht-B1b+Rht-*
26 *D1b*, *Rht-D1b+Rht-B1c*) were compared at one field site, but within different systems ('organic', O, 2005/6 to
27 2007/8 v 'intensive', I, 2005/6 to 2010/11). Further experiments at the site (2006/7 to 2008/9) compared
28 64 lines of a doubled haploid (DH) population [Savannah (*Rht-D1b*) × Renesansa (*Rht-8c+Ppd-D1a*)].
29 Gibberellin (GA) insensitive dwarfing alleles (*Rht-B1b*; *Rht-B1c*; *Rht-D1b*; *Rht-D1c*) could reduce α -amylase
30 activity and/or increase Hagberg falling number (HFN) but effects depended greatly on system, background
31 and season. Only *Rht-B1c* increased grain dormancy despite producing plants taller than *Rht-D1c*. The GA-
32 sensitive *Rht8c+Ppd-D1a* in Mercia was associated with reduced HFN but analysis of the DH population
33 suggested this was more closely linked with *Ppd-D1a*, rather than *Rht8c*. The severe GA-sensitive dwarfing
34 allele *Rht12* was associated with reduced HFN. Instability in HFN over season tended to increase with degree
35 of dwarfing. There was a negative association between mean grain weight and HFN that was in addition to
36 effects of *Rht* and *Ppd-D1* allele.

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38

39 1. Introduction

40

41 Hagberg falling number (HFN) is a quality criterion of bread-making wheat because of its negative
42 association with α -amylase activity (Perten, 1964). Doughs formed from flour with excessive α -amylase are
43 sticky and difficult to process, and when baked produce discoloured loaves that are poorly structured
44 (Chamberlain et al., 1982). Immoderate levels of α -amylase are most commonly produced in pre-harvest
45 sprouting (PHS) or as late-maturity α -amylase (LMA) (Lunn et al., 2001). Pre-harvest sprouting follows a loss
46 of grain dormancy and subsequent germination whilst in the ear, often in response to wet conditions
47 occurring between grain ripeness and harvest (Barnard, 2001), and also as might occur in lodged crops. Late
48 maturity α -amylase occurs in the absence of visible sprouting (Mares and Mrva, 2008). Low HFN or high α -
49 amylase activity in the absence of visible sprouting has been variously associated with: low temperatures
50 and/or high soil moisture during the linear phase of grain filling (Gooding et al., 2003; Gooding, 2010); slow
51 grain drying rate; abrupt temperature changes during grain filling; large grain size and mass; low specific
52 weights, and grain cavity characteristics (Clarke et al., 2004; Evers et al., 1995; Farrell and Kettlewell, 2008,
53 2009; Kindred et al., 2005; Mares and Mrva, 2008). There are strong genotype x environment (Gooding,
54 2010), and genotype x agronomy (Kindred et al., 2005) interactions on HFN.

55 Reduced height (*Rht*) alleles (Gale and Youssefian, 1985) are incorporated in wheat breeding programmes
56 to produce semi-dwarf wheats (Flintham et al., 1997a). The gibberellin (GA)-insensitive alleles *Rht-B1b* and
57 *Rht-D1b* (from Norin 10, syn. *Rht1* and *Rht2*), and the GA-sensitive allele *Rht8c* (from Akakomugi, often linked
58 with the photoperiod insensitivity allele *Ppd-D1a*) individually: reduce height by 10 to 15 %; reduce lodging
59 in fertile and humid conditions; and increase harvest index when added to excessively tall backgrounds
60 (Flintham et al., 1997a; Gooding et al., 2012). *Rht* alleles that confer reduced GA sensitivity have reduced
61 grain α -amylase activity and increased HFN (Flintham et al., 1997b; Gooding et al., 1999). Gibberellin activity
62 and sensitivity is implicated in PHS and in the production of LMA (Flintham et al., 1997b; Mares and Mrva,
63 2008). The benefit of GA-insensitivity for HFN has been particularly evident for the severe dwarfing allele
64 *Rht-B1c* (from Tom thumb, syn. *Rht3*) with reduced risk of PHS (Flintham et al., 1997b). *Rht-B1c*, unlike the
65 Norin 10 semi-dwarfing alleles, confers marked inhibition of aleurone activity when challenged with GA

66 (Fintham and Gale, 1982), possibly contributing to increased grain dormancy. A severe dwarfing allele is also
67 present at the *Rht-D1* locus (*Rht-D1c*, syn. *Rht10*, from Ai-Bian), but we are unaware of previous work
68 characterizing the effects of this allele on grain dormancy and HFN. Both *Rht-B1c* and *Rht-D1c* in the
69 homozygous state produce plants with statures sub-optimal for yield (Addisu et al., 2010), but it has been
70 suggested that *Rht-B1c* may have utility in the heterozygous state, or by controlling height in particularly tall
71 backgrounds, or even triticale (Flintham et al., 1997b).

