Theor Appl Genet (1990) 79:108–112

The effects of 'gibberellin-insensitive' dwarfing alleles in wheat on grain weight and protein content

M. J. Pinthus[1] **and M. D. Gale**[2]

[1] The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel
[2] AFRC Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB, UK

Received August 5, 1989; Accepted August 30, 1989
Communicated by J. MacKey

Summary. Three series of near-isogenic wheat lines differing in dwarfing alleles, in the varietal backgrounds of 'Maris Huntsman', 'Maris Widgeon' and 'Bersee', and the F_2 grain on intravarietal F_1 hybrids, produced with a chemical hybridising agent, were examined for grain size and protein content. Individual F_2 grains from *Rht1/rht*, *Rht2/rht* and *Rht3/rht* F_1 spikes were classified for *Rht* genotype by assaying embryo half grains in a gibberellic acid seedling response test, while the remaining half was used for protein determination. Mean grain weight and protein percentage were lower in all homozygous isogenic lines and the *Rht/rht* F_1 hybrids than in the respective tall lines, in an allele dose-dependent manner. In all the hybrids, the *Rht* genotype of individual F_2 grains, which segregated within the spikes of F_1 plants, had no significant effects on grain weight or protein. Consequently, the pleiotropic effects of the *Rht* alleles on these yield and quality components must be attributed to their presence in maternal plant tissues rather than in the endosperm or embryo tissues of individual grains.

Key words: Wheat – *Triticum* – Dwarfing genes – Grain weight – Grain protein

Introduction

The majority of modern high-yielding hexaploid wheat (*Triticum aestivum* L.) varieties are semi-dwarfs carrying the gibberellin-insensitive semi-dwarfing alleles, *Rht1* and/or *Rht2*. *Rht3*, the more extreme GA-insensitive 'Tom Thumb' dwarfing gene, has not been employed in commercial varieties but is allelic in the *Rht1* on chromosome 4BS (the chromosome designations are those agreed by the 7th International wheat Genetics Symposium in Cambridge 1988, where the chromosome previously known as 4A became 4B.) *Rht2* is carried at a homoeolocus on 4DS. The history, genetics, physiology and pleiotropic effects of these genes on agronomic characters is reviewed in Gale and Youssefian (1985). The high yield potential of semi-dwarf varieties has been attributed both to improved lodging resistance and consequent ability to respond to higher nitrogen applications without lodging, and to pleiotropic effects of the *Rht* genes on yield components. In high yielding conditions, these effects result in higher grain numbers by about 20% and smaller grains by about 10%, with a net yield improvement of about 10%.

The reduction in grain weight due to the effect of the *Rht* alleles has been observed also in cases in which the number of grains per spike was not affected (Fischer and Stockman 1986), or even if it was decreased (Pinthus and Levy 1983). Consequently, this reduction cannot always be ascribed to the competitive effect of a greater number of developing grains. Furthermore, reduction of grain protein percentage, attributable to the *Rht* alleles, has been found irrespective of whether grain yield of the *Rht* versus *rht* genotype was significantly increased (Gale 1979; Brandle and Knott 1986), hardly affected (McClung et al. 1986) or was reduced (Zaccai et al. 1987). Thus, the reduction in grain protein percentage cannot always be ascribed to the production of a greater total grain yield with similar nitrogen supply.

The objectives of the present study were: (1) to examine the effect of the GA-insensitive *Rht* alleles on size and protein content of individual grains of inbred isogenic lines and of F_1 hybrids between tall and *Rht* isogenics grown under drilled conditions in a high-yielding trial at Cambridge; and (2) to determine, by observation of grains on F_1 hybrid plants, whether these effects were exerted directly by the presence of these genes in the grains, or by their presence in the maternal plant tissues.

