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

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Abbreviations: DF, degrees of freedom; DH, doubled haploid; GA, gibberellic acid; HFN, Hagberg falling number; I, intensive farming system; LMA, late-maturity alpha-amylase; NIL, near isogenic line; O, organic farming system; PHS, pre-harvest sprouting; Ppd, photoperiod response allele; QTL, quantitative trait loci; REML, residual maximum likelihood; Rht, reduced height allele; SED, standard error of difference

ABSTRACT

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

1. Introduction

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

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

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

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

2. Experimental

2.1. Crop husbandry

All experiments were conducted within the same 10 ha site at the Crops Research Unit, Sonning, University of Reading, UK (51° 29' N, 0° 56' W), on a free-draining sandy loam. The site is split between an area receiving synthetic agrochemicals and fertilizers, managed intensively, and an area managed organically since 2001. Full details of the site, crop establishment and husbandry are available elsewhere (Addisu et al., 2009, 2010; Gooding et al., 2012). Untreated seeds were drilled between 21 September and 4 October at a nominal depth of 50 mm, on 120 mm rows in 2 m wide plots separated by 0.5 m double-width track wheelings. Weather data (Table 1) were recorded at an automated meteorological station at the site. Intensive management of the wheat typically involved: herbicide applications at growth stage (GS, Zadoks et al., 1974) 19 and/or 31-32; and fungicide applications at GS 30-31, 39 and 59. No plant growth regulators were applied. In each year, 100 kg N/ha + 40 kg S/ha was applied as a mixture of ammonium nitrate and ammonium sulphate at GS 30-31. A further 100 kg N/ha was applied as ammonium nitrate between GS 34-39. In the organic area, wheat was established after a three-year clover-rich ley. No agrochemicals or fertilizers were applied to the organic wheat. Replication, plot lengths and seed rate varied with experiment and year (Table 1).

2.2. Near-isogenic Lines

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

2.3. Doubled haploid population

Sixty-two lines were selected from a recombinant doubled haploid (DH) population of Savannah (*Rht-D1b*) x Renesansa (*Rht8c* + *Ppd-D1a*) (Simmonds et al., 2006). Savannah had high yield potential in NW Europe, low bread making quality, and was listed for the UK in 1998. Renesansa had high yield potential in southern Europe, good bread making quality, and was listed in 1995. Together with the parents, genotyping indicated 14 lines without either dwarfing allele, 15 lines with just *Rht-D1b*, seven lines with just *Rht8c*, and 21 lines with both *Rht-D1b* and *Rht8c*. Nine lines carried *Ppd-D1a* but not *Rht8c*, whereas three lines carried *Rht8c* but not *Ppd-D1a*. Seven lines with uncertain genotyping were also included. The 2007 harvest was mostly to multiply seed for use in subsequent years and had been, therefore, sown thinly (Table 1) in a single randomized block. In the two subsequent years the 64 lines were arranged in an 8 × 8 row + column design for two replicates.

2.4. Assessments

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

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

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

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

2.5. Statistical analysis

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

3. Results

3.1. Near isogenic lines

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

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

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

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

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

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

3.2. Doubled haploid population

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

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

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

4. Discussion

We confirm the association between GA-insensitivity conferred by dwarfing alleles and a positive effect on the HFN (and/or a negative effect on α-amylase) of wheat (Flintham et al., 1997b; Mares and Mrva, 2008; Tan et al., 2010). We report the mean effect of *Rht-D1c* to be consistent with this association. However, we also demonstrate this benefit of GA-insensitivity to be unstable over season, growing system and genetic background. Despite the similar mean effects of *Rht-B1c* and *Rht-D1c* there were differences between the two NILs in Mercia. We demonstrate the effect of *Rht-B1c* on grain dormancy (Flintham and Gale, 1982) and show that this is exhibited throughout grain filling and ripening. The lack of such an effect of *Rht-D1c*, or any other GA-insensitive allele, suggests that this is not due to GA-insensitivity *per se* rather that it is possibly a closely linked effect exhibited in all three backgrounds used here, or due to the nature of the mutation, specific to *Rht-B1c*. Acquired and retained dormancy may have been particularly beneficial in 2008 to prevent PHS. This year had the lowest mean HFN; pre-harvest sprouting may be implicated because harvest was delayed substantially (Table 1). It was only in 2008, when retained dormancy may have been particularly beneficial, that *Rht-B1c* produced grain of significantly higher HFN, and apparently lower α-amylase activity, than *Rht-D1c* in the intensive growing system.