72 The benefit of GA-insensitivity for HFN has raised concerns as to the effects of replacing the Norin 10
73 alleles with *Rht8c* in breeding programmes (Mares and Mrva, 2008). Here we use near isogenic lines (NILs) to
74 compare the effects of semi-, and severe-dwarfing alleles at the *Rht-B1* and *Rht-D1* loci with GA-sensitive
75 alleles conferring both semi- (*Rht8c+Ppd-D1a* linkage block on chromosome 2D) and severe- (*Rht12*, gamma
76 ray-induced allele from 'Karcagi 522') dwarfing. We also compare 62 doubled haploid (DH) progeny of cv.
77 Savannah (*Rht-D1b*) x Renesansa (*Rht8c + Ppd-D1a*) genotyped with markers for the dwarfing genes and *Ppd-*
78 *D1a* to assess the effects of the alleles individually and in combination. Allele effects on HFN are assessed for
79 stability over contrasting genetic backgrounds, seasons and systems ('intensive' vs 'organic') and interpreted
80 with reference to mean grain weight, grain specific weight, α -amylase activity, and the acquisition and
81 retention of grain dormancy.

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84 **2. Experimental**

85

86 **2.1. Crop husbandry**

87

88 All experiments were conducted within the same 10 ha site at the Crops Research Unit, Sonning,
89 University of Reading, UK (51° 29' N, 0° 56' W), on a free-draining sandy loam. The site is split between an
90 area receiving synthetic agrochemicals and fertilizers, managed intensively, and an area managed organically
91 since 2001. Full details of the site, crop establishment and husbandry are available elsewhere (Addisu et al.,
92 2009, 2010; Gooding et al., 2012). Untreated seeds were drilled between 21 September and 4 October at a

93 nominal depth of 50 mm, on 120 mm rows in 2 m wide plots separated by 0.5 m double-width track
94 wheelings. Weather data (Table 1) were recorded at an automated meteorological station at the site.
95 Intensive management of the wheat typically involved: herbicide applications at growth stage (GS, Zadoks et
96 al., 1974) 19 and/or 31-32; and fungicide applications at GS 30-31, 39 and 59. No plant growth regulators
97 were applied. In each year, 100 kg N/ha + 40 kg S/ha was applied as a mixture of ammonium nitrate and
98 ammonium sulphate at GS 30-31. A further 100 kg N/ha was applied as ammonium nitrate between GS 34-39.
99 In the organic area, wheat was established after a three-year clover-rich ley. No agrochemicals or fertilizers
100 were applied to the organic wheat. Replication, plot lengths and seed rate varied with experiment and year
101 (Table 1).

102

103 2.2. Near-isogenic Lines

104

105 Near-isogenic lines (NILs) of wheat varying for major dwarfing alleles were compared in complete
106 randomized blocks, harvested in each year from 2006 to 2011 (Table 1). In all six years, the experiments
107 included seven near isogenic lines (NILs) in a cv. Mercia background (*rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*,
108 *Rht8c+Ppd-D1a*, *Rht-D1c*, *Rht12*). In the last four years NILs with taller backgrounds were also included i.e.
109 Maris Widgeon and Maris Huntsman comprising *rht* (tall), *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht-B1b+Rht-D1b*, *Rht-*
110 *D1b+Rht-B1c*. Source of dwarfing alleles and markers used are in Addisu et al. (2009).

111

112 2.3. Doubled haploid population

113

114 Sixty-two lines were selected from a recombinant doubled haploid (DH) population of Savannah (*Rht-D1b*)
115 x Renesansa (*Rht8c + Ppd-D1a*) (Simmonds et al., 2006). Savannah had high yield potential in NW Europe, low
116 bread making quality, and was listed for the UK in 1998. Renesansa had high yield potential in southern
117 Europe, good bread making quality, and was listed in 1995. Together with the parents, genotyping indicated
118 14 lines without either dwarfing allele, 15 lines with just *Rht-D1b*, seven lines with just *Rht8c*, and 21 lines
119 with both *Rht-D1b* and *Rht8c*. Nine lines carried *Ppd-D1a* but not *Rht8c*, whereas three lines carried *Rht8c* but

120 not *Ppd-D1a*. Seven lines with uncertain genotyping were also included. The 2007 harvest was mostly to
121 multiply seed for use in subsequent years and had been, therefore, sown thinly (Table 1) in a single
122 randomized block. In the two subsequent years the 64 lines were arranged in an 8 × 8 row + column design
123 for two replicates.

124

125 2.4. Assessments

126

127 Crop height was calculated as the mean of three measurements with a rising disc of polystyrene (Peel,
128 1987): at anthesis, the end of grain filling, and at harvest maturity.

129 For each experiment, the central portion of each plot or sub-plot was combine-harvested at maturity with
130 a 1.3 m cutter bar. Mean grain weights were determined from a divided sample of at least 250 grains per plot.
131 Specific weight (SW) was measured using a chondrometer calibrated to ISO 7971:1995. To determine grain
132 viability and grain dormancy, two replicate fifty seed samples from each field plot, in each of two incubation
133 temperatures (10°C and 20°C) were placed on pre-water soaked and drained germination towels (3, each 230
134 x 310mm; Code 6803; Kimberley-Clark, Reigate, UK). A fourth towel was placed on top and towels were then
135 folded at 50mm from the bottom, rolled loosely, and enclosed in a polythene bag. Each bag was placed at
136 approximately 72° from the horizontal on a metal rack in incubators. Observations were made after seven
137 days for seeds incubated at 20°C and 14 days for seeds incubated at 10°C. The total number of germinated
138 seeds (ISTA, 1999) was derived from the seeds incubated at 10°C. Dormancy was assessed by comparing total
139 germination at 20°C with that at 10°C (Ellis et al., 1985). Germination data were angular transformed before
140 statistical analysis.