Materials and methods

Genotypes

Near-isogenic lines of *rht* (tall) and *Rht1, Rht2* and *Rht3* of three varietal series, 'Maris Huntsman', 'Maris Widgeon' and 'Bersee' and intravarietal F_1 hybrids, *Rht1/rht, Rht2/rht* and *Rht3/rht* in each series, were sown in a drilled plot randomised block experiment, as described in Gale et al. (1989). The F_1 hybrids were produced using the Shell chemical hybridising agent, WL 84811, kindly supplied and applied by the staff of Nickerson RPB plc, Rothwell, Lincs, UK. The male parent of the hybrids in each series was the tall (*rht*) line.

Plant material

Twenty main shoot spikes were sampled at random from each of the 21 plots in a single block of the trial. Eight grains, taken from the first and second florets of each of the four central spikelets on each spike, were individually weighed at 14% moisture.

Determination of F_2 genotype of individual grains from F_1 hybrids

The eight grains from each plant were halved, and the half containing the embryo was assayed for gibberellin (GA) response in a seedling test similar to that described by Gale and Gregory (1977). The eight grains were sown, in an order determined by their position in the spike, in vermiculite in trays and watered with a 25 ppm GA_3 solution. The seedlings were grown at 25 °C in continuous light (55 μEm-2 sec-1 PAR) until the third leaf had fully emerged. At this time, elongation of the first leaf was compared to that of the parents of each hybrid. Those with short leaves, i.e. about 30% of the tall in crosses involving *Rht1* and *Rht2,* or 20% of the tall in crosses involving *Rht3,* were classified as homozygous *Rht.* Those with long leaves similar to the tall parent were classified as *rht,* and the remainder, with leaves of intermediate length after GA treatment, were classified as *rht/Rht.*

The data for 3 of the 180 F_1 spikes, in which no segregation was observed, were assumed to have come from selfed grain and were discarded. The data for the parental 'Widgeon' *Rht1* isogenic line were also discarded, because the grains of this genotype segregated for GA response.

The frequency of the homozygous *rht* genotype in the F_2 population (averaged over the three *Rht* hybrids and the three varietal backgrounds) amounted to 25.1%, which fits the expected single gene segregation. However, the proportion of F_2 seedlings assessed as homozygous *Rht,* because of their resemblance to the respective homozygous *Rht* parental lines, amounted to 34.8%. The remaining seedlings which were considered as heterozygous *Rht/rht* comprised 40.1% of the F_2 population. This obvious discrepancy from the expected frequencies of 25% *Rht* and 50% *Rht/rht* casts some doubt on the identification of these genotypes. Nevertheless, this genotypic classification of the F_2 grains should still be adequate to enable detection of possible effects of the *Rht* alleles on their weight and protein percentage.

Protein determination

The protein levels of the endosperm half grains, calculated as *N* percentage × 5.7, were determined by a semi-micro Kjeldahl procedure, with a Tecator Kjeltec Auto 1030 Analyser. A small study on the relationship between protein levels in half grains and whole grains indicated that the protein concentration in endosperm halves was 93.4 ± 0.2% of that in intact whole grains.

In the parental homozygous lines, protein levels for each spike were measured on two combined samples each of four half-grains, one sample from the first florets and the other sample from the second florets. In the F_1 hybrids, protein levels were measured in groups of grain classified by *Rht* genotype for first and second florets pooled from ten spikes, i.e. three genotypes × two floret positions × two groups of ten spikes = 12 measurements per F_1 genotype.

Results

Floret position

In all parental and F_1 spikes, the grains from the second florets were heavier than those in the first florets. In addition, in all but 3 of the 20 genotypes tested, protein levels were higher in the second floret grains. The grain weight advantage was 6.5%, whereas the protein level advantage was 2.1%.

No grain position × genotype interaction was found in the analysis of either parameter. Thus, the first and second floret data were combined for further analysis.

Grain size and protein levels in individual F_2 grains from F_1 hybrid plants

Mean grain size and protein levels were reduced, relative to the comparable tall isogenic line, for all three F_2 populations in each of the three isogenic series (Fig. 1). These reductions were smaller than those found in the respective parental *Rht* lines. This difference between the hybrids and their maternal homozygous *Rht* parents excludes the possibility of a cytoplasmic inheritance of the effects of the *Rht* alleles on grain weight or protein percentage.

