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Dwarfing genes and cell dimensions in different organs of wheat

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Abstract

A field experiment was conducted under non-limiting water and nutritional conditions with three near-isogenic lines of spring wheat (dwarf, DD; semi-dwarf, SD and standard height, SH) to study the impact of the GA-insensitive alleles *Rht1* and *Rht2*, at the cellular level, on the growth of different vegetative organs and of the pericarp of grains. Cell length and width of blades of different leaves (3, 7 and flag leaf), the flag-leaf sheath and the penultimate internode as well as the pericarp of basal grains from central spikelets of the spike were evaluated. With the exception of the flag leaf, dwarfing genes produced a significant reduction in cell length in all the different vegetative organs analysed. There was no effect on the number of cells nor their width. Therefore, in vegetative organs, the effects of these alleles appeared to be exclusively due to a reduction in cell length. It would appear that dwarfing genes act on cell elongation without affecting cell division.

The *Rht* alleles did not modify cell length nor width in the pericarp. Grain weight was different between the lines and these differences were associated with grain volume at the beginning of linear grain growth. Thus, they reduced the size of individual grains by reducing the total number of cells in the pericarp.

It appears that *Rht* alleles reduced the final sizes of vegetative organs (such as internodes and leaves) and of tissues (pericarp) associated with reproductive structures (grains), but the modes of action in these different organs were different.

Key words: Cell dimensions, plant height, *Rht* alleles, *Triticum aestivum*, wheat.

Introduction

Genotypes carrying *Rht1* or *Rht2* alleles, derived from Norin 10, have contributed significantly to world-wide increases in potential grain yield (Gale and Youssefian, 1985; Slafer *et al.*, 1994; Calderini *et al.*, 1995). This impact has been associated with reductions in plant height, thus decreasing the risk of lodging (Allan, 1989). It has also been associated with increases in partitioning of assimilates to reproductive organs and in harvest indices (Slafer and Andrade, 1991; Miralles and Slafer, 1995*a*).

Previous reports have shown that dwarfing genes do not affect the timing of plant developmental events (Youssefian *et al.*, 1992; Miralles and Slafer, 1995*a*), with no differences in the rate of appearance or final numbers of leaves (Youssefian *et al.*, 1992; Calderini *et al.*, 1996) or in the final number of internodes (Borrell *et al.*, 1991). However, the final lengths of different organs are very susceptible to modification by *Rht* alleles, e.g. coleoptiles, leaves, sheaths, and internodes are consistently shorter in semi-dwarf than in tall lines (Nilson *et al.*, 1957; Pinthus *et al.*, 1989; Youssefian *et al.*, 1992; McCaig and Morgan, 1993). Bush and Evans (1988) and McCaig and Morgan (1993) found that *Rht* alleles decreased individual leaf length and area. Similar results were found for culm length by Borrell *et al.* (1991). Changes in both leaf (Keyes *et al.*, 1989; Calderini *et al.*, 1996) and internode lengths (Borrell *et al.*, 1991) were explained by changes

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in their extension rates rather than in the duration of their growth periods.

Although dwarfing genes affect the growth of a range of vegetative organs, there may be more than one mechanism involved. Calderini *et al.* (1996) observed that double allelic dosage decreased penultimate internode length by *c.* 60%, which is markedly higher than the reduction in leaf blade length (*c.* 17%) for the same phytomer, indicating that different mechanisms might be acting in the different organs.

There is evidence that a reduction in leaf length of Rht lines is mainly due to shortening of cell length while the number of cells is not modified (Gale *et al.*, 1985; Keyes *et al.*, 1989). Keyes *et al.* (1990) reported that gibberellic acid (GA) insensitivity conferred by *Rht* alleles was associated with changes in cell wall extensibility. The reduced cell size associated with GA-insensitivity conferred by *Rht* alleles could be the consequence of a reduction in wall extensibility (Keyes *et al.*, 1990) and/or reduced osmotic potential of the cell solutes (Hoogendoorn *et al.*, 1990). Most studies have only considered individual vegetative organs, and no generalization can be made of the impact of *Rht* alleles on the growth of different parts of the plant at the cellular level.

In spite of the positive effect of dwarfing genes in increasing grain number, this advantage is frequently partially offset by reductions in average grain size (Gale, 1979; Allan, 1989; Slafer and Andrade, 1989; Miralles and Slafer, 1995*a*). Flintham and Gale (1983) and Miralles and Slafer (1995*b*) observed that reduced average grain weights in semi-dwarf and dwarf lines were not only a consequence of a higher grain number produced in distal florets (with lower grain weight potential), but were also due to lighter grains at certain positions within the spike, compared with the tall line. Although Flintham and Gale (1983) suggested that reduced grain size in dwarf genotypes was associated with a competitive response to the increase in spikelet fertility, rather than a primary effect of dwarfing genes, the results of Miralles and Slafer (1995*b*) showed that differences in grain weight between tall and dwarf lines were independent of the availability of assimilates, indicating a direct effect of *Rht* alleles on the potential size of individual grains. An effect of *Rht* alleles on cell elongation of the caryopsis has been suggested as a possible explanation for differences in grain size between lines (Richards, 1996), but to the best of our knowledge there are no published studies to support this hypothesis.