141 Grain samples (20 g per plot) were dried at 80 °C for 48 h to determine moisture content, and to adjust
142 yields and mean grain weights to a dry matter basis. Samples of fresh grain (100 g per plot) were milled using
143 a Laboratory Mill 3100 (Perten Instruments AB, Huddinge, Sweden) and tested for HFN with a Perten
144 Instruments Falling Number 1500 machine assessed to ISO 3039, using 7g of flour adjusted for moisture
145 content to 15%. α -Amylase was assayed using the Ceralpha method (Megazyme, County Wicklow, Ireland; cat
146 no. K-CERA) with blocked p-nitrophenol maltoheptaoside (BPNPG7) (McCleary and Sheehan, 1987). The

147 results are expressed in Ceralpha units (CU), which correspond to the amount of enzyme required to release
148 one micromole of p-nitrophenol from BPNPG7 in one minute.

149 In 2009 the development and loss of dormancy before grain harvest was assessed from samples of ears
150 removed from each plot at weekly intervals from the milky-ripe growth stage (GS 81) to final harvesting. At
151 each harvest, ten ears were selected randomly from the central twelve rows of each plot leaving two guard
152 rows on each side and a 50 cm margin at each end. Immediately after cutting the ears, they were placed in a
153 polythene bag to prevent drying during transfer. Grains were then extracted from 1-2 cm in the middle of
154 each ear avoiding the tip and basal portions. Grains and ears were only removed from polythene bags for
155 short periods during this extraction to prevent moisture loss and to ensure that seed condition and dormancy
156 were preserved until germination and dormancy tests commenced, as described for the combined grain. All
157 samples were processed within seven to eight hours of collection from the field.

158

159 2.5. Statistical analysis

160

161 The NIL data from each year and system were subjected to appropriate analyses of variance (Block
162 structure = Block; Treatment structure = NIL). An analysis of residual maximum likelihood (REML; Genstat
163 10.1, Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK) used the data set
164 encompassing all years and comprised a fixed model of System x Background x Allele, and a random model of
165 Year/System/Block/NIL. Stability of the HFN results over season, from the intensively grown NILs was
166 assessed by the modified joint regression analysis of Digby (1979) using the RJOINT procedure in GenStat
167 Release 10.1. For the assessment of dormancy over time, there was no evidence of a Time x genotype effect. In
168 just this analysis, mean effects of allele over background were estimated in a REML analysis by including
169 Background in the random model, and fitting the effect of Time with a quadratic divided by quadratic
170 response. The combined REML analysis for the doubled-haploid population comprised a fixed model of *Rht8c*
171 (+/-) + *Rht-D1b* (+/-) + *Ppd-D1a* (+/-), and a random model of Year / System / Block / Column + Row.
172 Generalized linear models were tested to assess effects of allele and other potential explanatory variables
173 (height, dormancy, mean grain weight, specific weight) on HFN variation amongst the DH lines.

174

175

176

177 3. Results

178

179 3.1. Near isogenic lines

180

181 Averaged over Background, the semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b*, reduced height significantly
182 by about 15% (Table 2). The severe-dwarfing allele *Rht-B1c* reduced height by 48%. In the Mercia
183 background, the *Rht-D1c* NIL was shorter than *Rht-B1c*. The GA-sensitive combination of *Rht8c+Ppd-D1a*
184 produced heights comparable to the GA-insensitive semi-dwarfing alleles. *Rht12* produced the shortest plants
185 despite being GA-sensitive. In the taller backgrounds (M. Huntsman and M. Widgeon) the *Rht-B1b+D1b*
186 combination reduced height by about 45% and the *Rht-D1b+B1c* combination by about 58%. Grain yield was
187 optimized at crop heights of about 800 mm (Table 2). For M. Huntsman and M. Widgeon heights were supra-
188 optimal for yield for *rht*(tall) and addition of semi-dwarfing alleles tended to increase yield. For Mercia,
189 *rht*(tall) appeared near optimal, and addition of semi-dwarfing alleles *Rht-D1b* and *Rht8c+Ppd-D1a*
190 significantly reduced yield. Grain yields for all backgrounds were progressively reduced as heights declined
191 below 700 mm, irrespective of dwarfing allele or combination used.

192 The combined REML analysis on HFN revealed significant effects of System ($P=0.028$), Background
193 ($P<0.001$), Allele ($P<0.001$), System x Allele ($P<0.001$), and Background x Allele ($P<0.001$) (Table 3). Mean
194 HFN in the organic system was lower than that for the intensive system. Mercia maintained higher HFN than
195 Maris Huntsman or Maris Widgeon. Averaged over background and system there was an increase in HFN with
196 increasing GA-insensitivity, e.g. *rht*(tall) < *Rht-B1b* or *-D1b* < *Rht-B1c*. A contribution to the System x Allele
197 interaction was the reduced effect of *Rht-B1c* in the organic context. The significance of the Background x
198 Allele interaction can be partly attributed to the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* increasing HFN in
199 the two tallest backgrounds, but not in Mercia. Averaged over all seasons in the intensive context, severe
200 dwarfing with *Rht-B1c* increased HFN in all backgrounds. *Rht-D1c* produced similar effects to *Rht-B1c* in

201 Mercia. In Mercia, dwarfing with GA-sensitive alleles, whether semi- (*Rht8c + Ppd-D1a*), or severe-dwarfing
202 (*Rht12*) was detrimental to HFN, even when compared to *rht*(tall), i.e. GA-insensitive and GA-sensitive
203 dwarfing alleles have contrasting effects on HFN. There was a tendency for increasing dwarfism, by
204 whichever mechanism, to be associated with increased sensitivity (reduced stability) of HFN to environment.
205 In some cases this just reflected the size (rather than direction) of the effect varying with season, but in some
206 cases there were cross-overs in performance. For instance in intensively-grown Mercia, *Rht-D1c* produced
207 significantly lower HFN compared to *rht*(tall) in 2008, opposite to its average effect. *Rht-D1b+B1c* produced
208 comparable HFNs to *rht*(tall) in 2008, but very much greater HFNs in other years.