Analysis of individual grains of *rht/rht, Rht/rht* and *Rht/Rht* genotype within populations showed no significant effects of individual grain genotype on either parameter (Table 1). Consequently, the effects of the *Rht* alleles on grain size and protein level cannot be ascribed to the presence of these alleles in the embryo or endosperm tissues, but rather to the effects of these alleles on the maternal plant tissues.

Effects of Rht allele on grain size

Since all the effects of *Rht* alleles on grain characters are apparently manifest by the maternal genotype, the seven genotypes within isogenic series can be treated as lines differing only in dosage of three different *Rht* alleles. These dosage effects on grain weight in the three isogenic series are presented in Fig. 1 a along with the mean plant heights from Gale et al. (1989). The *Rht1/rht* and *Rht2/rht,* as well as the *Rht1/Rht1* and *Rht2/Rht2* data within each series, have been pooled because no significant differences were found between the effects of the two alleles, either as heterozygotes or homozygotes.

Within series, there are a few unusual results such as the apparent improvement in grain size for the shorter *Rht3/rht* and *Rht3/Rht3* genotypes of 'Bersee'. However,

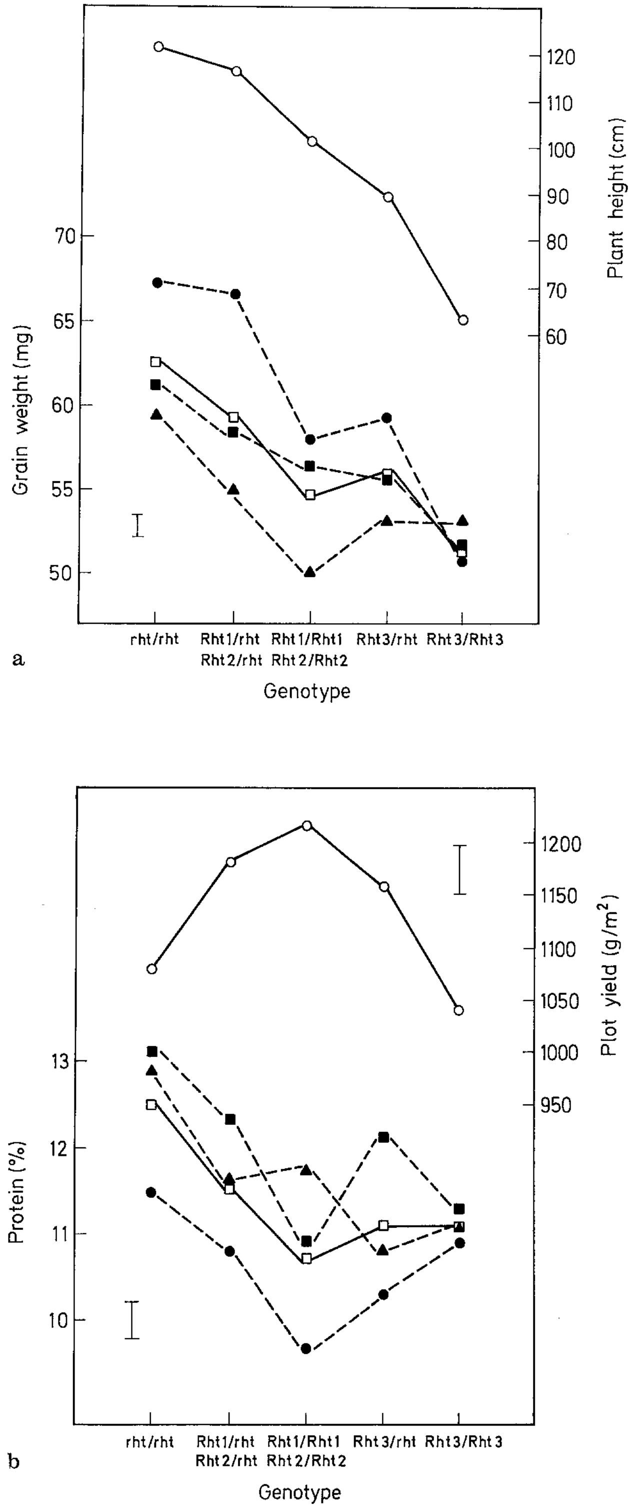

Fig. 1 a and b. The effects of the *rht* genotype on **a** grain weight and plant height, and **b** on protein percentage and plot yield, in three near-isogenic series: ● 'M. Huntsman', ■ 'M. Widgeon' and ▲ 'Bersee' (□ mean of the three series). The *Rht1/rht* and *Rht2/rht* and the *Rht1/Rht1* and *Rht2/Rht2* means have been pooled within each series. The **a** plant height and **b** plot yield data are means of the three series from Gale et al, (1989). The *vertical bars* indicate LSD (0.05). Plant height LSD = 1.88 cm, which is too small to be shown

Table 1. Grain weight and protein levels in segregating F_2 grains on *rht/Rht1*, *rht/Rht2* and *rht/Rht3* F_1 hybrids[a]

		F_1 hybrids			Tall
		Rht1/rht	*Rht2/rht*	*Rht3/rht*	*rht/rht*
