

# *Contrasting effects of dwarfing alleles and nitrogen availability on mineral concentrations in wheat grain*

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Accepted Version

Gooding, M., Fan, M., McGrath, S. P., Shewry, P. R. and Zhao, F.-J. (2012) Contrasting effects of dwarfing alleles and nitrogen availability on mineral concentrations in wheat grain. *Plant and Soil*, 360 (1-2). pp. 93-107. ISSN 0032-079X doi: <https://doi.org/10.1007/s11104-012-1203-x> Available at <http://centaur.reading.ac.uk/27336/>

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To link to this article DOI: <http://dx.doi.org/10.1007/s11104-012-1203-x>

Publisher: Springer

Publisher statement: The original publication is available at [www.springerlink.com](http://www.springerlink.com)

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1 **Contrasting effects of dwarfing alleles and nitrogen availability on mineral**  
2 **concentrations in wheat grain**

3

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25 **Keywords:** Rht alleles • Wheat grain • Mineral concentration • Nitrogen fertilizer • Green revolution

26

27

28 **Abstract**

29 *Background and aim* Concentrations of essential minerals in plant foods may have declined in modern  
30 high-yielding cultivars grown with large applications of nitrogen fertilizer (N). We investigated the effect  
31 of dwarfing alleles and N rate on mineral concentrations in wheat.

32 *Methods* Gibberellin (GA)-insensitive reduced height (*Rht*) alleles were compared in near isogenic  
33 wheat lines. Two field experiments comprised factorial combinations of wheat variety backgrounds,  
34 alleles at the *Rht-B1* locus (*rht-B1a*, *Rht-B1b*, *Rht-B1c*), and different N rates. A glasshouse experiment  
35 also included *Rht-D1b* and *Rht-B1b+D1b* in one background.

36 *Results* In the field, depending on season, *Rht-B1b* increased crop biomass, dry matter (DM) harvest  
37 index, grain yield, and the economically-optimal N rate ( $N_{opt}$ ). *Rht-B1b* did not increase uptake of Cu, Fe,  
38 Mg or Zn so these minerals were diluted in grain. Nitrogen increased DM yield and mineral uptake so  
39 grain concentrations were increased (Fe in both seasons; Cu, Mg and Zn in one season). *Rht-B1b* reduced  
40 mineral concentrations at  $N_{opt}$  in the most N responsive season. In the glasshouse experiment, grain yield  
41 was reduced, and mineral concentrations increased, with *Rht* allele addition.

42 *Conclusion* Effects of *Rht* alleles on Fe, Zn, Cu and Mg concentrations in wheat grain are mostly due to  
43 their effects on DM, rather than of GA-insensitivity on  $N_{opt}$  or mineral uptake. Increased N requirement in  
44 semi-dwarf varieties partly offsets this dilution effect.

45

46

## 47 **Introduction**

48 Deficiencies of the micronutrients iron (Fe) and zinc (Zn) affect more than 2 billion people worldwide  
49 (WHO 2002). Other minerals such as magnesium (Mg) and copper (Cu) may also be inadequate in the  
50 diet of some people (White and Broadley 2009). Cereals are an important source of minerals in the human  
51 diet; providing 44% of the daily intake of Fe, 27% of Mg, 25% of Zn and 31% of Cu in the UK adult  
52 population (Henderson et al. 2003). There are concerns that the concentrations of essential minerals in  
53 plant foods may have declined as a result of increased yield (Davis et al. 2004; Garvin et al. 2006; White  
54 and Broadley 2005). Analysis of the archived wheat grain samples from the Broadbalk long-term  
55 experiment at Rothamsted has shown that the concentrations of Fe, Zn, Cu and Mg have all declined since  
56 the 1960s, coinciding with the introduction of modern short-straw cultivars (Fan et al. 2008).

57 Rapid increases in wheat yields in major wheat growing areas of the world from the 1960s through to  
58 the 1990's were associated with a reduction in wheat stature. This shortening was accompanied by an  
59 increase in dry matter harvest index, and also permitted sufficient nitrogen (N) fertilizer to be applied to  
60 the crop without the crop lodging such that canopy size could be optimized for capture of  
61 photosynthetically active radiation, and thus biomass yield (Gooding 2009). In the UK during the 1970s  
62 and 1980s yield increases were concurrent with final crop heights declining to around an apparently  
63 optimal stature of between 800 and 900 mm (Flintham et al. 1997; Austin 1999). Reductions in height  
64 were achieved either by the combined effect of many minor genes, and/or by the inclusion of major genes  
65 for reduced height (*Rht*). The commonly used *Rht-B1b* and *Rht-D1b* (previously named *Rht1* and *Rht2*)  
66 semi-dwarfing alleles from the Japanese wheat 'Norin 10' reduce sensitivity to endogenous gibberellins  
67 (GA) (Murase et al. 2008), and individually often reduce height by about 15% (Addisu et al. 2010). In the  
68 UK, *Rht-D1b* has been widely used in elite lines since the 1970's (e.g. cvs. Hobbit (1977) and Mardler  
69 (1978) were early examples (Austin et al. 1980)), although *Rht-B1b* has also been used more recently (e.g.  
70 cv. Robigus). More potent alleles, conferring severe dwarfism (>30 % height reduction) are available e.g.  
71 *Rht-B1c* (previously *Rht3*) from 'Tom Thumb' (Borner et al. 1997). The last widely used winter wheat  
72 cultivar suited for bread-making in the UK which did not have a major semi-dwarfing gene was cv.  
73 Mercia (introduced in 1983). Despite the lack of an *Rht* allele, the height of Mercia can be considered to  
74 be within the range commonly observed for modern commercial releases in the UK, that have an *Rht*  
75 allele (Gooding et al. 2012).

76 There is little information as to whether the reductions seen in mineral concentrations in UK wheat  
77 are due to effects of gibberellin insensitivity conferred by the adoption of the Norin 10 semi-dwarfing  
78 alleles, or due to yield increases associated with shortening *per se*. Here we use near isogenic lines  
79 varying for GA sensitivity conferred by alleles at the *Rht-B1* locus (*rht-B1a* (tall); *Rht-B1b* (semi-dwarf);

80 *Rht-B1c* (severe dwarf) in backgrounds that vary for height (Mercia, Maris Widgeon (1964) and Maris  
81 Huntsman (1969)) in three different environments: field plots in two contrasting seasons, and pots under  
82 glass (the Maris Huntsman series only).

83 Adoption of semi-dwarfing alleles increases the amount of N fertilizer that can be justified to be  
84 applied by farmers on economic grounds (Loddo and Gooding 2012). Nitrogen availability has a  
85 significant impact on mineral uptake and partitioning to, and concentrations in, the grain (Cakmak et al.  
86 2010; Kutman et al. 2011a, b). To more fully evaluate the impact of the adoption of semi-dwarf cultivars  
87 on the mineral concentrations on UK wheat it is, therefore, necessary to investigate any interaction  
88 between dwarfing alleles and N fertilizer application rate, and more specifically, to evaluate mineral  
89 concentrations at the economically optimal N rate for individual lines.

90 The aim of the present study was to quantify the effects of dwarfing alleles and the application rate  
91 of N on the uptake of minerals and their concentrations in grain, especially Fe, Zn, Cu and Mg which are  
92 often inadequate in the diet.

93

## 94 **Materials and methods**

95

### 96 **Field experiments**

97

98 Plots of winter wheat were maintained on a free-draining sandy loam soil (9% clay, particle size <0.2 µm;  
99 19% silt, 2-20 µm; 72% sand, 20-2000 µm) overlying coarse red-brown sand, principally of the Sonning  
100 series (Jarvis, 1968), at the Crop Research Unit, University of Reading, UK (51°29'N, 0°56'W, 35m  
101 above sea level), in the 2009/10 and 2010/11 growing seasons. In both seasons, the experiments followed  
102 a three year rye grass and chicory ley, destroyed with glyphosate before mouldboard ploughing to 300  
103 mm (Table 1). Before cultivation, the soil was tested for pH, phosphorous, potassium and magnesium  
104 availability (Anon. 1986; Anon. 2010; Table 1) and corrective nutrients applied accordingly. Following  
105 cultivation in autumn, total soil carbon and mineral N content (0 - 900 mm) averaged 0.69% DM and 55  
106 kg N/ha respectively. Soil mineral N content in the following February averaged 35 Kg N/ha. For this  
107 rotational position at this site over five previous seasons the N uptake by the above ground wheat  
108 receiving full crop protection but no N fertilizer averaged 75 kg N/ha, i.e. a soil N supply index of 1  
109 (Anon. 2010). Crop husbandry was indicative of local commercial practice (Table 1), and achieved a high  
110 level of control of weeds, pests and diseases. Rainfall and air temperature were recorded at an automatic  
111 weather station within 400 m of the experiments (Table 1).