209 There was a degree of scatter in the relationship between HFN and α -amylase activity (Fig. 1), no doubt
210 partly associated with sampling errors in both assessments. Nonetheless, the effects of Year, Background, and
211 the larger effects of Allele on HFN are demonstrably associated with effects on α -amylase activity. Also
212 evident in the α -amylase assessment is the instability for some of the shorter lines; giving higher activity in
213 2008, and lower activity in 2010 compared with taller lines.

214 There was inconsistency between the effects of dwarfing alleles on grain dormancy after harvest, and their
215 effects on GA-insensitivity, height and HFN (Table 2). Only lines containing *Rht-B1c* significantly increased
216 grain dormancy, whether alone or in combination with *Rht-D1b*. The most notable discontinuity is that of *Rht-*
217 *D1c* in Mercia which failed to increase grain dormancy despite having a greater dwarfing effect on height than
218 *Rht-B1c*. The GA-sensitive semi-dwarfing allele *Rht8c-Ppd-D1a* significantly reduced dormancy in Mercia but
219 the much shorter GA-sensitive line, *Rht12* had no effect on grain dormancy. The results from 2009 (Fig. 2)
220 demonstrate that the effect of *Rht-B1c* was evident throughout grain filling, but that there was no significant
221 effect of any other allele in the time series.

222 Mean grain weight, and grain specific weight tended to decline with degree of dwarfism, whether achieved
223 by GA-insensitivity or not (Table 2). An exception to this trend was the high mean grain weight for M.
224 Huntsman *Rht-D1b+B1c*. Significant lodging was only seen in *rht*(tall) for M. Huntsman and M. Widgeon.

225 3.2. Doubled haploid population

226

227 In the additive model for the Savannah x Renesansa progeny, over all four season and system
228 combinations, the marker for *Ppd-D1a* was associated with significantly ($P<0.001$) reduced HFN, *Rht-D1b* was
229 associated with increased HFN ($P<0.001$), and the marker for *Rht8c* was not associated with any effect on
230 HFN ($P=0.239$; Table 3). Similar effects (or lack of) were evident within each of the four individual
231 experiments.

232 The markers for both *Rht8c* and *Rht-D1b* were associated with reduced heights, but only *Rht-D1b* was
233 associated with increased grain yields (Table 2). *Ppd-D1a* was associated with lower grain yields. *Rht-D1b*
234 was associated with reduced mean grain weight and lower specific weights. There was no association among
235 the alleles at the three loci and grain dormancy.

236 In a generalized linear model to explain the variation in HFN among the individual lines, the effects of
237 adding height, grain yield, mean grain weight, or specific weight individually to the main effects of *Rht-D1* and
238 *Ppd-D1* alleles was tested. In this analysis, the addition of mean grain weight to the model was justified
239 significantly ($P=0.007$ for the change; compared with 0.062, 0.95 and 0.051 for adding height, yield, or
240 specific weight respectively). The main effect of mean grain weight was -4.25 s/mg (Fig. 3; s.e. = 1.19).
241 Variance accounted for (r^2_{adj}) by *Rht-D1* + *Ppd-D1* + mean grain weight = 34.5%, compared with 24% for just
242 *Rht-D1* + *Ppd-D1*. The interaction between *Rht-D1b* and height on HFN was not statistically significant
243 ($P=0.129$).

244

245

246 4. Discussion

247

248 We confirm the association between GA-insensitivity conferred by dwarfing alleles and a positive effect on
249 the HFN (and/or a negative effect on α -amylase) of wheat (Flintham et al., 1997b; Mares and Mrva, 2008; Tan
250 et al., 2010). We report the mean effect of *Rht-D1c* to be consistent with this association. However, we also
251 demonstrate this benefit of GA-insensitivity to be unstable over season, growing system and genetic
252 background. Despite the similar mean effects of *Rht-B1c* and *Rht-D1c* there were differences between the two
253 NILs in Mercia. We demonstrate the effect of *Rht-B1c* on grain dormancy (Flintham and Gale, 1982) and show

254 that this is exhibited throughout grain filling and ripening. The lack of such an effect of *Rht-D1c*, or any other
255 GA-insensitive allele, suggests that this is not due to GA-insensitivity *per se* rather that it is possibly a closely
256 linked effect exhibited in all three backgrounds used here, or due to the nature of the mutation, specific to
257 *Rht-B1c*. Acquired and retained dormancy may have been particularly beneficial in 2008 to prevent PHS. This
258 year had the lowest mean HFN; pre-harvest sprouting may be implicated because harvest was delayed
259 substantially (Table 1). It was only in 2008, when retained dormancy may have been particularly beneficial,
260 that *Rht-B1c* produced grain of significantly higher HFN, and apparently lower α -amylase activity, than *Rht-*
261 *D1c* in the intensive growing system.