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

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

We confirm the negative relationship that is often observed between mean grain weight and Hagberg falling number (Evers et al., 1995; Farrell and Kettlewell, 2008). Although GA-insensitivity was again associated with reduced mean grain weight (Flintham et al., 1997b), the regression analysis of the DH population would suggest that the beneficial effects of GA-insensitivity on HFN involved mechanisms in addition to associations with grain size. *Rht-D1b+B1c* produced higher HFN and larger grain than several other lines in M. Huntsman. It is also notable that dwarfing through mechanisms other than GA-insensitivity reduced mean grain weight, but had negative effects on HFN, contrary to the average trend between mean grain weight and HFN in the DH population.

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

5. Conclusions

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

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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
Experiments comparing Near Isogenic Lines (NILs)						
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
Experiments comparing Double Haploid (DH) population						
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		

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)
Means from Near Isogenic Lines (2006 to 2011)						
Mercia						
rht(tall)	816	6.51	41.3	79.2	0.4	12.7
Rht-B1b	755	6.61	38.6	77.7	0.2	9.5
Rht-D1b	696	5.91	36.1	76.3	0.4	4.9
Rht8c+Ppd-D1a	720	5.28	38.2	76.9	0.2	2.8
Rht-B1c	447	5.02	32.3	72.2	0.0	34.9
Rht-D1c	360	3.08	32.8	70.3	0.0	10.6
Rht12	322	2.35	27.0	64.8	0.0	12.1
Maris Huntsman						
rht(tall)	982	5.73	48.9	75.8	10.9	6.2
Rht-B1b	801	6.67	45.5	75.2	0.4	6.4
Rht-D1b	778	6.59	45.8	74.1	2.9	6.9
Rht-B1b+D1b	557	5.45	43.2	71.9	0.0	7.2
Rht-B1c	461	4.82	41.8	70.1	0.0	39.9
Rht-D1b+B1c	384	3.33	46.1	70.1	0.0	41.8
Maris Widgeon						
rht(tall)	1020	4.14	46.1	78.4	17.9	4.6
Rht-B1b	831	5.53	42.2	77.2	3.1	5.1
Rht-D1b	844	5.82	43.8	76.6	1.3	1.1
Rht-B1b+D1b	560	4.37	37.1	72.6	0.0	11.2
Rht-B1c	538	4.27	42.3	73.5	0.0	22.2
Rht-D1b+B1c	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
REML-predicted means from Doubled Haploids of Savannah x Renesansa						
Rht8c						
-	892	5.61	47.1	76.2		7.1
+	794	5.58	48.6	75.9		9.6
Rht-D1b						
-	913	5.44	49.8	76.6		8.8
+	773	5.76	45.9	75.6		7.9
Ppd-D1a						
-	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

[a] for comparing alleles within Mercia; [b]for comparing alleles within Maris Huntsman and Maris Widgeon (290 DF)

Table 3. Effect of reduced height (*Rht*) and photoperiod (*Ppd-D1a*) alleles on Hagberg falling number of wheat in organic (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
Means from Near Isogenic Lines											
Mercia											
rht(tall)	380	438	295	332	214	244	340	354	332	339	0.68
Rht-B1b	392	414	298	322	129	205	343	333	347	329	0.77
Rht-D1b	413	428	279	336	137	255	347	370	380	352	0.66
Rht8c+Ppd-D1a	345	402	278	265	95	142	253	343	264	276	1.00
Rht-B1c	410	435	333	415	201	235	378	417	408	382	0.84
Rht-D1c	407	434	268	408	92	187	371	447	406	377	1.09
Rht12	357	368	219	264	66	133	289	361	276	281	0.97
Maris Huntsman											
rht(tall)					68	77	229	240	211	212	0.80
Rht-B1b					85	111	260	330	264	261	1.00
Rht-D1b					131	121	234	357	306	271	1.27
Rht-B1b+D1b					65	66	260	288	348	266	1.10
Rht-B1c					93	113	307	442	383	332	1.57
Rht-D1b+B1c					88	73	300	449	398	329	1.83
Maris Widgeon											
rht(tall)					106	121	234	262	175	218	0.57
Rht-B1b					142	199	269	288	304	285	0.49
Rht-D1b					91	164	316	316	279	292	1.29
Rht-B1b+D1b					72	105	239	370	339	281	0.73
Rht-B1c					109	147	350	431	401	355	1.41
Rht-D1b+B1c					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
REML-predicted means from Doubled Haploids of Savannah x Renesansa											
Rht8c											
-				197	165	174	224			198	
+				200	175	177	244			209	
Rht-D1b											
-				176	150	153	189			173	
+				220	190	199	279			233	
Ppd-D1a											
-				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	