The objective of this work was to analyse the action of *Rht1* and *Rht2* alleles at the cellular level (number and size) on the growth of different vegetative organs. In addition, the hypothesis of a direct action of *Rht* alleles in limiting cell length in the pericarp, thus reducing potential grain size was evaluated.

Materials and methods

General conditions and treatments

An experiment was carried out at the experimental field of the Department of Plant Production, University of Buenos Aires (34°35′ S, 58°29′ W) during the 1993 growing season. The soil was a silty clay loam classified as Aeric Argiudol (Slafer and Andrade, 1989). Three near-isogenic lines from the Brazilian cultivar 'Maringá' were used. These lines were produced by Dr MD Gale at The Plant Breeding Institute, Cambridge, UK, through seven backcrosses from CIMMYT lines carrying the semi-dwarf genes, followed by selfing to produce Rht and rht homozygous lines of Maringá, and were generously supplied by Dr EY Suarez (INTA-Castelar, Argentina). These lines, which are expected to be homozygous at over 99% of the loci unlinked for Rht alleles, included the standard height cultivar (SH; *rht1 rht1, rht2 rht2*) and its near near-isogenic semi-dwarf (SD; *Rht1 Rht1, rht2 rht2*) and dwarf (DD; *Rht1 Rht1, Rht2 Rht2*) lines.

The genotypes were arranged in a completely randomized block design with three replicates. Each plot consisted of ten rows, 0.15 m apart and 2 m long, which were oriented in a north–south direction. The crop was hand-sown on 8 August 1993 at a rate of 185 plants m^{-2}. When the first leaf was fully expanded, the plots were thinned to 160 plants m^{-2}. The crop was irrigated, fertilized with urea (80 kg N ha^{-1}) and treated with fungicides and insecticides as required. Weeds were removed by hand throughout the growing season. In order to prevent lodging, nets were installed during stem elongation.

Measurements of dimensions in vegetative organs

Five plants per plot within the central rows were tagged and leaves corresponding to three different positions on the main stem were selected to represent different positions in the canopy. From the bottom to the top, the blade of leaves 3 (basal leaf), 7 (middle position leaf) and 10 (flag leaf) were sampled when fully expanded. Total length and maximum width were measured and leaf blade areas were calculated as length × width × 0.835 (Miralles and Slafer, 1991).

The length and width of the sheath of the flag leaf were also measured in order to calculate the area (length × width). The length and diameter of the internode immediately below the peduncle (P-1 internode) were measured. Internode surface area was calculated as for a cylinder. At maturity, plant height (from the soil surface to the base of the spike) was measured in five randomly chosen main shoots per plot.

Cell number and cell dimension in vegetative organs

Central sections of fully expanded organs were taken and fixed in formalin–acetic alcohol (FAA). Epidermal peels were prepared from the median region of the adaxial surface of leaf blades, sheath and internode of the plants sampled, using the potassium hydroxide technique (D'Ambrogio, 1986). To isolate the adaxial epidermal tissue of different vegetative organs they were placed in potassium hydroxide solution (4%) at 60 °C for 15, 30 and 45 s for the blades of leaf 3, 7 and flag-leaf, respectively, and for 3 min for sheath and peduncle. After this treatment the adaxial epidermis was isolated under a binocular microscope. These epidermal peels were attached to a microscope slide, stained with safranin and mounted in clear resin. Following the classification of cell types made by Wenzel *et al.* (1997) in barley, all cells between veins, lateral cells and cells of the stomatal rows were measured at two different positions on each peel (i.e. two microscope fields per peel and four fields for each replicate). Therefore, on average, 1500 cells per organ (range 600 to 3000) were measured in each line (*c.* 100 cells per

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slide). Cells lying over the veins (Wenzel *et al.*, 1997) were not considered in this study. Within each sample, cell length for different cell types did not show a wide variation within the files taken in each peel (data not shown).

Epidermal cell length, width and number were measured using a projected microscope field (Projectina 4041 BK–2). The areas of blade, sheath and internode cells were calculated as length × width for each sample. The total epidermal cell number in the external surface of the internodes was calculated dividing the area of the different vegetative organs by the average of cell area measured in each organ.

Grain size and pericarp cell dimensions

The two basal grains from two central spikelets of the spike of five main shoots per plot were sampled at 15 d after anthesis (i.e. approximately at half of total grain growth period) and fixed in FAA solution. To measure the dimensions of pericarp cells, scanning electron micrographs (SEM) were made. Grains were dehydrated using an ascending graded series of ethyl alcohol concentration (from 60% to 100%) for 30 min at each concentration. The grains were affixed to aluminium stubs with copper paint, then sputter coated with gold–palladium and viewed with a Phillips microscope.