Grain weight (mg)					
F_2	*rht/rht*	60.1	60.2	56.1	
genotypes	*Rht/rht*	59.8	59.5	55.8	
	Rht/Rht	60.0	60.8	56.1	
S.E.		0.46	0.45	0.53	
Mean		60.0	59.8	56.0	62.7
Mean reduction (% tall)		4.3	4.6	8.9	
Protein (%)					
F_2	*rht/rht*	11.7	11.6	11.1	
genotypes	*Rht/rht*	11.8	11.4	11.1	
	Rht/Rht	11.8	11.3	11.0	
S.E.		0.10	0.13	0.07	
Mean		11.7	11.4	11.1	12.5
Mean reduction (% tall)		6.4	8.8	11.2	

[a] F_2 genotypes were classified in selected individual grains from F_1 plants (see text). The data for talls, F_1 hybrids and F_2 genotypes have been meaned over the 'M. Huntsman', 'M. Widgeon' and 'Bersee' near-isogenic series

the mean trend mirrors that of the effects of the genes on plant height.

In addition, it is clear that the effect of the *Rht* alleles on grain size, like that on height, is not recessive and for the heterozygotes lies close to the midpoints between the talls and homozygous *Rht* lines.

Effects of Rht alleles on protein content

Protein levels in the isogenic allelic series are shown in Fig. 1 b. Although there are some differences between the varietal backgrounds, it is clear that the largest protein penalty is observed with the homozygous *Rht1* and *Rht2* lines and that there is a trend towards recovery of protein levels in the two shorter genotypes (*Rht3/rht* and *Rht3/Rht3*). This may be related to the apparent inverse relationship between protein content and overall plot yield also shown in Fig. 1 b.

Although the grains analysed here were selected in order to reduce non-genetic variability and were the largest in the plots, i.e. the grains from the first two florets of central spikelets from main tillers, the results correlate very closely with grain sizes measured in the total plot yield bulks (which were on the average 15% smaller). Thus, the product of protein level of the selected grains × plot yield is likely to provide a meaningful estimate of protein yield. This estimate, shown in Fig. 2, demonstrates an additive effect of the *Rht* alleles on the protein amount recovered per plot.