262 The organic system provided less nitrogen during stem extension and grain filling than did the intensive
263 system (Gooding et al., 2012). The reduced HFN in the organic system is therefore consistent with positive
264 relationships between the amount of nitrogen made available in the spring and HFN (Kettelwell, 1999;
265 Kindred et al., 2005). Other factors, however, may have contributed. The organic system suffered significant
266 weed pressures that became progressively worse with degree of dwarfing (Addisu et al., 2010). Hand
267 weeding in organic and other systems has increased HFN (Awan, 2002; Cosser, 1996) and any effect of weeds
268 on HFN in these experiments would have contributed to the System x Allele interaction.

269 The results from both the NILs and DHs confirm the benefit to HFN of the semi-dwarfing GA-insensitivity
270 conferred by the *Rht-B1b* and *Rht-D1b* alleles (Flintham et al., 1997b). The lack of this effect in Mercia,
271 however, contrasts with the previous work. Mercia *rht*(tall) had the highest HFN (and least α -amylase
272 activity) of all the backgrounds so there was less potential for the dwarfing alleles to give improvements.
273 Additionally, Mercia did not lodge whereas, *Rht-B1b* and *Rht-D1b* controlled lodging in both M. Widgeon and
274 M. Huntsman. Any α -amylase production associated with lodging would, therefore, have contributed to the
275 Background x Allele interaction. Nonetheless, the lack of a benefit of any dwarfing allele on the HFN of Mercia
276 in the challenging conditions of 2008 requires further explanation.

277 We confirm the negative relationship that is often observed between mean grain weight and Hagberg
278 falling number (Evers et al., 1995; Farrell and Kettlewell, 2008). Although GA-insensitivity was again
279 associated with reduced mean grain weight (Flintham et al., 1997b), the regression analysis of the DH
280 population would suggest that the beneficial effects of GA-insensitivity on HFN involved mechanisms in

281 addition to associations with grain size. *Rht-D1b+B1c* produced higher HFN and larger grain than several
282 other lines in M. Huntsman. It is also notable that dwarfing through mechanisms other than GA-insensitivity
283 reduced mean grain weight, but had negative effects on HFN, contrary to the average trend between mean
284 grain weight and HFN in the DH population.

285 We demonstrate that dwarfing with GA-sensitive alleles can be detrimental to HFN, consistent with the
286 concerns expressed by Mares and Mrva (2008). In the case of the *Rht8c+Ppd-D1a* linkage block, however, the
287 analysis of the DHs suggests that negative effects are more closely linked to *Ppd-D1a* rather than *Rht8c*. It is
288 possible that this is at least partly due to the photoperiod-insensitivity bringing forward the grain filling
289 period into wetter and cooler conditions (Addisu et al., 2010), which would be possibly more conducive to
290 LMA and/or PHS. Against this, however, is the consistency of the *Ppd-D1a* effect over the four DH experiments
291 over three contrasting seasons, i.e. a negative effect of about 40s in each case. Alternative explanations are
292 possible and others have found significant QTLs for PHS and/or LMA on 2D (Ren et al., 2008; Tan et al., 2010)
293 and other group 2 chromosomes (Munkvold et al., 2009).

294

295

296 **5. Conclusions**

297

298 GA-insensitive dwarfing alleles, including *Rht-D1c*, can help maintain HFN in wheat but this effect depends on
299 background, season and system. Increased grain dormancy during and after crop maturation is not a
300 universal consequence of reduced GA-insensitivity even when dwarfing, as with *Rht-D1c*, is severe: rather, it
301 appears associated only with *Rht-B1c*. The *Rht8c+Ppd-D1a* linkage block is associated with reduced HFN,
302 although the association appears closer to *Ppd-D1a* than with *Rht8c*. The negative association between mean
303 grain weight and HFN is in addition to effects that are associated with major dwarfing and *Ppd-D1* alleles.

304

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306

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311

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313 **References**

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Table 1. Experimental details on intensive (I) and organic (O) areas comparing reduced height (*Rht* alleles).

Growing Season	2005/6	2006/7	2007/8	2008/9	2009/10	2010/11
Rainfall (mm)/mean temperature (°C)						
March	45.6/5.3	44.4/7.1	82.6/6.5	31.0/7.8	46.2/6.3	13.8/6.5
April	25.7/9.1	1.8/11.4	59.0/8.1	34.0/10.1	22.0/9.2	2.8/12.2
May	79.7/12.6	92.2/12.4	66.4/13.7	30.8/12.5	12.0/11.2	30.0/12.3
June	11.1/16.4	93.7/16.1	49.4/14.9	40.2/15.3	20.8/16.1	89.8/14.2
July	32.0/20.6	115.6/16.3	77.6/16.5	69.2/16.7	31.6/18.4	41.1/16.2
August	36.2/16.8	40.5/16.3	74.6/16.8	27.4/16.9	108.0/16.1	125.2/15.9
<u>Experiments comparing Near Isogenic Lines (NILs)</u>						
Systems included	Intensive and organic	Intensive and organic	Intensive and organic	Intensive	Intensive	Intensive
Number of complete blocks per system	4	4	3	5	3	4
NILs included	Mercia	Mercia	Mercia M. Huntsman M. Widgeon	Mercia M. Huntsman M. Widgeon	Mercia M. Huntsman M. Widgeon	Mercia M. Huntsman M. Widgeon
Plot lengths (m)	10	10	7.5	7.5	7.5	5
Seeds sown/m ²	300	300	250	300	300	300
Harvest date (day.month)	02.8	10.8	03.9	20.8	06.8	15.8
<u>Experiments comparing Double Haploid (DH) population</u>						
Systems included		Intensive	Intensive and organic	Intensive		
Number of complete blocks per system		1	2	2		
Plot lengths (m)		5	7.5	5		
Seeds sown/m ²		150	250	250		
Harvest date (day.month)		08.8	03.9	25.8		