The caryopsis of each grain was photographed at two different dorsal sectors chosen randomly (Fig. 1). Pericarp cell length and width were measured and the area was calculated in a similar manner to that of the sheath.

Grain surface area and volume were calculated assuming the grain as an ellipsoid.

$$\text{Grain surface area} = 2\pi w^2 + \frac{2\pi aw}{e} \text{arc sin } e \text{ (Granville, 1952)}$$

$$\text{Grain volume} = 4/3\ \pi abc \text{ (Jenner, 1979)}$$

where: $\pi = 3.1416$; a is 0.5 grain length; w is the average between 0.5 dorsal width (b) and 0.5 lateral width (c), and e is the ellipse eccentricity calculated as $(\sqrt{a^2+b^2})/a$.

Total pericarp epidermal cell number was determined from the surface area of each grain divided by the average of all pericarp cell areas.

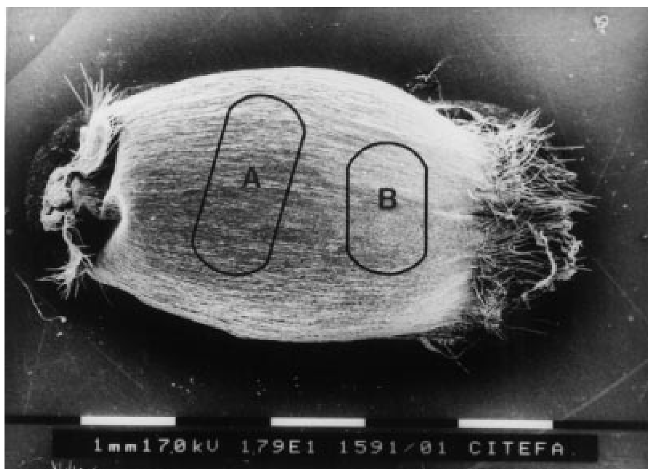

Fig. 1. SEM view of the dorsal position of the grain indicating the two sectors (A and B) where photographs were taken 15 d after anthesis (× 180). Black and white lines indicate the scale (i.e. 1 mm).

Results

Effect of Rht alleles on the dimensions of vegetative organs

Dwarfing genes did not affect the final numbers of leaves and internodes. As expected, the presence of *Rht* alleles reduced the final length of the main culm. One dose of *Rht* shortened the culm by 30% while the double dose reduced plant height by 60% (Table 1). Therefore, final culm length was negatively associated with the allelic dosage ($r = -0.99$; $P < 0.05$). In addition, the chosen P-1 internode showed a similar proportional reduction as that observed for the whole culm (Table 1) suggesting that the *Rht* alleles affect all internodes proportionally. On the other hand, the *Rht* alleles reduced the length of leaf blades in different proportions (Table 1). While the most significant decrease in length was observed in leaf blade 7 (*c.* 30% for the DD compared with the SH line), the blades of flag leaves were virtually the same for all lines (Table 1) and leaf blade 3 showed an intermediate reduction (16%). The length of the flag-leaf sheath, however, was sensitive to *Rht* alleles being reduced by 13% and 24% for one and two *Rht* gene doses, respectively (Table 1). The presence of *Rht* alleles produced greater dwarfing in internodes than in leaves.

The impact of *Rht* alleles on the surface area of vegetative organs was almost completely explained by their effect on length ($r = 0.96$, $P < 0.001$, Fig. 2).

Effect of Rht alleles on grain dimensions

Grains from different floret positions within the two central spikelets showed different final weights. In the SH line, basal grains proximal to the rachis (G1 and G2) showed no differences in weight, but they were significantly heavier than the distal grain (Table 2). The DD and SH line had the lightest and heaviest grains, respectively, while the SD line showed intermediate values (Table 2). All lines exhibited similar weights for G3 (Table 2). A positive linear association ($r = 0.93$, $P < 0.001$) was found between final grain weight and grain volume 15 d after anthesis.

Fifteen days after anthesis, G1 and G2 had greater volumes than G3 in all lines (Table 2). At the same time, the presence of *Rht* alleles did not modify the volume of distal grains, but the size of basal grains was significantly reduced by the introgression of these alleles. In basal grains one dose of *Rht* alleles reduced grain volume by 22%, and by 28% when the dose was doubled (Table 2). Grain surface area showed a similar pattern to that observed for grain volume, but the differences were less marked (Table 2). The DD line had the lowest grain area in basal positions showing a reduction, relative to the SH line, of 17%. The grain surface area/volume relationship only showed significant differences ($P < 0.05$) in G2 (data not shown) indicating that *Rht* alleles affected the grain shape only for this grain position.