112

113 Near isogenic lines (NILs) comprised a factorial combination of background varieties (Maris  
114 Huntsman, Maris Widgeon and Mercia in 2009/10 and just Maris Widgeon and Mercia in 2010/11) and  
115 three alleles at the *Rht-B1* locus: *rht-B1a* (tall), *Rht-B1b* (semi-dwarf, syn. *Rht1*) and *Rht-B1c* (severe-  
116 dwarf, syn. *Rht3*). Untreated seeds (300/m<sup>2</sup>) were drilled into 120 mm rows in 2 x 6 m sub-plots,  
117 separated by a 500 mm double track wheeling. Main plots, randomized in three complete blocks,  
118 comprised the background x allele combinations; each contained randomized sub-plots allocated to  
119 receive different rates of N fertilizer applied as granular ammonium nitrate. In 2009/10 the total N rates  
120 were 0, 100, 200 and 350 kg N/ha. For the three sub-plots receiving N, 50kg N/ha was applied at the first  
121 node detectable growth stage (GS 31, Zadoks et al. 1974) and again at flag leaf emergence (GS 39). The  
122 200 and 350 kg N/ha treatments received a further 100 and 250 kg N/ha respectively at the second node  
123 stage (GS 32). In 2010/11 the total N rates were 0, 50, 100, 200, 350 and 450 kg N/ha applied in equal  
124 splits at GS 31 and 33.

125 Crop height was measured five days before harvest with a polystyrene rising disc (Addisu et al.  
126 2009). During the week before harvest, above ground whole crop rows either side of three randomly  
127 placed 0.5 m lengths (total area equivalent of 0.36 m<sup>2</sup>) were collected from each subplot. Samples were  
128 partitioned into grain, chaff and straw. After oven drying at 80°C, dry weight of each component was  
129 estimated and mineral content assessed. Plot combine harvesting was with a 1.4 m cutter bar such that  
130 effective separation of subplots was 0.8 m to reduce edge effects. Information from sample areas was  
131 used to calculate harvest indices of dry matter and minerals.

132

### 133 Pot experiment

134

135 Soil (0 – 200 mm) was collected from the Rothamsted farm, air dried and sieved through a 5 mm sieve.  
136 Plastic pots (200 mm diameter) were filled with 3.5 kg air-dried soil each. Fertilizers (0.7 g N as NH<sub>4</sub>NO<sub>3</sub>  
137 and 0.175 g P as KH<sub>2</sub>PO<sub>4</sub>) were mixed into the soil. Seeds of the NILs of different *Rht* alleles (*rht*-  
138 *B1a/D1a*, *Rht-B1b*, *Rht-D1b*, *Rht-B1b+D1b*, *Rht-B1c*) in the Maris Huntsman background were  
139 germinated on perlite and vernalized at 5°C in a controlled environment room for 8 weeks. Seedlings  
140 were then transplanted into the soil (5 per pot). Each NIL was planted in four pots. Pots were placed on  
141 benches in a randomized block design inside a glasshouse lit with sunlight supplemented with sodium  
142 vapor lamps to maintain a light intensity of >350 μmol/m<sup>2</sup>/s. Plants were watered daily with deionized  
143 water to approximately 70% of the water holding capacity. At maturity, plants were harvested by cutting  
144 at 1 cm above the soil surface. Grain was separated from the straw and dried at 80°C for 48 h.

145

146

147 Chemical analysis

148

149 Grain and straw were ground to fine powder and digested with HNO<sub>3</sub>/HClO<sub>4</sub> (85/15, v/v) in a heating  
150 block (Zhao et al. 1994). The concentrations of minerals, including the major nutrients K, P, S, Ca and  
151 Mg, the micronutrients Fe, Zn, Cu and Mn, and the soil contamination indicator mineral Ti, were  
152 determined using inductively-coupled plasma atomic emission spectrometry. A certified reference  
153 material (NIST 1567a wheat flour) was included in the analysis for quality assurance. The results  
154 obtained were in good agreement with the certified values; mean and standard deviation from 18  
155 digestion repeats were 14.6 ± 1.5 mg/kg for Fe (certified value 14.1 ± 0.5), 2.3 ± 0.2 mg/kg for Cu  
156 (certified value 2.1 ± 0.2), 12.6 ± 0.8 mg/kg for Zn (certified value 11.6 ± 0.4) and 386 ± 9 mg/kg for Mg  
157 (certified value 400 ± 2). Nitrogen concentration was determined with the Dumas combustion method  
158 (LECO FP-328, LECO, Stockport, UK).

159

160 Statistical analysis

161

162 For the field experiments, appropriate split plot analyses of variance (ANOVA) included a treatment  
163 structure of Background\*Allele\*pol(N rate;3) (GENSTAT 13; VSN International, Hemel Hempstead,  
164 UK) so as to split N effects into polynomial contrasts. Titanium (Ti) on the above-ground crop  
165 (straw+chaff+grain) was used as a covariate for above ground yields and harvest indices of DM and  
166 minerals to help correct for error associated with any soil contamination. The effect of the covariate was  
167 significant ( $P < 0.05$ ) in all these cases. Ti has been used as an indicator of soil contamination because it is  
168 abundant in soil but hardly taken up by plants (Cook et al. 2009). There were no significant effects of the  
169 covariate on any assessments of the grain (yields, mass per grain, and concentration) and therefore, the  
170 covariate was omitted from grain ANOVAs. There was little evidence of Background x Allele x N rate  
171 interactions so data are presented for the two factor interactions including N rate. The economically  
172 optimal level of N fertilizer was calculated on the simple basis of the N rate fitted to give the greatest  
173 financial margin over N cost. These calculations were based on N:grain price ratios of 5:1 and 10:1  
174 (Sylvester-Bradley and Kindred 2009) with grain traded on a 15% moisture content basis. Grouped  
175 polynomial regression analysis was employed to assess the relationships between mineral and N  
176 concentrations. For the pot experiment, ANOVA was performed to test the significance of the differences  
177 between the NILs.

178

179

180 **Results**

181

182 Field experiment 2009/10

183

184 The 2009/10 growing season was characterized by comparatively dry conditions during stem extension  
185 (April and May; Table 1) and grain filling (June and July).

186 Averaged over background and N rate, the inclusion of the Norin 10 semi-dwarfing *Rht-B1b* allele  
187 produced crop heights in the 800-900 mm range i.e. that predicted to be optimal for yield (Table 2). The  
188 *rht-B1a* and *Rht-B1c* alleles were associated with crop heights which would be considered to be supra-  
189 and sub- optimal respectively. *Rht-B1b* increased grain yield of DM (Fig. 1c) because it increased both  
190 above-ground crop biomass (Fig. 1a), and harvest index (Fig. 1b). Relative to *rht-B1a* (tall), *Rht-B1b* was  
191 associated with significantly ( $P<0.05$ ) reduced concentrations of Cu in grain (Fig. 1i; Main Effect = -2.6  
192 mg/kg; s.e.d. = 0.0768 (d.f.=16)), Fe (Fig. 1n; -2.7; 0.71), Mg (Fig. 1s; -98.4; 12.77), and Zn (Fig. 1x; -  
193 2.6; 0.50). *Rht-B1b* also significantly reduced the concentrations of N (Fig. 2), manganese (Mn),  
194 phosphorous (P), and sulfur (S), but not of calcium (Ca), potassium (K), molybdenum (Mo) or sodium  
195 (Na) (data not presented). The decreased concentrations of Cu, Fe, Mg and Zn can only be ascribed to  
196 dilution due to increased yields of dry matter, as there was no significant effect of *Rht-B1b* on total  
197 mineral uptake, whether expressed in terms of the above ground biomass or grain per unit area, or on a  
198 per grain basis (Fig. 1).