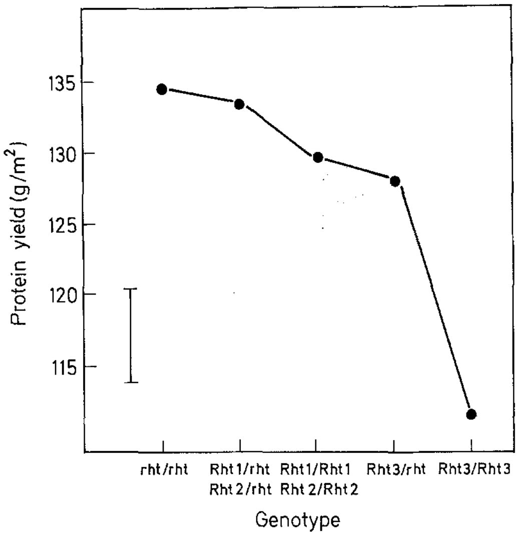

Fig. 2. The effects of allelic differences at the *Rht* loci on protein yield meaned over the three isogenic series of 'M. Huntsman', 'M. Widgeon' and 'Bersee'. Protein yield was calculated as the product of protein percentage in the present study and overall grain yield from Gale et al. (1989). The *vertical bar* indicates LSD (0.05)

Discussion

The results demonstrate that, under the high yielding conditions of field trials in the UK designed to measure yield and yield component potential, the GA-insensitive *Rht* alleles have very marked decreasing effects on grain size and protein levels.

The novel features of this investigation are a measurement of these parameters in individual grains and the use of near-isogenic lines, and F_1 hybrids between lines differing in few genes other than those at the *Rht* loci. The F_2 grain, on F_1 plants grown under drilled conditions, have provided for the first time an opportunity to investigate the effect of embryo and endosperm genotype relative to that of the maternal plant. The results show clearly that the pleiotropic effects of the *Rht* alleles are not a direct effect of their action in the grain, but rather an indirect consequence of the genes' effects on the vegetative plant. Indeed, other genotypic effects, not associated with *Rht,* on grain size and on grain protein have been previously shown to be related to the maternal tissues (Millet and Pinthus 1980; Millet et al. 1984).

Since both carbohydrates and storage proteins are synthesised in the developing grain, this effect is likely to be related to the supply of sugars and nitrogen to the grain. The *Rht* genotypes in this experiment also had higher grain numbers per spike (Gale et al. 1989), and thus any effects via limitations to the supply of assimilates to individual grains should be enhanced by increased competition between grains.

Carbohydrate source limitations during grain filling, which have been shown to affect grain weight (Fischer and Hille Ris Lambers 1978), would be expected to affect short-straw types more than tall types mainly because of the smaller photosynthesising area of their short peduncles. However, the possibility should not be excluded that the effects of the *Rht* alleles on grain weight and grain protein may be due to their pleiotropic effects on various characters of different maternal tissues which have been shown to affect grain size or grain protein. Such effects may be associated with the supply of phytohormones (particularly cytokinin) from vegetative plant parts to the developing grain (Herzog 1986), with the size of the floral cavity (Millet 1986) or even with pericarp characters (Millet and Pinthus 1984), all of which have been shown to affect grain size. Moreover, recently the weight increase of cultured wheat grains has been found to depend on the presence of an attached rachis fragment (Millet and Jones 1988). Effects on grain protein exerted via maternal plant tissues seem very plausible indeed since all the plant parts, including the glumes of the spikelets, have been shown to contribute to the translocation of nitrogen to the developing grain (Simpson et al. 1983).

The effect of the *Rht* alleles on grain size has been noted many times in genetically defined lines (Gale 1979; Allan and Pritchett 1980; Pinthus and Levy 1983), although the additive effects of the alleles on this parameter in *Rht/rht* heterozygotes is demonstrated here for the first time. The results are comparable, however, with those of Allan and Pritchett (1980) who observed, again in isogenic lines, that the reduction in grain size in *Rht1* + *Rht2* homozygotes was about twice that found for the single gene homozygotes. The additive effect in heterozygotes for grain size and plant height contradicts the many reports that the Norin 10 genes are recessive in their effects. This difference is almost certainly related to the experimental genotypes employed. Results for intervarietal crosses, such as are commonly used to assess dominance, will be confounded with the effects of heterosis on grain weight and plant height. Indeed, the mean grain weights for the six intervarietal *Rht1/rht* and *Rht2/rht* genotypes between the three isogenic series investigated here were higher than the tall inbreds, due to heterotic effects (Gale et al. 1989).