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Table 1. *Plant height (cm), penultimate internode length (cm), leaf blade length (cm) for leaves 3, 7 and 10 (the flag leaf) and flag leaf sheath length (cm) in three near-isogenic lines. DD, SD and SH indicate dwarf, semi-dwarf and standard height lines*

Lines	Plant height (cm)	P-1 internode length (cm)	Leaf blade length (cm)			Sheath length (cm)
			3	7	10	
DD	44.9	12.8	12.2	20.7	21.5	15.1
SD	77.3	22.8	13.1	27.7	20.8	17.2
SH	111.3	30.8	14.5	29.0	20.5	19.8
l.s.d* (0.05)	9.1	2.4	2.2	2.7	1.1	1.2

*l.s.d indicates the least significant difference at$P \leq 0.05$.

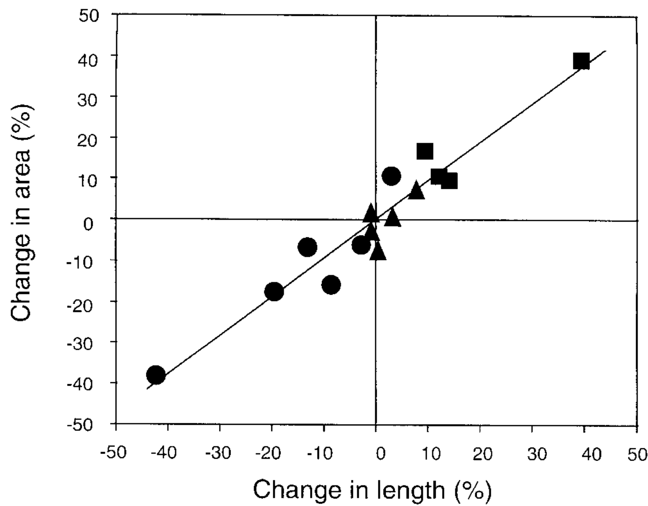

Fig. 2. Relative changes in the area of different vegetative organs (i.e. blades, sheath and penultimate P-1 internode) as a function of relative changes in their respective lengths in three near-isogenic lines, DD (circles), SD (triangles) and SH (squares). Both variables are expressed as the proportional increase (positive values) or reduction (negative values) produced by different doses of *Rht* alleles for each particular vegetative organ in relation to the mean values (average for all lines) of length and area of each specific organ. Solid line was fitted by regression.

Effect of Rht *alleles on cellular dimensions*

The *Rht* alleles did not affect the total number of epidermal cells produced in any vegetative organ investigated (Table 3). Only the flag-leaf sheath showed differences in cell number but they were not consistently related to the dose of *Rht* alleles (Table 3). In addition, no relationship was found between the area of vegetative organs and their number of cells ($r = 0.36$, $P > 0.1$); therefore, differences in area of vegetative organs were associated with changes in cell dimensions rather than in cell number.

With the exception of leaf blade 3 and sheath, the presence of *Rht* alleles did not significantly affect cell width in vegetative organs (Table 3). Differences in cell width was *c.* 7% (averaged across different vegetative organs) and the significant differences observed in leaf blade 3 and sheath were not consistently related to the dose of *Rht* alleles. The effect of *Rht* alleles on cell length was, on the other hand, significant. Blades of leaves 3 and 7 and the sheath of the flag leaf showed a considerable reduction in cell length (17%, 21% and 21%, respectively) with the introgression of double dosage of *Rht* alleles (Table 3). Leaf-blade 10 did not show differences in this trait (Table 3). For leaves, changes in the average surface area of the cells were associated with changes in cell

Table 2. *Individual grain area (mm^2) and grain volume (mm^3) calculated at 15 d after anthesis and final grain weight (mg) at harvest of different grain positions in the central spikelets in three near-isogenic lines of wheat*

G1 indicates the most proximal grain to the rachis, and G3 the most distal grain. DD, SD and SH indicate dwarf, semi-dwarf and standard height lines.

Grain position	Lines	Grain area (mm^2)	Grain volume (mm^3)	Grain weight (mg)
G1	DD	47.7	26.9	31.7
	SD	49.1	29.1	35.6
	SH	60.3	38.5	44.2
	l.s.d* (0.05)	11.4	9.8	3.1
G2	DD	48.2	26.9	37.3
	SD	52.4	30.8	39.4
	SH	59.1	37.1	45.9
	l.s.d* (0.05)	10.6	8.6	7.0
G3	DD	35.5	17.1	30.0
	SD	34.0	16.2	31.6
	SH	37.0	18.5	30.8
	l.s.d* (0.05)	16.5	10.9	11.4

*l.s.d indicates the least significant difference at $P \leq 0.05$.