[a] for comparing alleles within Mercia; [b] for comparing alleles within Maris Huntsman and Maris Widgeon

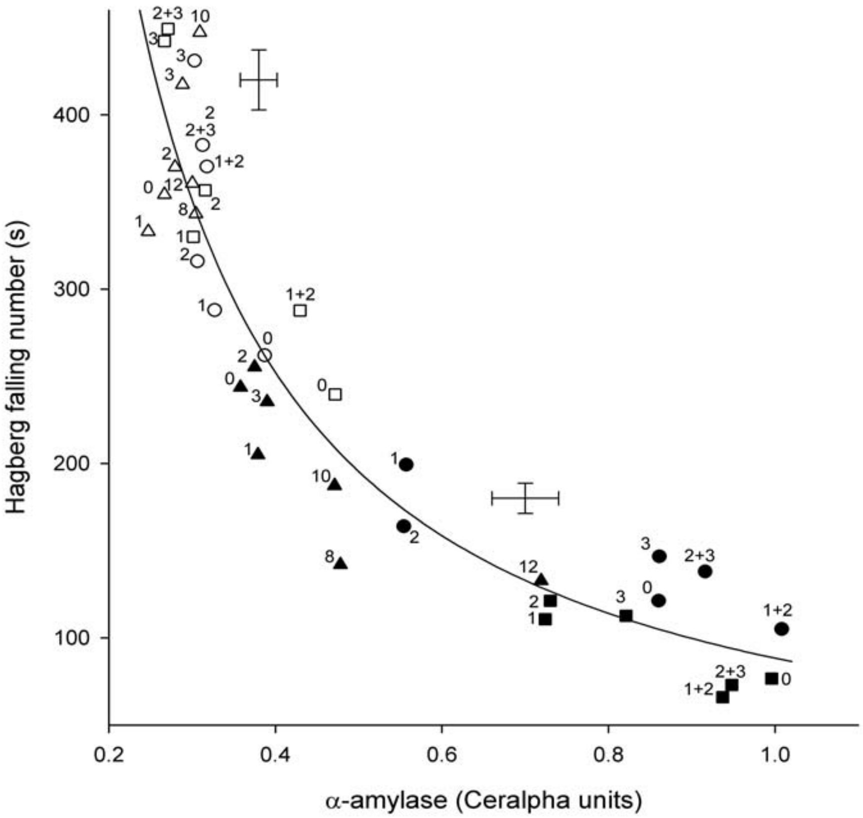

Fig. 1. Relationship between α-amylase activity and Hagberg falling number of wheat near isogenic lines varying for background (Mercia = △,▲; Maris Huntsman = □,■; Maris Widgeon = ○,●) and dwarfing allele (numerals correspond: 0 = *rht*(tall); 1 = *Rht-B1b*; 2 = *Rht-D1b*; 3 = *Rht-B1c*; 8 = *Rht8c*+*Ppd-D1a*; 10 = *Rht10*; 12 = *Rht12*), grown intensively in two years (2008 = closed symbols; 2010 = open symbols). Error bars are 1 SED. (DF = 35).