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Table 2. Near isogenic lines varying in reduced height alleles (*Rht*) on crop height, yield and seed quality of winter wheat. Values are REML-predicted means from field experiments harvested between 2006 and 2011 under intensive management.

	Final crop height (mm)	Grain yield (t DM/ha)	Mean grain weight (mg DM)	Specific weight (kg/hl)	Lodging score (%)	Seed dormancy (% angular transform)
<u>Means from Near Isogenic Lines (2006 to 2011)</u>						
Mercia						
<i>rht</i> (tall)	816	6.51	41.3	79.2	0.4	12.7
<i>Rht-B1b</i>	755	6.61	38.6	77.7	0.2	9.5
<i>Rht-D1b</i>	696	5.91	36.1	76.3	0.4	4.9
<i>Rht8c+Ppd-D1a</i>	720	5.28	38.2	76.9	0.2	2.8
<i>Rht-B1c</i>	447	5.02	32.3	72.2	0.0	34.9
<i>Rht-D1c</i>	360	3.08	32.8	70.3	0.0	10.6
<i>Rht12</i>	322	2.35	27.0	64.8	0.0	12.1
Maris Huntsman						
<i>rht</i> (tall)	982	5.73	48.9	75.8	10.9	6.2
<i>Rht-B1b</i>	801	6.67	45.5	75.2	0.4	6.4
<i>Rht-D1b</i>	778	6.59	45.8	74.1	2.9	6.9
<i>Rht-B1b+D1b</i>	557	5.45	43.2	71.9	0.0	7.2
<i>Rht-B1c</i>	461	4.82	41.8	70.1	0.0	39.9
<i>Rht-D1b+B1c</i>	384	3.33	46.1	70.1	0.0	41.8
Maris Widgeon						
<i>rht</i> (tall)	1020	4.14	46.1	78.4	17.9	4.6
<i>Rht-B1b</i>	831	5.53	42.2	77.2	3.1	5.1
<i>Rht-D1b</i>	844	5.82	43.8	76.6	1.3	1.1
<i>Rht-B1b+D1b</i>	560	4.37	37.1	72.6	0.0	11.2
<i>Rht-B1c</i>	538	4.27	42.3	73.5	0.0	22.2
<i>Rht-D1b+B1c</i>	477	3.28	41.6	73.1	0.0	17.0
SED ^a	10.8	0.250	1.02	0.56		4.76
SED ^b	13.8	0.310	1.26	0.76		4.76
<u>REML-predicted means from Doubled Haploids of Savannah x Renesansa</u>						
<i>Rht8c</i>						
-	892	5.61	47.1	76.2		7.1
+	794	5.58	48.6	75.9		9.6
<i>Rht-D1b</i>						
-	913	5.44	49.8	76.6		8.8
+	773	5.76	45.9	75.6		7.9
<i>Ppd-D1a</i>						
-	805	5.80	47.2	76.0		8.5
+	881	5.40	48.5	76.1		8.2
SED (260 DF)	18.3	0.178	0.78	0.33		2.03

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^a for comparing alleles within Mercia; ^bfor comparing alleles within Maris Huntsman and Maris Widgeon (290 DF)

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406 **Table 3.** Effect of reduced height (*Rht*) and photoperiod (*Ppd-D1a*) alleles on Hagberg falling number of wheat in organic
 407 (O) and intensive (I) growing systems.