199 *Rht-B1c* improved harvest index relative to *Rht-B1b* (Fig. 1b) but this was insufficient to counter a  
200 negative effect on above ground biomass (Fig. 1a), such that severe dwarfing was associated with reduced  
201 grain yields (Fig. 1c). *Rht-B1c* was not, however, significantly ( $P>0.05$ ) detrimental to the accumulation  
202 of Cu (Fig. 1e,g), Fe (Fig. 1j,l), or Zn (Fig. 1t,v) so relative to *Rht-B1b*, the grain concentrations of all  
203 three mineral were increased (Fig. 1i,n). For Mg, as for other minerals, *Rht-B1c* did not influence uptake  
204 into the above-ground crop (Fig. 1o). In contrast to effects on DM, however, severe dwarfing was  
205 associated with a reduced harvest index of Mg (Fig. 1p) such that the yields of Mg per grain were reduced  
206 by *Rht-B1c* (Fig. 1q), contributing to reduced concentrations of Mg in the grain (Fig. 1s).

207 In the absence of *Rht*, the height of Mercia would be considered optimal and Maris Widgeon supra-  
208 optimal (Table 2). The two shorter cultivars had the highest DM harvest indices and apparently, also the  
209 higher grain yields ( $P=0.044$  for the main effect of background). Similar to the results for *Rht-B1b*, there  
210 was no significant effect of background on mineral uptake (Cu, Fe, Mg or Zn) into the above-ground  
211 biomass, and mineral concentrations tended to be more dilute in Mercia compared with Maris Widgeon  
212 (Table 2,  $P<0.001$ , 0.077,  $<0.001$  for the main effects of background on Cu, Mg and Zn respectively).  
213 Additional effects of cultivar were, however, evident: the harvest indices and grain yields per unit area for

214 all four minerals varied significantly among the backgrounds, and were always highest for Maris  
215 Huntsman (Table 2).

216 In contrast to the effects of *Rht-B1b* which diluted mineral concentrations as grain yields of dry  
217 matter were increased, N rate was associated with both increased grain DM yields, *and* increased  
218 concentrations of Cu and Fe. The quantity of Fe per grain was also increased by N applications (Fig. 1*h*,  
219 *m*, *r*, *w*) even though such applications also reduced mean grain weight (Fig. 1*d*). Nitrogen application  
220 increased the quantities of all four minerals in the above ground crop biomass (Fig. 1*e*, *j*, *o*, *t*), which  
221 largely explains similar effects on quantities in the grain on a unit area basis (Fig. 1*g*, *l*, *q*, *v*), although N  
222 application also increased the harvest index of Fe (Fig. 1*k*). There was a strong association between the  
223 grain Fe concentration and that of grain N (Fig. 2); to the extent that when grain N concentration was  
224 included in the regression model, there was no statistical justification for including effects of Allele as an  
225 explanatory factor. This was not the case for Cu, Mg and Zn (Fig. 2).

226 The economically optimal level (Table 3) of N fertilizer varied with allele because there was a  
227 significant Allele x N rate interaction on grain yield of DM (Fig. 1*c*). The grain yield of *Rht-B1b* was the  
228 most responsive to N (Fig. 1*c*), partly because of a combination of comparatively high biomass yield (Fig.  
229 1*a*) and harvest index (Fig. 1*b*) at the highest N rate. Using the quadratic fits in Fig. 1*c*,  $N_{opt}$  was between  
230 40 and 50 kg N/ha higher for *Rht-B1b* compared with *rht-B1a* (Table 3). Despite the benefit of N  
231 application for some mineral concentrations, however, this level of increase in N application was  
232 insufficient to compensate for the diluting effect of *Rht-B1b* (Table 3) i.e. semi-dwarfing reduced the  
233 concentrations of all four minerals whether compared at a single average N rate, or at the different  $N_{opt}$  for  
234 the different alleles. Increasing N cost relative to grain value reduced  $N_{opt}$  and therefore tended to reduce  
235 Fe concentration at  $N_{opt}$ .

236

237 Field experiment 2010/11

238

239 Conditions were particularly dry during the start of stem extension (March and April, Table 1), and also  
240 exceptionally warm during April. The period of below average rainfall continued in May. The grain  
241 filling period (June and July) was associated with above average rainfall and comparatively cool  
242 temperatures. The notably dry conditions during spring, coinciding with the timing of the N application,  
243 probably contributed to plants being shorter than in the previous season (Tables 2 and 3), and also to the  
244 crop being much less N responsive in terms of above ground biomass and grain yield (Fig. 3*a,c*). In  
245 contrast to the previous year, crop heights of *rht-B1a* would not be considered excessive for optimizing  
246 grain yields (Table 2). Also in contrast to 2009/10, there were no significant main effects of *Rht-B1b* on  
247 above-ground dry matter or grain yield despite a small positive effect on harvest index (Fig. 3*a,b,c*).

248 Relative to *rht-B1a* (tall), the size of effects of *Rht-B1b* on grain mineral concentrations were much  
249 reduced compared with the previous year. Indeed, for the 2011 harvest there was no statistically  
250 significant main effect of *Rht-B1b* on the grain concentrations of Cu, Fe, Zn (Fig. 3*i, n, x*), Ca, Mn, Na or  
251 S (data not presented). *Rht-B1b* was associated with small but significant reductions in the concentrations  
252 of Mg (Fig. 1*s*; Main Effect = -31 mg/kg; s.e.d. = 9.6 (d.f.=16)), N (Fig. 2; -0.84; 0.366) and P (-110; 35),  
253 and an increase in the concentration of K (+139; 34.1). Where effects were statistically significant, it is  
254 difficult to ascribe these directly to effects on dilution with DM, mineral uptake, or partitioning given the  
255 very marginal effects of *Rht-B1b* on any of these variables.

256 As in 2010, *Rht-B1c* improved harvest index relative to *Rht-B1b* (Fig. 3*b*) but in 2011 this was  
257 sufficient to counter the negative effect on above ground biomass (Fig. 3*a*), such that severe dwarfing was  
258 associated with comparable grain yields to taller lines, when taking the averages over N rates (Fig. 3*c*). As  
259 well as above ground dry matter accumulation *Rht-B1c* was detrimental to the accumulation of Mg (Fig.  
260 3*o*) and Zn (Fig. 3*t*), particularly at high rates of N fertilizer. In contrast to the situation with DM, *Rht-B1c*  
261 did not increase the harvest index of minerals, and this failure contributed to the reduction in  
262 concentration of Mg in the grain (Fig. 3*s*).

263 In 2011, grain yields of Mercia tended to be higher than Maris Widgeon, associated with  
264 significantly reduced crop height and increased DM harvest index (Table 3). Harvest index of Cu was  
265 increased in Mercia, but this was insufficient to prevent a dilution of Cu in the grain. Zn was also more  
266 dilute in Mercia grain, again not because of reduced uptake or partitioning, but because effects did not  
267 match those on DM.

268 As in 2010, uptake of Cu, Fe, Mg and Zn increased with N rate, both in the above ground biomass  
269 and in the grain (Fig. 3). Increases in mineral quantity in the grain tended to be greater than the grain DM  
270 yield response to N application such that, for at least part of the range of rates used, mineral concentration  
271 increased with N application: for Cu to about 100 kg N/ha (Fig. 3*i*); for Fe and Mg, to about 300 kg N/ha  
272 (Fig. 3*n, s*); and for Zn to about 400 kg N/ha (Fig. 3*x*). The concentration of Fe in the grain was again  
273 closely associated with N concentration (Fig. 2). There were also positive relationships between grain N  
274 concentration and the concentrations of Mg and Zn, but not to the extent that N concentration could  
275 account for *Rht* allele effects.

276 Although there was no main effect of *Rht* allele on grain DM yield, there was a significant Allele x N  
277 rate interaction (Fig. 3*c*). *Rht-B1b* was again the most N-responsive allele, having a comparatively low  
278 yield at low N rates, but the highest yield at high N rates. The derived  $N_{opt}$  for a 5:1 N:grain price ratio  
279 suggested that it would have been justified to apply 64 kg more N/ha on *Rht-B1b* than on *rht-B1a* (Table  
280 4). Given the shallow response of grain yield to N in this season, however, the statistical error around this  
281 figure must be substantial. Nonetheless, if an extra 64 kg N was applied to *Rht-B1b* this would have been

282 sufficient to negate the diluting effect of the allele on the Mg concentration in the grain. It should also be  
283 noted, however, that the grain yield response was so shallow, that there was no economic justification for  
284 applying any N fertilizer to any allele if the N:grain price ratio was as high as 10:1 (Table 4), and hence  
285 the diluting effect of *Rht-B1b* on Mg concentration would have persisted at high N cost.