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Table 3. *Total adaxial epidermal cell number, number of cells mm^{-2}, cell length and width in different vegetative organs and on basal grains (G1 + G2) in three near-isogenic lines (standard-height, SH; semi-dwarf, SD and dwarf, DD) of wheat*

Measurements on vegetative organs were made at the end of their growth and those on grains were made 15 d after anthesis.

	Blade 3	Blade 7	Blade 10	Sheath	Internode	Grain
Total cell number						
DD	105 788	466 754	899 308	293 040	580 290	30 661
SD	121 045	653 109	772 138	1 303 924	576 629	30 322
SH	102 646	532 642	886 108	992 614	564 710	38 106
l.s.d* (0.05)	34 821	227 611	113 045	130 595	133 993	13 555
Number of cells mm^{-2}						
DD	137	176	243	284	375	608
SD	135	188	250	377	231	610
SH	103	148	281	253	159	595
l.s.d* (0.05)	18.8	47.8	37.6	25.1	79.6	219.5
Cell length (μm)						
DD	146.5	120.3	115.8	69.4	86.7	94.3
SD	161.0	129.7	117.8	65.7	164.7	94.7
SH	177.4	151.7	112.9	83.4	250.7	93.7
l.s.d (0.05)	14.5	31.4	5.9	3.5	61.5	18.4
Cell width (μm)						
DD	34.6	33.0	23.0	28.7	20.3	18.4
SD	39.4	32.3	21.8	22.9	20.0	17.8
SH	38.2	30.0	22.1	26.3	20.3	18.7
l.s.d* (0.05)	2.7	4.3	1.2	2.7	1.2	1.9

*l.s.d indicates the least significant difference at $P \leq 0.05$.

length, which explained as much as 90% of variability observed in the average surface area of the cells ($r = 0.95$; $P < 0.001$). For each line, cell average surface area and length decreased as leaves appeared in upper positions in the canopy.

In the P-1 internode, the *Rht* alleles produced the highest impact on cell length, and a significant negative relationship between cell length and the dosage of *Rht* alleles was found ($r = -0.99$; $P < 0.05$). This reduction of cell length reached a value of 34% and 51% with the presence of one or two doses of *Rht* alleles, respectively (Table 3; Fig. 3).Therefore, changes in cell area were associated with changes in cell length (r = 0.99, $P < 0.001$), but not with changes in cell width ($r = 0.04$, $P > 0.1$).

In contrast to vegetative organs, *Rht* alleles did not affect cell length in grain pericarp (Table 3; Fig. 4). In this reproductive tissue, although the differences were not statistically significant, the presence of *Rht* alleles decreased the total cell number by 20% when comparing the extreme lines, i.e. DD and SH lines. In addition the number of cells per unit area was not modified by *Rht* alleles (Table 3).

Cell dimensions shown in Table 3 represent the average of all cells measured in each organ, but the impact of *Rht* alleles was also evident on the frequencies of cell length categories for different organs (Fig. 5). Leaf cells of the SH line showed the highest frequency within the 150–200 μm range, while those of lines with *Rht* alleles showed the highest frequency within 100–150 μm (Fig. 5a). Thus, while the SH line had 25% of its leaf cells longer than 150 μm, the DD and SD lines had just 10% of their cells longer than 150 μm (Fig. 5a). In the sheath, the SH line concentrated 75% of the cells in 0–100 μm range, while the DD and SH lines had 89 and 91%, respectively, of their cells in the same range (Fig. 5b).

The most important differences in cell length frequency distribution between lines were found in the internode, where the DD line showed *c.* 96% of its cells within 0–150 μm range, while the SH line exhibited only 10% of those cells in that range. The SD line recorded intermediate values (Fig. 5c).

Consistent with the lack of effect on average cell length, the frequency of cell length distribution in grains did not show differences between the lines (Fig. 5d).

Discussion

This study is part of a series of experiments carried out to investigate and obtain a better understanding of the effects of *Rht1* and *Rht2* dwarfing genes on different physiological attributes of the wheat crop. The effects of *Rht* alleles on leaf and internode length, and on grain size, are consistent under different environmental conditions, indicating high penetrance (Miralles and Slafer, 1995*a*; Calderini *et al.*, 1996).

In this study, an effect of *Rht* alleles on plant height was found that agrees with previous reports in the literature (Youssefian *et al.*, 1992; Borrell *et al.*, 1991; Miralles and Slafer, 1995*a*). In addition, it is known that dwarfing genes produce a significant reduction in the length of other vegetative organs such as leaves (McCaig and Morgan, 1993), but the relative impact of *Rht* alleles appears to depend on the organ, such as leaves and internodes (Bush and Evans, 1988; Calderini *et al.*, 1996).