	2006		2007		2008		2009	2010	2011	REML mean	Sensi- tivity
	O	I	O	I	O	I	I	I	I	I	I
<u>Means from Near Isogenic Lines</u>											
Mercia											
<i>rht</i> (tall)	380	438	295	332	214	244	340	354	332	339	0.68
<i>Rht-B1b</i>	392	414	298	322	129	205	343	333	347	329	0.77
<i>Rht-D1b</i>	413	428	279	336	137	255	347	370	380	352	0.66
<i>Rht8c+Ppd-D1a</i>	345	402	278	265	95	142	253	343	264	276	1.00
<i>Rht-B1c</i>	410	435	333	415	201	235	378	417	408	382	0.84
<i>Rht-D1c</i>	407	434	268	408	92	187	371	447	406	377	1.09
<i>Rht12</i>	357	368	219	264	66	133	289	361	276	281	0.97
Maris Huntsman											
<i>rht</i> (tall)					68	77	229	240	211	212	0.80
<i>Rht-B1b</i>					85	111	260	330	264	261	1.00
<i>Rht-D1b</i>					131	121	234	357	306	271	1.27
<i>Rht-B1b+D1b</i>					65	66	260	288	348	266	1.10
<i>Rht-B1c</i>					93	113	307	442	383	332	1.57
<i>Rht-D1b+B1c</i>					88	73	300	449	398	329	1.83
Maris Widgeon											
<i>rht</i> (tall)					106	121	234	262	175	218	0.57
<i>Rht-B1b</i>					142	199	269	288	304	285	0.49
<i>Rht-D1b</i>					91	164	316	316	279	292	1.29
<i>Rht-B1b+D1b</i>					72	105	239	370	339	281	0.73
<i>Rht-B1c</i>					109	147	350	431	401	355	1.41
<i>Rht-D1b+B1c</i>					66	138	301	383	359	317	1.21
SED	20.2	13.3	23.8	23.8	23.3	17.3	29.0	34.4	24.6	13.4 ^a 17.9 ^b	0.202 ^a 0.243 ^b
DF	18	18	18	18	36	36	65	35	54	380	48
<u>REML-predicted means from Doubled Haploids of Savannah x Renesansa</u>											
<i>Rht8c</i>											
-				197	165	174	224			198	
+				200	175	177	244			209	
<i>Rht-D1b</i>											
-				176	150	153	189			173	
+				220	190	199	279			233	
<i>Ppd-D1a</i>											
-				213	188	195	257			223	
+				184	152	157	211			183	
SED				18.9	13.6	12.6	17.6			9.4	
DF				53	109	106	100			261	

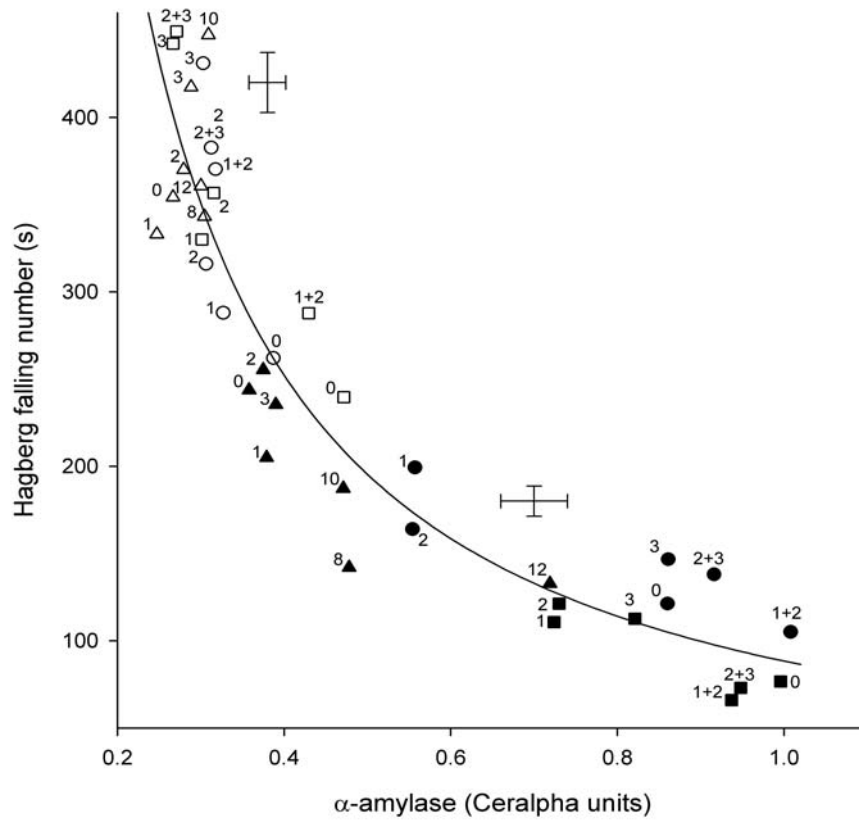
408 ^a for comparing alleles within Mercia; ^bfor comparing alleles within Maris Huntsman and Maris Widgeon