286

287 Pot experiment

288

289 Both plant height and grain yield decreased with the introduction of dwarfing alleles in the Maris  
290 Huntsman background in the order of *rht-B1a/D1a* (tall) > *Rht-B1b* > *Rht-D1b* > *Rht-B1B+D1b* > *RhtB1c*  
291 (Fig. 4a). In contrast to the field experiments, *Rht-B1b* did not increase the grain yield compared with *rht-*  
292 *B1a/D1a* (tall). Plant heights of all lines were substantially shorter than the corresponding ones in the  
293 field experiments.

294 The concentrations of Fe, Zn and Cu differed significantly ( $P < 0.001$ ) between the dwarfing alleles,  
295 but, in contrast to the field experiments, they increased with the severity of the dwarfing phenotype (Fig.  
296 4b). Note that the concentrations of Fe and Zn were considerably larger than those from the field trial. In  
297 addition to the three minerals presented here, the concentrations of Mg, P and S also showed significant  
298 increasing trends with the degree of dwarfing (data not shown). There were highly significant negative  
299 correlations between the concentrations of Fe, Zn or Cu and grain yield ( $r = -0.80, -0.79$  and  $-0.71$  for Fe,  
300 Zn and Cu, respectively,  $P < 0.001$ ; Fig. 5). The amounts of Fe, Zn and Cu taken up by the plants (straw  
301 and grain) did not differ significantly between the different *Rht* alleles (Fig. 4c), nor were there any  
302 significant differences in the amounts of these minerals contained in the grain (data not shown). The  
303 harvest indices for both the biomass and the three minerals were broadly similar, showing a decreasing  
304 trend with the dwarfing severity (Fig. 4d).

305

## 306 Discussion

307

308 We confirm the shortening effects of alleles at the *Rht-B1* and *Rht-D1* loci (Flintham et al. 1997). Also  
309 consistent with previous studies, we find semi-dwarfing is beneficial for grain yield when it reduces final  
310 crop height to around 800-900 mm in UK conditions (Flintham et al. 1997; Austin 1999), and that semi-  
311 dwarfing increases the response to N fertilizer in such circumstances (Anderson et al. 1991). That *Rht-*  
312 *B1c* reduces grain yield because increases in harvest index are insufficient to compensate for reductions in  
313 above ground biomass was also reported by Flintham et al. (1997). The glasshouse pot experiment here  
314 was consistent with other studies where semi-dwarfing with *Rht* alleles have had neutral or negative

315 effects on yield in situations where *rht-B1a/D1a* is associated with plant heights already optimal or sub-  
316 optimal for yield (Flintham et al. 1997; Chapman et al. 2007; Gooding et al. 2012).

317 The main objective of the present study was to test if the introduction of the GA-insensitive dwarfing  
318 alleles in wheat affects the concentrations of minerals in grain that are particularly important for human  
319 nutrition. Our results suggest that the dwarfing alleles have comparatively little direct effects on the  
320 uptake and translocation to grain of Fe, Zn, Cu and Mg; however, the concentrations of these minerals in  
321 grain were affected indirectly mainly as a result of the changes in grain yield. That the dilution of  
322 minerals by the incorporation *Rht-B1b* was not due to more direct physiological effects of gibberellin  
323 insensitivity is further supported by: a lack of progression of effects through *rht-B1a* to *Rht-B1b* to *Rht-*  
324 *B1c* on mineral concentration despite the progression in GA insensitivity and degree of dwarfing; the  
325 contrasting effects of *Rht* alleles in the two field experiments, and between the field and pot experiments,  
326 consistent with the contrasting effects on grain yield; and a similar dilution of minerals between Maris  
327 Widgeon and Mercia, associated with reduced height but not mediated through differences in GA-  
328 sensitivity.

329 Consistent with the effects of *Rht-B1b* and Mercia in the field, mineral (e.g. Zn, Fe or Mg)  
330 concentrations have been found to correlate negatively with grain yield among diverse wheat cultivars  
331 (McDonald et al. 2008; Morgounov et al. 2007; Oury et al. 2006; Zhao et al. 2009). The decreasing trend  
332 of mineral concentrations in wheat grain, either with the cultivar release date (Garvin et al. 2006; Zhao et  
333 al. 2009) or in the Broadbalk long-term experiment (Fan et al. 2008), can be largely attributed to the  
334 dilution effect brought about by increased grain yield. This effect may arise because the distribution of  
335 minerals to grain does not match the increased biomass allocation to grain in the higher yielding cultivars.

336 Whilst yield gain through cultivar improvement results in a dilution of mineral concentrations in  
337 wheat grain, yield increase in response to increasing the application rate of N did not. In fact, increasing  
338 N rate increased the concentrations of Fe and, depending on season and to a lesser extent, Cu, Mg and Zn  
339 in grain. Similar effects have been reported for Fe and Zn in durum (*Triticum durum*) and bread (McGrath  
340 1985; Cakmak et al. 2010; Shi et al. 2010) wheats. Increasing N supply was found to increase the uptake,  
341 root-to-shoot translocation and remobilization from vegetative tissues to grain of Zn and Fe in durum  
342 wheat, possibly because of more transporter proteins and an increased pool of nitrogenous chelators (e.g.  
343 nicotianamine) that are important for the phloem transport of these metals (Kutman et al. 2011a, b). Our  
344 results relating grain mineral concentration to grain N concentration suggest that any effect of plant N  
345 compounds on Fe is much more direct than that for Cu or Zn. Our results would suggest that the benefits  
346 of N rate for Zn are more reliable as grain N concentrations exceed 20 g N/kg. Grain N concentrations  
347 were in a lower range in the 2009-10 than in the 2010-11 trial, which may explain the lack of a significant  
348 N effect on grain Zn in the former season.

349 We demonstrate that the greater yield responses of appropriately-statured cultivars justify the use of  
350 larger N rates. This has the effect of offsetting some of the dilution effect on grain mineral concentration  
351 but at the economically optimal level of N the concentrations of minerals were still about 10% lower for  
352 the semi-dwarf *Rht-B1b* allele than in the tall allele in the most responsive field season of 2009/10 (Table  
353 3). It is well known, however, that wheat responses to N rate, and its interaction with genotype varies with  
354 site and season, so further experiments would be necessary to quantify this offset more generally. Our  
355 field experiments were conducted in two comparatively dry seasons for the site, which would be expected  
356 to reduce the wheat response to N (Gooding 2009). A comparison of the two seasons reported here  
357 strongly supports the importance of adequate moisture for large responses of wheat grain yield to N rate.  
358 The comparison also suggests that offsetting of the *Rht-B1b* mediated dilution of minerals with the extra  
359 N fertilizer is less likely in wetter seasons. Future benefits of N for mineral concentrations (particularly  
360 for Fe) are likely to be curtailed if increased cost and restriction of N fertilizer continues, and/or genetic  
361 improvements in N utilization efficiency (crop DM / crop N; Gooding et al. 2012) are realized. There is,  
362 therefore, increasing need to explore further the genetic variability in grain mineral concentration to  
363 achieve both high yields and sufficient levels of minerals in wheat (White and Broadley 2009; Zhao et al.  
364 2009).

365  
366

### 367 **Acknowledgements**

368  
369 The authors are grateful to Mr RJ Casebow and Mr S Loddio for technical assistance for the field  
370 experiments, to Mr A Crosland for ICP analysis and to the John Innes Centre for providing the seeds.  
371 Rothamsted Research receives strategic funding from the Biotechnology and Biological Sciences  
372 Research Council of the UK. M. S. Fan was supported by a Rothamsted International Fellowship.

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### 376 **References**

377 Addisu M, Snape JW, Simmonds JR, Gooding MJ (2009) Reduced height (*Rht*) and photoperiod  
378 insensitivity (*Ppd*) allele associations with establishment and early growth of wheat in contrasting  
379 production systems. *Euphytica* 166:249-263

380 Addisu M, Snape JW, Simmonds JR, Gooding MJ (2010) Effects of reduced height (*Rht*) and photoperiod  
381 insensitivity (*Ppd*) alleles on yield of wheat in contrasting production systems. *Euphytica* 172:169-  
382 181

383 Anderson WK, Seymour M, D'Antuono MF (1991) Evidence for differences between cultivars in  
384 responsiveness of wheat to applied nitrogen. *Aust J Agric Res* 42: 363-377.