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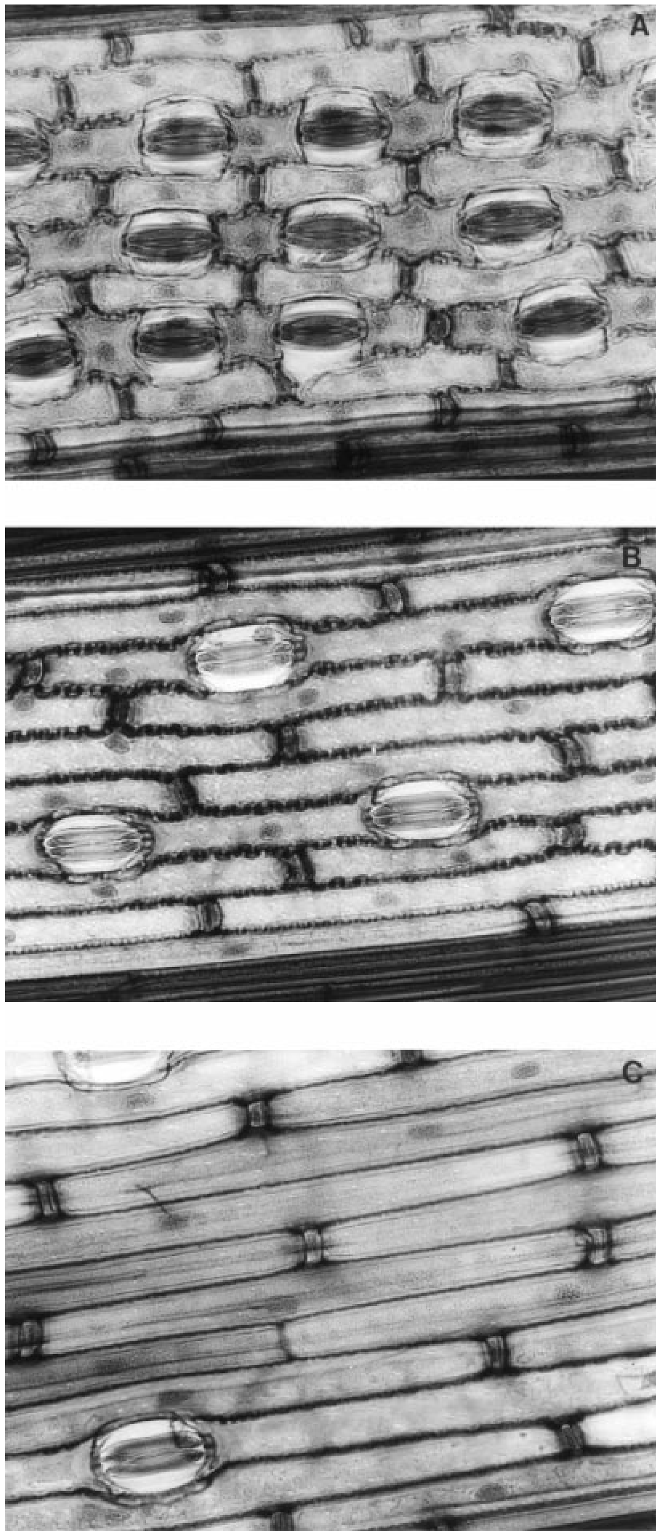

Fig. 3. Epidermal cells in the penultimate internode for (a) DD, (b) SD and (c) SH isogenic lines (× 400).

The results of this experiment confirmed the differential effect of *Rht* alleles on different vegetative organs. In leaves the reduction ranged between *c.* 0 and 30% while in the penultimate internode this effect doubled. In agreement with the results of King *et al.* (1983) and McCaig and Morgan (1993), the *Rht* alleles affected the flag leaf characteristics less than those of earlier expanded leaves.

Rht alleles did not modify the number of cells in vegetative organs. Therefore, changes in area and length of the leaf blade, flag-leaf sheath and penultimate internode produced by the introgression of *Rht* alleles were associated with reductions in cell length. In addition, when cell length was unmodified by *Rht* alleles, (e.g. those in the blade of leaf 10), there were no differences between lines in organ size. Therefore, it is concluded that for vegetative organs, dwarfing genes operate upon cell elongation and have no direct effect on cell division (Fig. 6).

Although cell length reduction explained the broad effect of *Rht* alleles on the reduction in size of different vegetative organs, the underlying physiological basis for this reduction is still unknown. Keyes *et al.* (1989) showed an inverse relationship between the dosage of *Rht* alleles and cell wall extensibility suggesting that they affected the plastic components of the cell wall. Further results reported by Keyes *et al.* (1990) confirmed that GA insensitivity conferred by *Rht* alleles limits cell wall extensibility. In oak internodes, Montague (1995) found that gibberellins promoted cell wall loosening and internode elongation rate.