The effect of the *Rht* alleles on protein levels in genetically defined lines have also been recorded before, although not often in suitably defined genetic stocks (see Table 1.2 in Gale and Youssefian 1985). In the present experiment, the reduction in protein level seems to be related to overall grain yield, to the extent that the lowest-yielding extreme dwarf genotypes had higher protein contents than the highest-yielding semi-dwarf genotypes (Fig. 1). However, the estimated protein yields per unit area demonstrate clearly that the *Rht* alleles cause a reduction in the protein level over and above the often observed negative relationship between grain yield and protein content.

Acknowledgements. Thanks are due to Mr. D. Bonfil (Rehovot) and Mr. A. M. Salter (Cambridge) for technical assistance and statistical analysis.

References

Allan RE, Pritchett JA (1980) Registration of 16 lines of club wheat germ plasm. Crop Sci 20:832–833

Brandle JE, Knott DR (1986) The effect of a gene for semidwarfism (*Rht1*) on various characters in a spring wheat cross. Can J Plant Sci 66:529–533

Fischer RA, Hille Ris Lambers D (1978) Effect of environment and cultivar on source limitation to grain weight in wheat. Aust J Agric Res 29:443–458

Fischer RA, Stockman YM (1986) Increased kernel number in Norin 10-derived dwarf wheat: Evaluation of the cause. Aust J Plant Physiol 13:767–784

Gale MD (1979) The effects of the Norin 10 dwarfing genes on yield in wheat. In: Ramanajam S (ed) Proc 5th Int Wheat Genet Symp. IARI, New Delhi, pp 978–987

Gale MD, Gregory RS (1977) A rapid method for early generation selection of dwarf genotypes in wheat. Euphytica 26: 733–738

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. In: Russell GE (ed) Progress in plant breeding, vol 1. Butterworths, London, pp 1–35

Gale MD, Salter AM, Angus WJ (1989) The effect of dwarfing genes on the expression of heterosis for grain yield in F_1 hybrid wheat. In: Maluszynski M (ed) Current options for cereal improvement. Kluwer Academic, Dordrecht, pp 49–62

Herzog H (1986) Source and sink during the reproductive period of wheat development and its regulation with special reference to cytokinins. Adv Agron Crop Sci 8:1–104 (Suppl 8 to J Agron Crop Sci)

McClung AM, Cantrell RG, Quick JS, Gregory RS (1986) Influence of the *Rht1* semidwarf gene on yield, yield components and grain protein in durum wheat. Crop Sci 26:1095–1099

Millet E (1986) Relationships between grain weight and the size of floret cavity in the wheat spike. Ann Bot 58:417–423

Millet E, Jones RJ (1988) Long-term in vitro culture of wheat grains. Plant Physiol 88:409–411

Millet E, Pinthus MJ (1980) Genotypic effects of the maternal tissues of wheat on its grain weight. Theor Appl Genet 58: 247–252

Millet E, Pinthus MJ (1984) Effects of removing floral organs, light penetration and physical constraint on the development of wheat grains. Ann Bot 53:261–269

Millet E, Levy AA, Zamir R, Feldman M (1984) Evidence for maternal effect in the inheritance of grain protein in crosses between cultivated and wild tetraploid wheats. Theor Appl Genet 67:521–524

Pinthus MJ, Levy AA (1983) The relationship between the *Rht1* and *Rht2* dwarfing genes and grain weight in *Triticum aestivum* L. spring wheat. Theor Appl Genet 66:153–157

Simpson RJ, Lambers H, Dalling MJ (1983) Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.). IV. Development of a quantitative model of the translocation of nitrogen to the grain. Plant Physiol 71:7–14

Zaccai M, Pinthus MJ, Levy AA (1987) The effect of the *Rht1* gene on grain protein content in tetraploid wheat *Triticum turgidum*. J Cer Sci 6:27–32