385 Anon. (1986) *The Analysis of Agricultural Materials*, MAFF Reference Book 427, 3rd edn. HMSO,  
386 London.

387 Anon. (2010) *Fertiliser Manual (RB209)* 8th edn. TSO, Norwich.

388 Austin RB (1999) Yield of wheat in the United Kingdom: Recent advances and prospects. *Crop Sci*  
389 39:1604-1610

390 Austin RB, Bingham J, Blackwell RD, Evans LT, Ford MA, Morgan CL, Taylor M. (1980) Genetic  
391 improvements in winter-wheat yields since 1900 and associated physiological changes. *J Agric Sci*  
392 94:675-689

393 Borner A, Roder M, Korzun V (1997) Comparative molecular mapping of GA insensitive *Rht* loci on  
394 chromosomes 4B and 4D of common wheat (*Triticum aestivum* L.). *Theor Appl Genet* 95:1133-1137

395 Cakmak I, Pfeiffer WH, McClafferty B (2010) Biofortification of durum wheat with zinc and iron. *Cereal*  
396 *Chem* 87:10–20.

397 Chapman SC, Mathews KL, Trethowan RM, Singh RP (2007) Relationships between height and yield in  
398 near-isogenic spring wheats that contrast for major reduced height genes. *Euphytica* 157:391-397

399 Cook LL, McGonigle TR, Inouye RS (2009) Titanium as an indicator of residual soil on arid-land plants.  
400 *J Environ Qual* 38:188-199.

401 Davis DR, Epp MD, Riordan HD (2004) Changes in USDA food composition data for 43 garden crops,  
402 1950 to 1999. *J Am Coll Nutr* 23:669-682

403 Fan MS, Zhao FJ, Fairweather-Tait SJ, Poulton PR, Dunham SJ, McGrath SP (2008) Evidence of  
404 decreasing mineral density in wheat grain over the last 160 years. *J Trace Elem Med Biol* 22:315-  
405 324

406 Flintham JE, Borner A, Worland AJ, Gale MD (1997) Optimizing wheat grain yield: Effects of *Rht*  
407 (gibberellin-insensitive) dwarfing genes. *J Agric Sci* 128:11-25.

408 Garvin DF, Welch RM, Finley JW (2006) Historical shifts in the seed mineral micronutrient  
409 concentration of US hard red winter wheat germplasm. *J Sci Food Agric* 86:2213-2220

410 Gooding MJ (2009) The wheat crop. In: K Khan, P R Shewry (eds) *Wheat: Chemistry and Technology*,  
411 4th edn. AACC International, Minnesota. pp 35-70.

412 Gooding MJ, Addisu M, Uppal RK, Snape JW, Jones HE (2012) Effect of wheat dwarfing genes on  
413 nitrogen use efficiency. *J Agric Sci* (available online June 2011 doi: 10.1017/S0021859611000414).

414 Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G, Farron M (2003) The National  
415 Diet & Nutrition Survey: adults aged 19 to 64 years, Volume 3. Her Majesty's Stationery Office,  
416 London

417 Kutman UB, Yildiz B, Cakmak I (2011a) Effect of nitrogen on uptake, remobilization and partitioning of  
418 zinc and iron throughout the development of durum wheat. *Plant Soil* 342:149-164

419 Kutman UB, Yildiz B, Cakmak I (2011b) Improved nitrogen status enhances zinc and iron concentrations  
420 both in the whole grain and the endosperm fraction of wheat. *J Cereal Sci* 53:118-125

421 Loddo S, Gooding MJ (2012) Semi-dwarfing (*Rht-B1b*) improves nitrogen-use efficiency in wheat, but  
422 not at economically optimal levels of nitrogen availability. *Cereal Res Commun* (accepted May  
423 2011)

424 McDonald GK, Genc Y, Graham RD (2008) A simple method to evaluate genetic variation in grain zinc  
425 concentration by correcting for differences in grain yield. *Plant Soil* 306:49-55

426 McGrath SP (1985) The effects of increasing yields on the macroelement and microelement  
427 concentrations and offtakes in the grain of winter wheat. *J Sci Food Agric* 36:1073-1083

428 Morgounov A, Gomez-Becerra HF, Abugalieva A, Dzhususova M, Yessimbekova M, Muminjanov H,  
429 Zelenskiy Y, Ozturk L, Cakmak I (2007) Iron and zinc grain density in common wheat grown in  
430 Central Asia. *Euphytica* 155:193-203

431 Murase K, Hirano Y, Sun TP, Hakoshima T (2008) Gibberellin-induced DELLA recognition by the  
432 gibberellin receptor GID1. *Nature* 456:459-U15

433 Oury FX, Leenhardt F, Remesy C, Chanliaud E, Duperrier B, Balfourier F, Charmet G (2006) Genetic  
434 variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. *Eur J*  
435 *Agron* 25:177-185

436 Shi RL, Zhang YQ, Chen XP, Sun QP, Zhang FS, Römheld V, Zou CQ (2010) Influence of long-term  
437 nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum* L.). *J*  
438 *Cereal Sci* 51:165–170

439 Sylvester-Bradley R, Kindred DR 2009 Analysing nitrogen response of cereals to prioritize routes to the  
440 improvement of nitrogen use efficiency. *J Exp Bot* 60:1939-1951

441 White PJ, Broadley MR (2005) Historical variation in the mineral composition of edible horticultural  
442 products. *J Hort Sci Biotech* 80:660-667.

443 White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in  
444 human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol* 182:49-84

445 WHO (2002) The World Health Report (2002) Reducing risks, promoting healthy life. pp 1-230. World  
446 Health Organization, Geneva, Switzerland.

- 447 Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Res*  
448 44:415-421
- 449 Zhao F, McGrath SP, Crosland AR (1994) Comparison of three wet digestion methods for the  
450 determination of plant sulphur by inductively coupled plasma atomic emission spectroscopy (ICP-  
451 AES). *Commun Soil Sci Plant Anal* 25:407-418
- 452 Zhao FJ, Su YH, Dunham SJ, Rakszegi M, Bedo Z, McGrath SP, Shewry PR (2009) Variation in mineral  
453 micronutrient concentrations in grain of wheat lines of diverse origin. *J Cereal Sci* 49:290-295  
454

**Table 1** Monthly weather data, soil analyses, and crop husbandry common to all treatments for the two field plot experiments

	2010		2011		Long term average (47 years)	
	Rainfall (mm)	Mean temp. (°C)	Rainfall (mm)	Mean temp. (°C)	Rainfall (mm)	Mean temp. (°C)
March	46.2	6.3	13.8	6.5	47.2	6.5
April	22.0	9.2	2.8	12.2	47.9	8.7
May	12.0	11.2	30.0	12.3	47.9	12.0
June	20.8	16.1	89.8	14.2	49.3	15.0
July	31.6	18.4	41.1	16.2	44.6	17.2
August	108.0	16.1	125.2	15.9	60.1	17.0
Treatment or operation	Growth stage (GS, Zadoks et al. 1974)					
Soil test 0-90cm						
pH	6.3		6.4			
P mg/l (index)	49.0 (4)		26.4 (3)			
K mg/l (index)	99 (1)		88 (1)			
Mg mg/l (ind.)	48 (1)		52 (2)			
Herbicide	Destruction of previous sward		Glyphosate 1.8kg/ha			
Sub-soiled	Pre-ploughing	16 September	Not applicable			
Nutrition	Pre-ploughing	120 kg/ha K <sub>2</sub> O equ. as KCl	90 kg/ha K <sub>2</sub> O equ. as KCl			
Ploughed		8 October	23 September			
Harrowed		21 October	8 October			
Drilled		22 October	8 October			
Rolled		22 October	11 October			
Herbicide	13	1.2 kg/ha pendimethalin + 20 g/ha carfentrazone-ethyl + 10 g/ha flupyr-sulfuron-methyl				
Nutrition	30	40 kg/ha SO <sub>3</sub> equ. as K <sub>2</sub> SO <sub>4</sub>	32 kg/ha SO <sub>3</sub> equ. as K <sub>2</sub> SO <sub>4</sub>			
Herbicide	31	50 g/ha thifensulfuron-methyl + 5 g/ha metsulfuron-methyl				
Fungicide	32	125 g/ha epoxiconazole + 150 g/ha quinoxifen + 1 kg/ha chlorothalonil		125 g/ha epoxiconazole + 150g/ha metrafenone + 1 kg/ha chlorothalonil		
Fungicide	39	125 g/ha epoxiconazole + 250 g/ha azoxystrobin				
Fungicide	59	250 g/ha tebuconazole				
Insecticide	59	5 g/ha deltamethrin				
Harvest		9 August	17 August			

**Table 2** The effect of allele at the *Rht-B1* locus and nitrogen fertilizer application rate (N, kg/ha) on the final crop height (mm) of winter wheat.