In reproductive organs (i.e. grains), there is evidence of a negative association between individual grain weight and the dosage of *Rht* alleles (Pinthus and Levy, 1983; McClung *et al.*, 1986; Allan, 1989; Miralles and Slafer, 1995*a*). Miralles and Slafer (1995*b*) postulated that the reduction in grain weight produced by *Rht* alleles was an indirect effect of an increased proportion of grains from distal positions within the spike (with smaller weight potential) together with a direct effect of these alleles on the potential size of the grains. Additionally, the close association found between the final weight and the grain volume 15 d after anthesis could imply that the potential weight of the grains would already be determined at the beginning of the linear grain growth period (Miralles *et al.*, 1996). The straightforward hypothesis for explaining the direct effect of *Rht* alleles on grain size with which this study began, was that dwarfing genes would operate through a similar cellular mechanism to that shown in vegetative organs by reducing the length of pericarp cells (Richards, 1996). However, the results of the present study indicated that these alleles do not affect cell length or width of the pericarp. It is inferred that they modified grain size through reducing the total number of cells in the pericarp in clear contrast to the effects observed in the vegetative organs.

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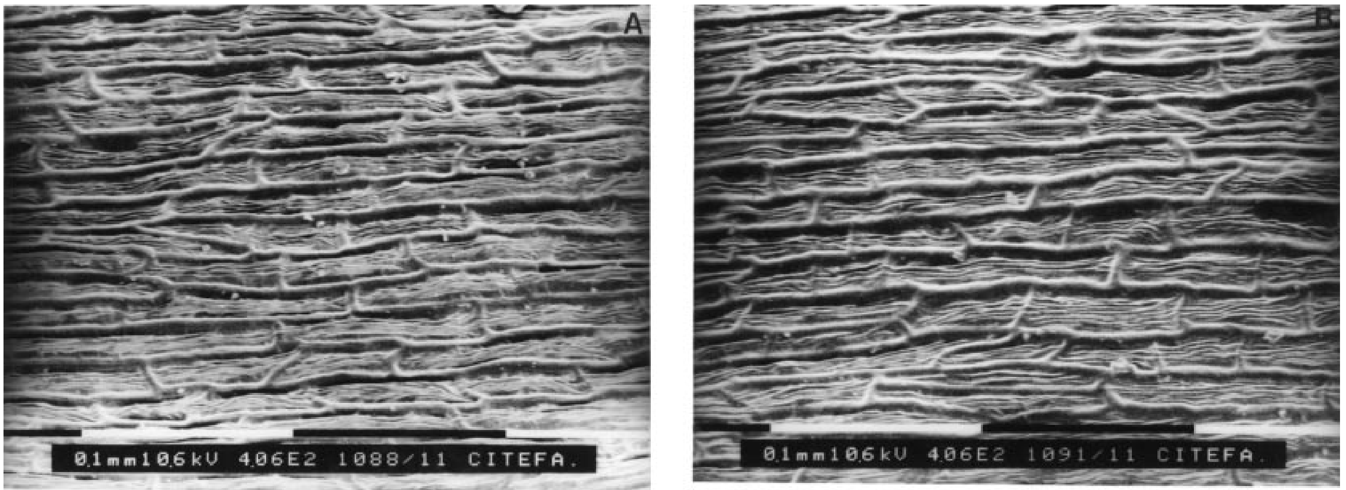

Fig. 4. SEM photographs of pericarp cells in basal grains taken 15 d after anthesis for the (a) double dwarf and (b) standard height isogenic line (×400). Black and white lines indicate the scale (i.e. 0.1 mm).