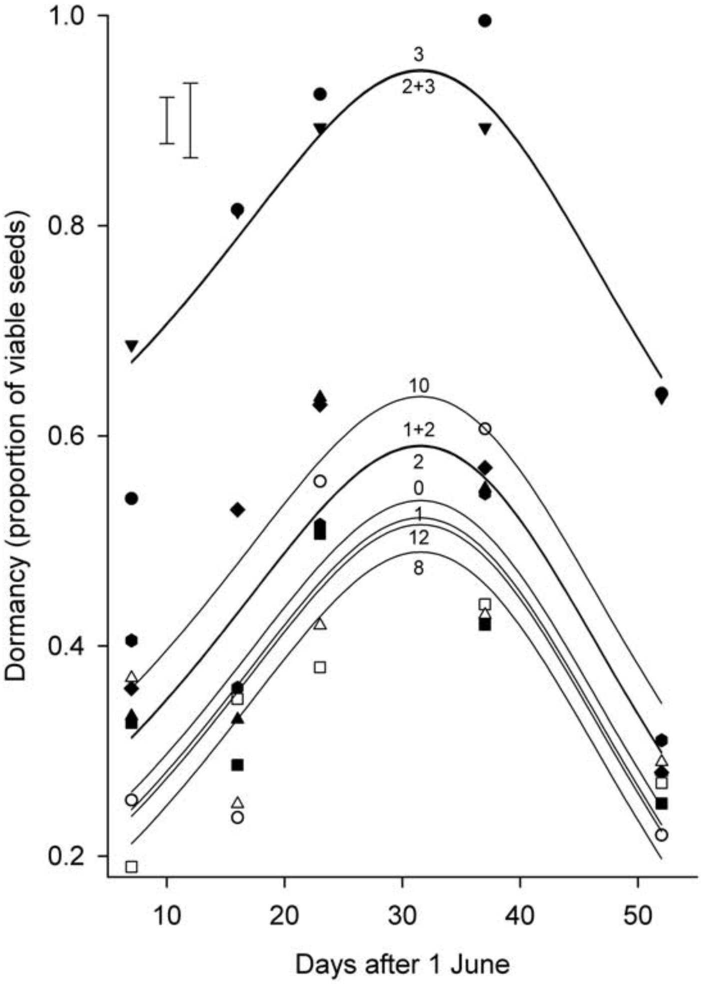

Fig. 2. Effect of dwarfing allele on the acquisition and loss of dormancy during grain filling and ripening of intensively-grown winter wheat in 2009. Numerals denote fits for individual alleles: 0 = *rht-B1a+D1a*, ○; 1 = *Rht-B1b*, ■; 2 = *Rht-D1b*, ▲; 1+2 = *Rht-B1b+D1b*, ⬢; 3 = *Rht-B1c*, ▼; 2+3 =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

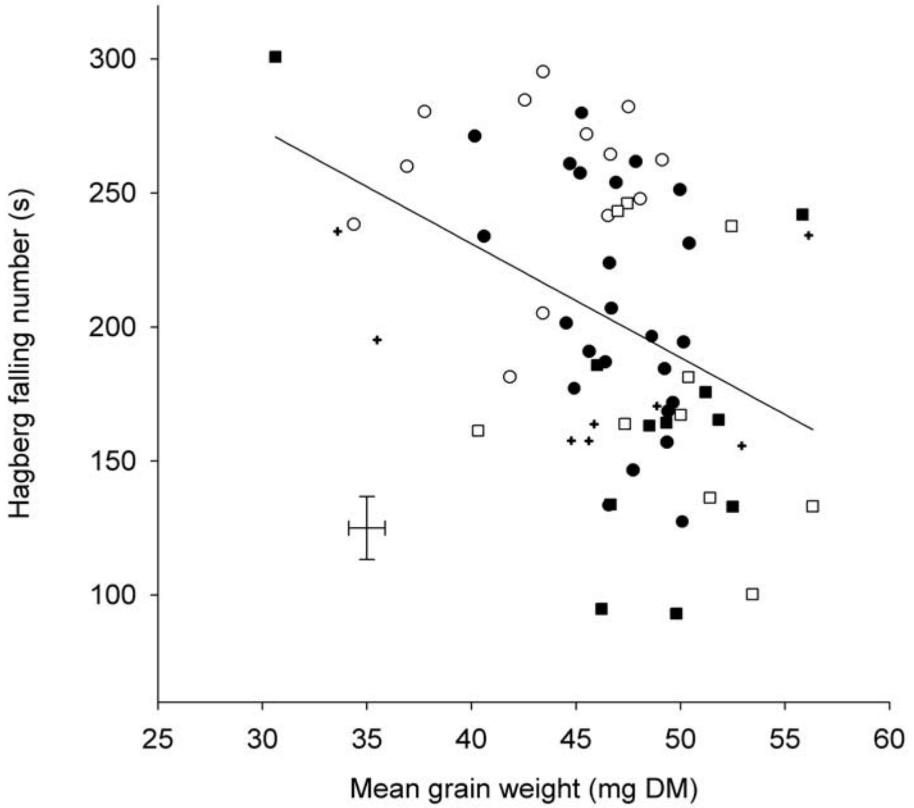

Fig. 3. Relationship between mean grain weight and Hagberg falling number of wheat. Points are means (from four experiments, Table 1) for individual doubled haploid progeny of Savannah x Renesansa, marked as either with or without *Rht-D1b* (circles or squares, respectively), and with or without *Ppd-D1a*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