2010 (Mean of Maris Huntsman, Maris Widgeon and Mercia backgrounds)				2011 (Mean of Maris Widgeon and Mercia backgrounds)			
N	<i>B1a</i>	<i>B1b</i>	<i>B1c</i>	N	<i>B1a</i>	<i>B1b</i>	<i>B1c</i>
0	910	720	420	0	840	700	450
100	1020	830	460	50	850	720	460
200	1020	840	480	100	830	700	440
350	1000	830	470	200	820	730	440
				350	830	750	460
				450	810	740	450
S.E.D. <sup>a</sup>			11				13
d.f.			54				55
Mean	990	810	460		830	720	450
S.E.D. <sup>b</sup>			12				19
d.f.			16				10

459 <sup>a</sup>for comparing N levels within allele; <sup>b</sup>for comparing allele means

**Table 3** Effect of cultivar on the height, yields and partitioning of dry matter and minerals in winter wheat.

	2010				2011		
	Maris Huntsman	Mercia	Maris Widgeon	S.E.D. (16 d.f.)	Mercia	Maris Widgeon	S.E.D. (10 d.f.)
Crop height (mm)	1010	820	1120	20	750	910	27
<b>Dry Matter</b>							
Above ground crop (t/ha)	13.9	13.0	15.0	1.23	9.9	8.8	1.50
Harvest_index	0.47	0.47	0.39	0.013	0.54	0.48	0.007
Grain yield (t/ha)	6.85	6.23	5.82	0.535	5.33	4.24	0.556
Mass per grain (mg)	49.5	40.7	48.9	1.28	54.8	59.1	0.74
<b>Copper (Cu)</b>							
Above ground crop (mg/m <sup>2</sup> )	4.32	3.85	4.25	0.411	2.88	2.96	0.439
Harvest_index	0.70	0.63	0.60	0.021	0.66	0.62	0.011
Grain yield (mg/m <sup>2</sup> )	3.10	2.41	2.54	0.201	1.91	1.88	0.199
Mass per grain (µg)	0.22	0.16	0.21	0.006	0.20	0.26	0.006
Grain concentration (mg/kg)	4.52	3.87	4.32	0.133	3.60	4.42	0.105
<b>Iron (Fe)</b>							
Above ground crop (mg/m <sup>2</sup> )	53.4	51.8	57.6	7.02	184.1	184.9	9.90
Harvest_index	0.41	0.36	0.33	0.026	0.11	0.09	0.013
Grain yield (mg/m <sup>2</sup> )	20.6	18.5	18.3	1.88	15.8	12.7	1.68
Mass per grain (µg)	1.42	1.16	1.48	0.068	1.61	1.75	0.057
Grain concentration (mg/kg)	28.8	28.7	30.1	1.23	29.2	29.5	0.99
<b>Magnesium (Mg)</b>							
Above ground crop (g/m <sup>2</sup> )	1.13	1.03	1.15	0.119	0.96	0.80	0.164
Harvest_index	0.59	0.59	0.52	0.013	0.63	0.58	0.028
Grain yield (g/m <sup>2</sup> )	0.68	0.61	0.59	0.046	0.60	0.46	0.062
Mass per grain (µg)	50.0	40.4	49.9	1.00	61.3	64.4	1.11
Grain concentration (g/kg)	1.01	0.99	1.02	0.022	1.12	1.09	0.014
<b>Zinc (Zn)</b>							
Above ground crop (mg/m <sup>2</sup> )	15.6	14.6	16.1	1.63	12.4	11.6	1.52
Harvest_index	0.85	0.85	0.79	0.017	0.92	0.90	0.008
Grain yield (mg/m <sup>2</sup> )	13.3	12.2	12.6	1.09	11.2	10.2	1.02
Mass per grain (µg)	0.97	0.80	1.07	0.035	1.15	1.42	0.063
Grain concentration (mg/kg)	19.6	19.8	21.9	0.86	20.9	24.0	1.16

Values are means over N fertilizer rates and three replicates.

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**Table 4** The effect of allele at the *Rht-B1* locus on the fitted economically optimal level of nitrogen ( $N_{opt}$ ) for two N:Grain price ratios, and the fitted (Figs. 1 & 3) mineral concentrations at  $N_{opt}$ .

N:Grain price ratio	2010			2011		
	<i>B1a</i>	<i>B1b</i>	<i>B1c</i>	<i>B1a</i>	<i>B1b</i>	<i>B1c</i>
$N_{opt}$ (kg/ha)						
5.0:1	253	299	243	115	179	97
10.0:1	213	252	197	0	0	0
Grain yield at $N_{opt}$						
5.0:1	7.8	9.2	7.3	4.7	4.9	4.9
10.0:1	7.6	9.0	7.1	4.1	3.9	4.4
mg Cu/kg at $N_{opt}$						
5.0:1	4.3	4.0	4.9	4.2	4.3	4.2
10.0:1	4.3	4.0	4.8	3.8	3.9	4.1
mg Fe/kg at $N_{opt}$						
5.0:1	33.9	30.4	33.9	28.9	30.7	28.3
10.0:1	32.8	29.3	31.8	24.0	24.0	24.0
g Mg/kg at $N_{opt}$						
5.0:1	1.01	0.88	0.88	1.10	1.10	1.01
10.0:1	1.00	0.87	0.88	1.05	1.02	0.97
mg Zn/kg at $N_{opt}$						
5.0:1	19.6	17.4	20.4	21.5	22.7	21.2
10.0:1	19.5	16.9	19.8	18.8	18.8	18.8