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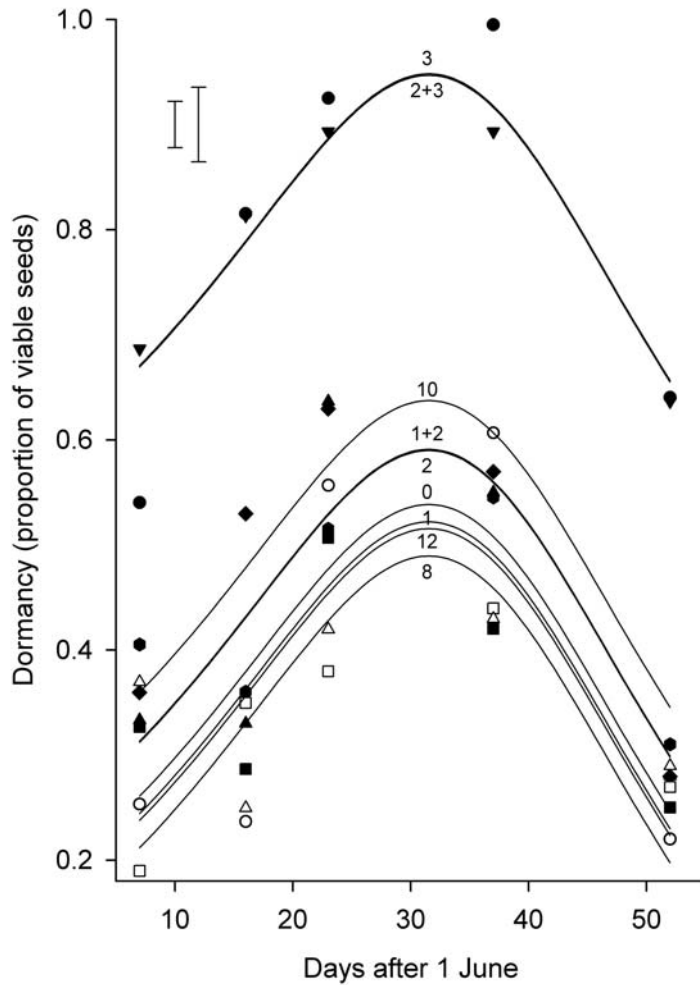
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415 **Fig. 1.** Relationship between α -amylase activity and Hagberg falling number of wheat near isogenic lines varying for
 416 background (Mercia = $\triangle, \blacktriangle$; Maris Huntsman = \square, \blacksquare ; Maris Widgeon = \circ, \bullet) and dwarfing allele (numerals correspond: 0
 417 = *rht*(tall); 1 = *Rht-B1b*; 2 = *Rht-D1b*; 3 = *Rht-B1c*; 8 = *Rht8c+Ppd-D1a*; 10 = *Rht10*; 12 = *Rht12*), grown intensively in two
 418 years (2008 = closed symbols; 2010 = open symbols). Error bars are 1 SED. (DF = 35).

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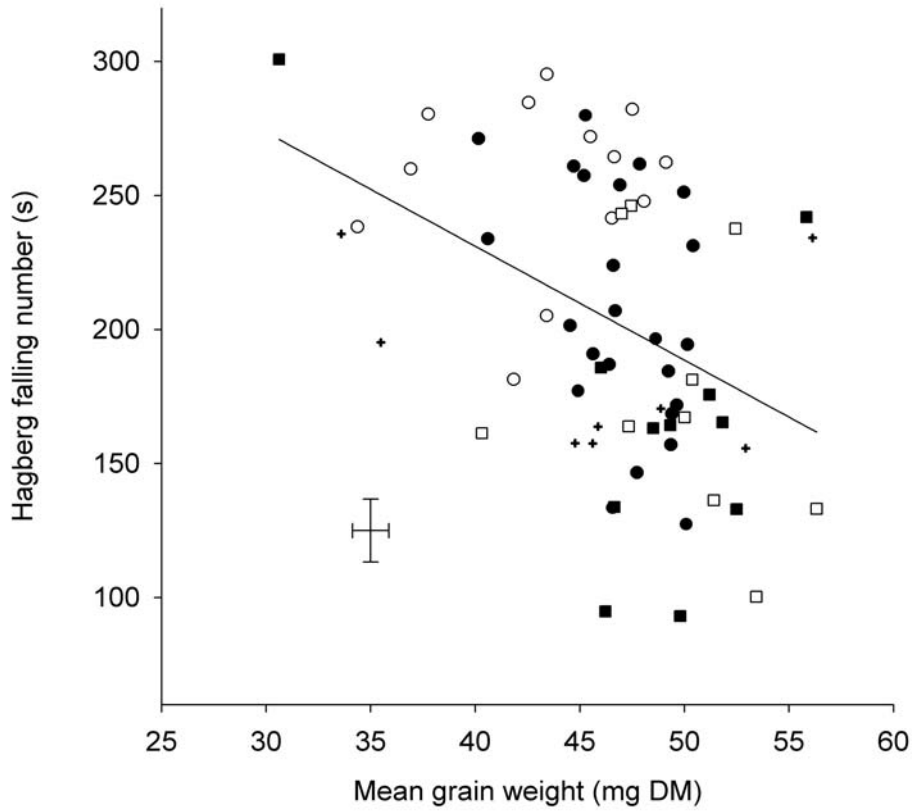
420

421 **Fig. 2.** Effect of dwarfing allele on the acquisition and loss of dormancy during grain filling and ripening of intensively-
 422 grown winter wheat in 2009. Numerals denote fits for individual alleles: 0 = *rht-B1a+D1a*, ○; 1 = *Rht-B1b*, ■; 2 = *Rht-D1b*,
 423 ▲; 1+2 = *Rht-B1b+D1b*, ●; 3 = *Rht-B1c*, ▼; 2+3 = *Rht-B1c+D1b*, ●; 10 = *Rht-D1c*, ◆; 8 = *Rht8c+Ppd-D1a*, □; *Rht12*=△.
 424 Solid symbols are GA-insensitive alleles. Error bars are SEDs for comparing main effects of Allele; left = minimum (among
 425 0,1,2 and 3), right = maximum (among 8, 10 and 12). Points are means of two blocks and three (0,1,2 and 3), two (1+2 and
 426 2+3) or one background (8, 10, 12). Effects of background have been removed (see text for details).

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431 **Fig. 3.** Relationship between mean grain weight and Hagberg falling number of wheat. Points are means (from four
 432 experiments, Table 1) for individual doubled haploid progeny of Savannah x Renesansa, marked as either with or without
 433 *Rht-D1b* (circles or squares, respectively), and with or without *Ppd-D1a* (solid or open symbols, respectively). + denotes
 434 uncertain genotyping. Error bars are one SED. (334 DF).

435