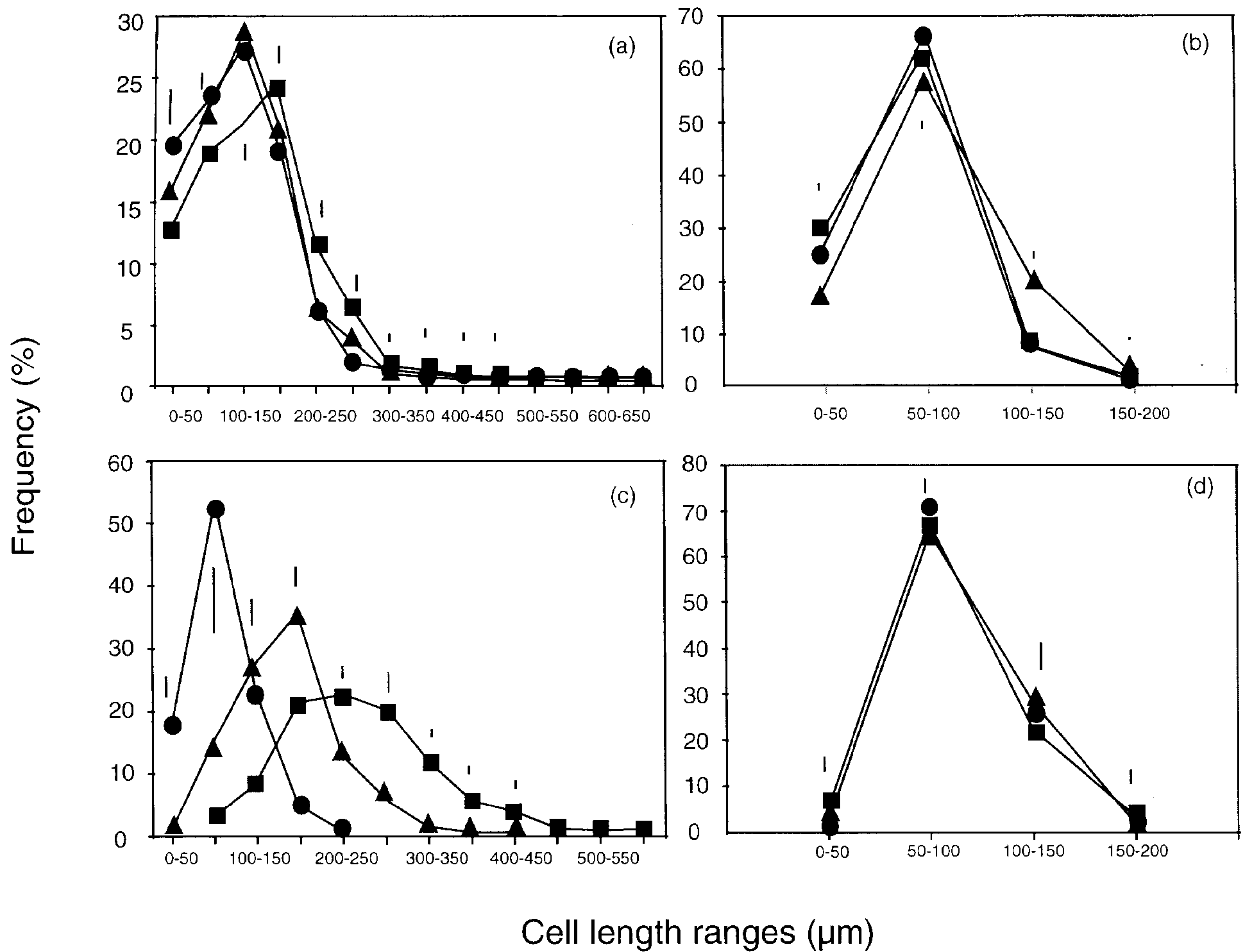

Fig. 5. Frequency of appearance of different cell length ranges for (a) blade of leaf 7, (b) flag leaf sheath, (c) penultimate internode, and (d) pericarp of basal grains in three near-isogenic lines, DD (circles), SD (triangles) and SH (squares). Vertical bars indicate the standard error of means.

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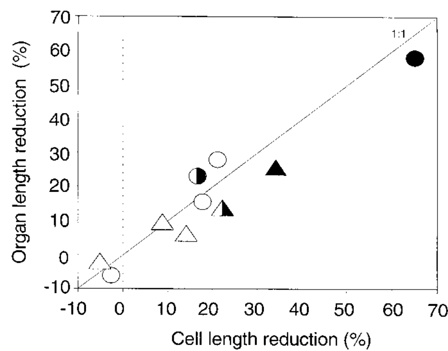

Fig. 6. Relationship between the proportion of organ length reduction and the proportion of cell length reduction in the DD (circles) and SD (triangles) relative to the SH line for leaves (open symbols), flag leaf sheath (half-toned symbols) and the penultimate internode (closed symbols). Solid line indicates the 1 : 1 relationship.

Differences between cell length responses of vegetative and reproductive organs due to *Rht* alleles could be related to differences in the types of GAs present in these organs. Radley (1970) and Gale *et al.* (1987) showed that in vegetative tissues the level of GA_1 and GA_3 was higher in DD than in SH lines.

In developing grains where the predominant gibberellin is GA_{54} (Gaskin *et al.*, 1980; Lenton and Gale, 1987), Gale *et al.* (1987) showed that apparently the *Rht* alleles do not affect the levels of this gibberellin. This suggests that GA metabolism could be different in vegetative than in reproductive organs. Thus, the final reduction in grain size due to a direct effect of *Rht* alleles does not appear to be associated with the same mechanism found in vegetative organs.

The effects of *Rht* alleles in vegetative and reproductive organs in general have been discussed in this paper but, in some vegetative organs (e.g. flag leaf-blade), *Rht* alleles did not modify cell length. In the present work, a positive correlation was found between the relative reduction in cell length due to the presence of *Rht* alleles and cell length in the SH line ($r=0.87$; $P<0.05$) for all organs. It is possible that the *Rht* alleles have a stronger effect in those organs where cell elongation is less restricted. It is possible that the lack of response to GAs where cell elongation is restricted, is because these organs do not initiate cell types which produce elongation in response to GA or the magnitude of cell elongation depends on the type and quantities of levels of endogenous GAs in each organ.

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