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468 **Figures:**

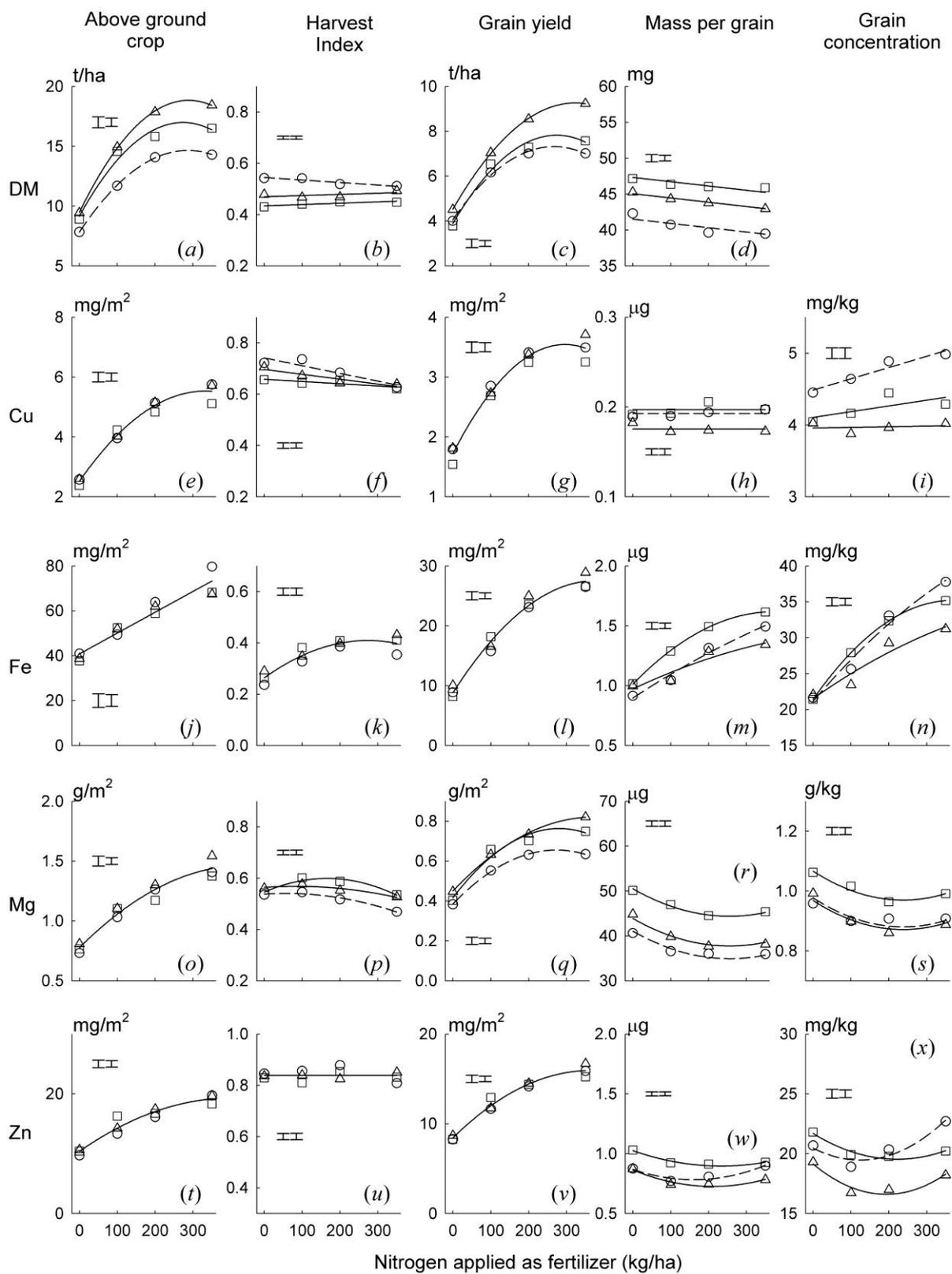
469 **Fig. 1** Effect of nitrogen and allele at the *Rht-B1* locus on yield and mineral content of winter wheat near  
470 isogenic lines. □=*rht-B1a* (tall); △ = *Rht-B1b* (semi-dwarf); ○, dashed line = *Rht-B1c* (severe dwarf).  
471 Points are means of three replicate blocks and three wheat backgrounds (Maris Huntsman, Mercia, Maris  
472 Widgeon). Left and right error bars are S.E.D. for comparing between and within alleles respectively (sub  
473 plot error d.f. = 53). Main and interacting effects of Allele, Linear N and Quadratic N only fitted if effect  
474 is statistically significant ( $P<0.05$ ).

475 **Fig. 2** Relationships between grain mineral concentration and grain nitrogen concentration in wheat  
476 harvested in 2010 (left) and 2011 (right). Points are means of three replicate blocks and wheat  
477 backgrounds (Mercia, Maris Huntsman and Maris Widgeon in 2010; Mercia and Maris Widgeon in  
478 2011), and vary for allele as near isogenic lines at the *Rht-B1* locus (□=*rht-B1a* (tall); △ = *Rht-B1b*  
479 (semi-dwarf); ○, dashed line = *Rht-B1c* (severe dwarf)) and for nitrogen fertilizer application rate (N  
480 rate). Numerals above inverted triangles in Fe panels are the N rates (kg/ha) associated with the nitrogen  
481 concentrations achieved (averaged over allele). Error bars are maximum S.E.D. for comparing points (sub  
482 plot error d.f. = 53 and 55 for 2010 and 2011 respectively). Main and interacting effects of Allele, Linear  
483 N and Quadratic N only fitted if effect is statistically significant ( $P<0.05$ ).

484 **Fig. 3** Effect of nitrogen and allele at the *Rht-B1* locus on yield and mineral content of winter wheat near  
485 isogenic lines harvested in 2011. □=*rht-B1a* (tall); △ = *Rht-B1b* (semi-dwarf); ○, dashed line = *Rht-B1c*  
486 (severe dwarf). Points are means of three replicate blocks and two wheat backgrounds (Mercia, Maris  
487 Widgeon). Left and right error bars are S.E.D. for comparing between and within alleles respectively (sub  
488 plot error d.f. = 55). Main and interacting effects of Allele, Linear N and Quadratic N only fitted if effect  
489 is statistically significant ( $P<0.05$ ).

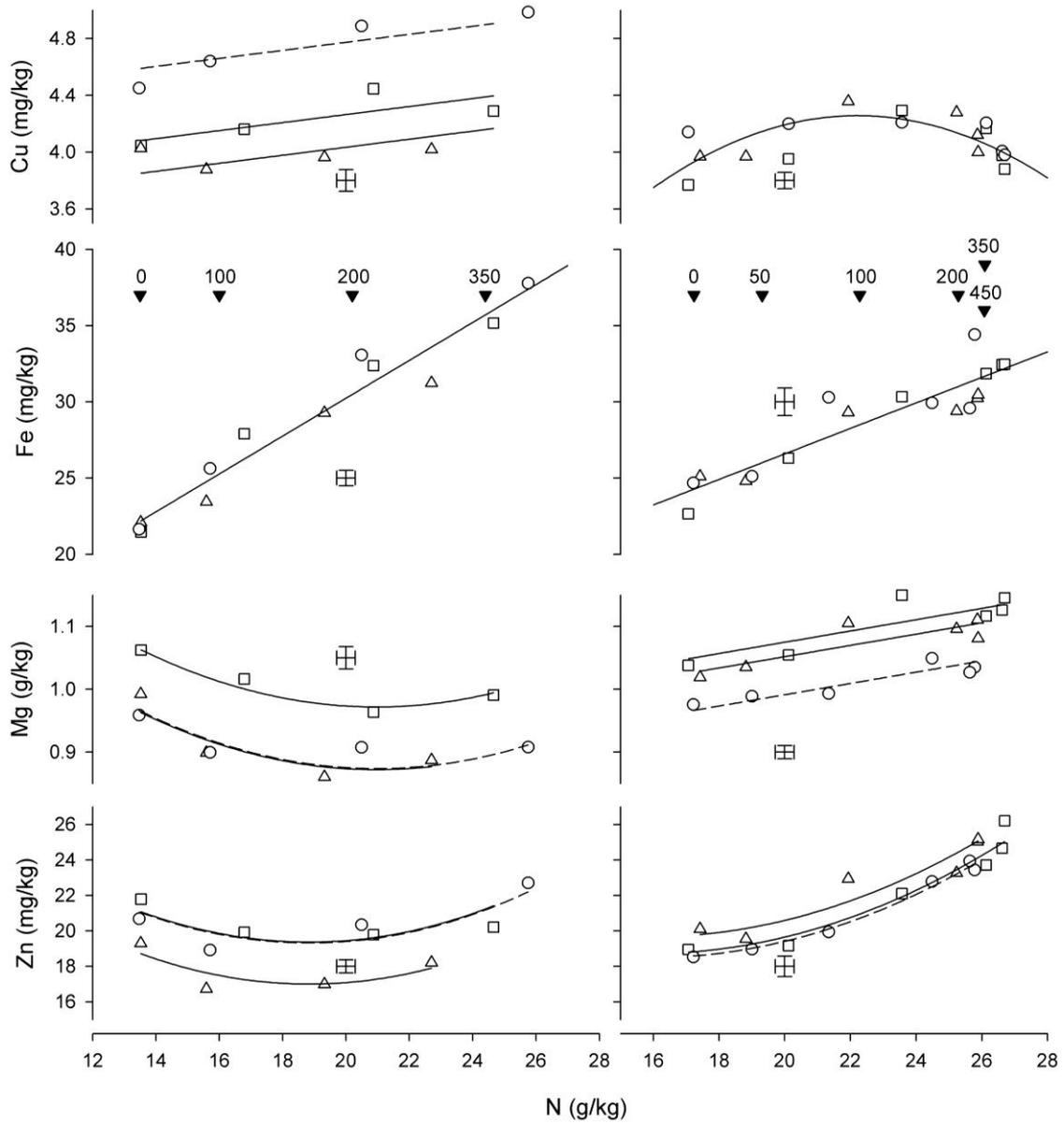
490 **Fig. 4** Effects of dwarfing alleles in the Maris Huntsman background on grain yield and height (a), grain  
491 micronutrient concentrations (b), uptake (c) and harvest indices in the pot experiment. Error bars are  
492 S.E.D. (d.f. = 12) for comparing different alleles; the error bars from left to right in each graph correspond  
493 to the variable from top to bottom in the legend box.

494 **Fig. 5** Correlation between grain Fe, Zn or Cu concentrations and grain yield in the pot experiment.



495

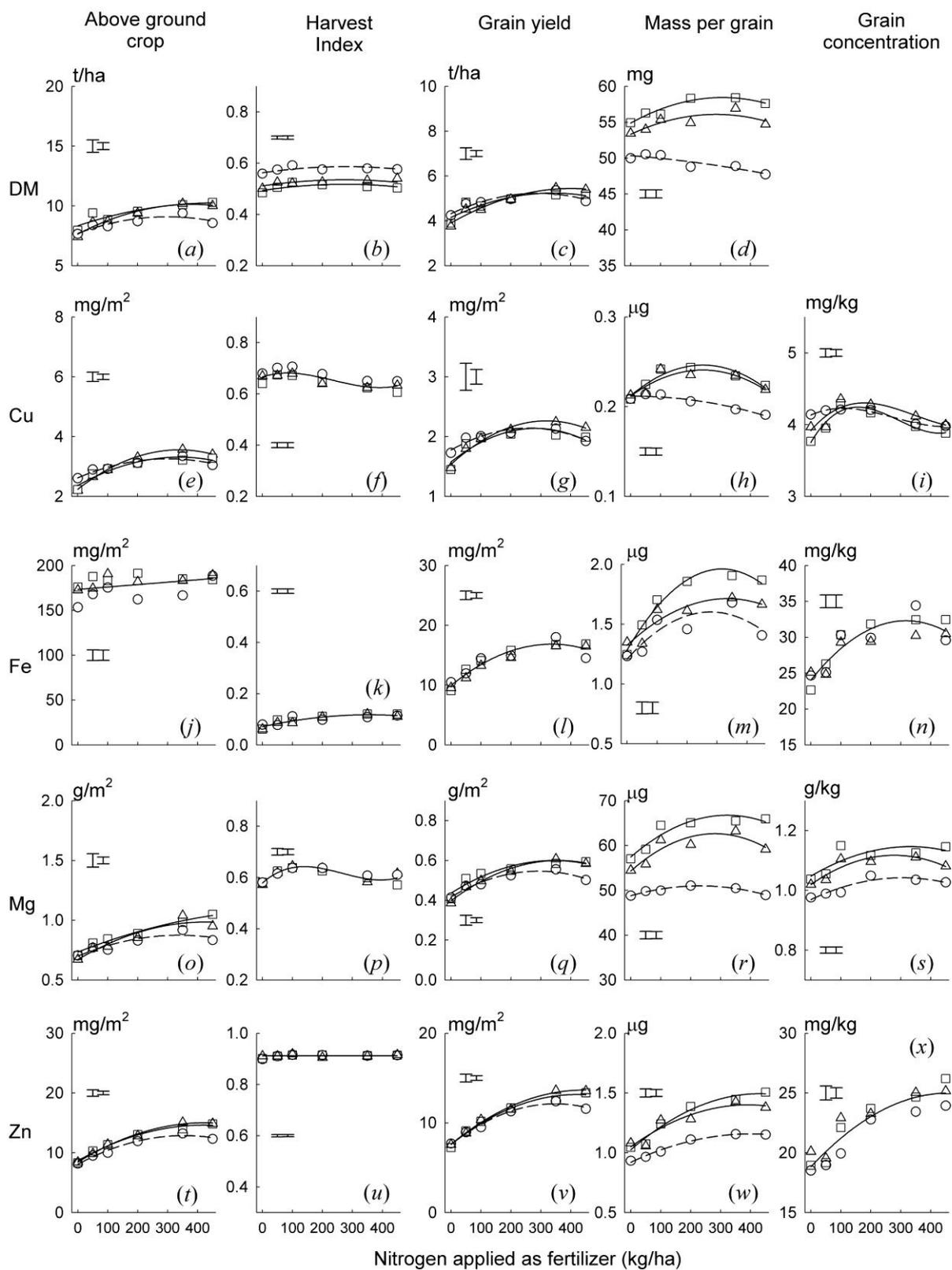
496 **Figure 1.**



497

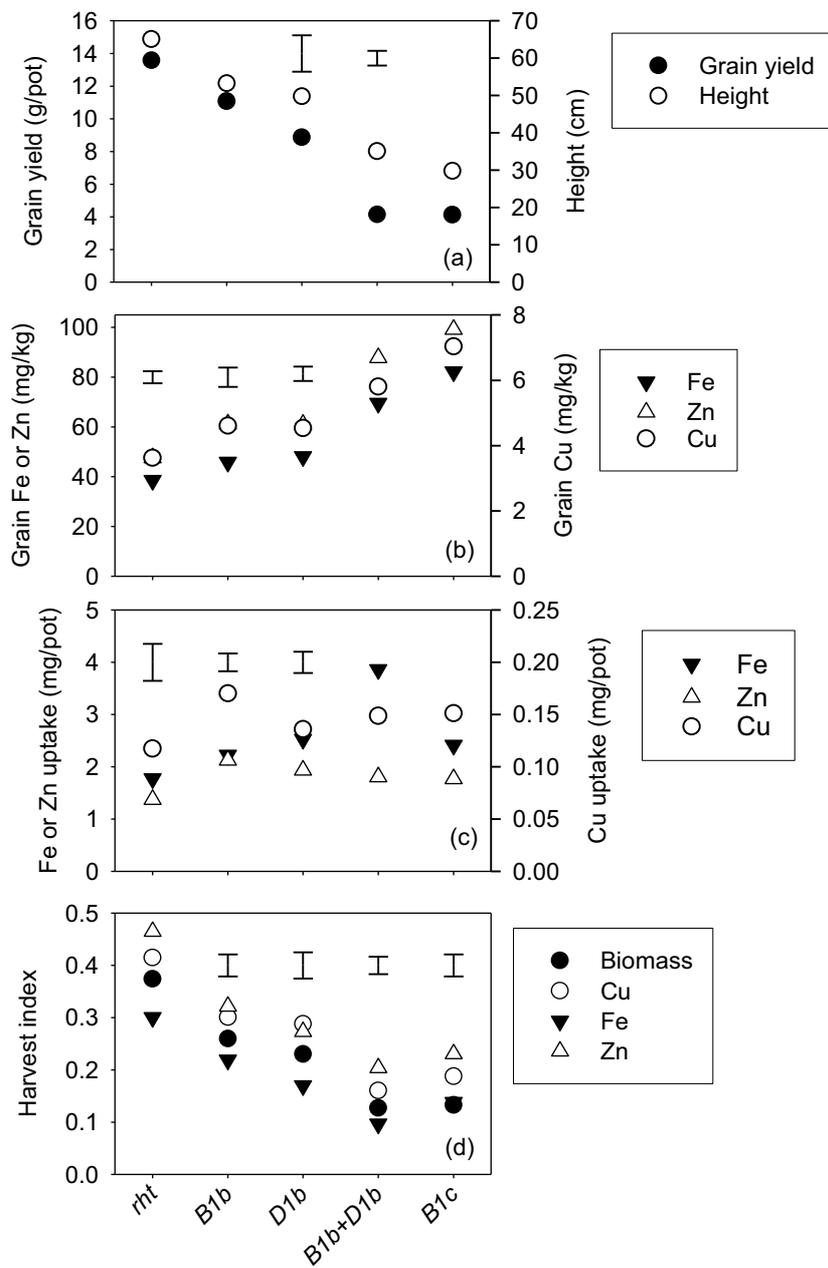
498 **Figure 2.**

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501 Figure 3.

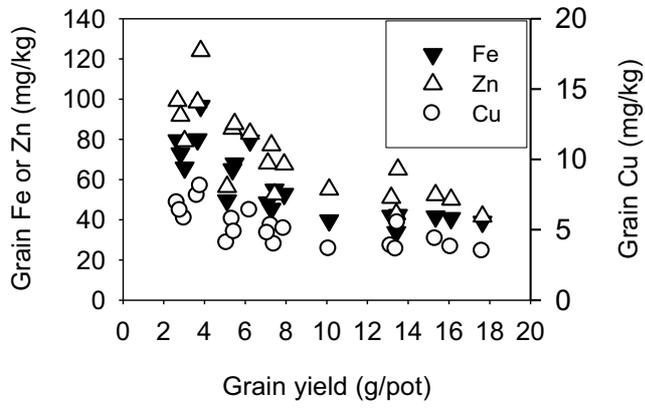


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503 Figure 4.

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507 Figure 5.