

Effect of Temperature on Gibberellin (GA) Responsiveness and on Endogenous GA₁ Content of Tall and Dwarf Wheat Genotypes

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ABSTRACT

Near-isogenic wheat (*Triticum aestivum* L.) lines differing in height-reducing (*Rht*) alleles were used to investigate the effects of temperature on endogenous gibberellin (GA) levels and seedling growth response to applied GA₃. Sheath and lamina lengths of the first leaf were measured in GA treated and control seedlings, grown at 11, 18, and 25°C, of six *Rht* genotypes in each of two varietal backgrounds, cv Maris Huntsman and cv April Bearded. Endogenous GA₁ levels in the leaf extension zone of untreated seedlings were determined by gas chromatography-mass spectrometry with a deuterated internal standard in the six Maris Huntsman *Rht* lines grown at 10 and 25°C. Higher temperature increased leaf length considerably in the tall genotype, less so in the *Rht1* and *Rht2* genotypes, and had no consistent effect on the *Rht1+2*, *Rht3* and *Rht2+3* genotypes. In all genotypes, endogenous GA₁ was higher at 25°C than at 10°C. At 10°C the endogenous GA₁ was at a similar level in all the genotypes (except *Rht2+3*). At 25°C it increased 1.6-fold in the tall genotype, 3-fold in *Rht1* and *Rht2*, 6-fold in *Rht3*, and 9-fold in *Rht1+2*. Likewise, the genotypic differences in leaf length were very conspicuous at 25°C, but were only slight and often insignificant at 11°C. The response of leaf length to applied GA₃ in the *Rht1*, *Rht2*, and *Rht1+2* genotypes increased significantly with lowering of temperature. These results suggest the possibility that the temperature effect on leaf elongation is mediated through its effect on the level of endogenous GA, and that leaf elongation response to endogenous or applied GAs is restricted by the upper limits set by the different *Rht* alleles.

Most modern high-yielding wheat cultivars have short straw and are referred to as 'dwarf' or 'semi-dwarf.' The majority of these cultivars owe their short stature to the presence of one or both of the Norin 10-derived, height-reducing genes, *Rht1* and *Rht2* (5). Almost no commercial cultivars carry the more potent height-reducing 'Tom Thumb' allele, *Rht3*, and the combination of *Rht2* + *Rht3* alleles has been generated only recently in experimental lines. The *Rht1* and *Rht2* alleles are carried on chromosomes 4BS¹ and 4DS, respectively, the *Rht3* is an allele at the *Rht1* locus on chromosome 4BS (5).

¹ At the 7th International Wheat Genetics Symposium in 1988 a decision was taken to reverse the designations of chromosomes 4A and 4B. This new nomenclature is used in this paper.

Culm and leaf elongation of tall (*rht*) cultivars that do not carry any of these *Rht* alleles are promoted by application of GA₃, whereas genotypes carrying these *Rht* alleles are relatively insensitive to applied GA₃ (4, 12). In near-isogenic lines in the genetic background of cv Maris Huntsman the presence of the *Rht1* allele has been shown to reduce the responsiveness of the second leaf sheath to applied GA₃, whereas the line containing the *Rht3* allele was totally unresponsive compared with the normal tall *rht* line (9). This information regarding responses to GA₃ application was obtained at temperatures of about 20°C.

Work with GA-deficient mutants of maize and pea has shown that GA₁ is essential for shoot elongation (10). The concentration of biologically active GAs in wheat cultivars containing *Rht1*, *Rht2*, *Rht1+2*, and *Rht3* alleles, however, is much greater than that of *rht*, tall cultivars (12, 18). In addition, in Maris Huntsman near-isogenic lines, the level of GA₁ has been shown to be 4.5-fold and 25-fold greater (compared with the tall line) in expanding stem internodes containing *Rht1* and *Rht3* alleles, respectively. Thus, GA₁ accumulates in proportion to the potency of the dwarfing gene (9). Stoddart (18) has shown that when corrections are made for differences in endogenous pool sizes of GA₁ in *rht* and *Rht3* lines, the rate of metabolism of GA₁ in shoots of the dwarf was 66% that of the tall line. The increase in GA₁ in the dwarf lines has been explained in terms of reduced metabolism in nonexpanding tissue (6).

The rate of leaf extension was faster with increasing temperature (up to 30°C) and greater for the *rht* cultivar Capelle Desprez than for an *Rht3* dwarf line, particularly above 15°C (19). Applied GA₃ increased the growth rate of the tall cultivar, and it was proportionally more effective at temperatures lower than 15°C. Although there was no effect of applied GA₃ on the extension rate of *Rht3* dwarf seedlings above 20°C, there was some indication of a growth stimulation by GA₃ at lower temperatures (19). However, Stoddart and Lloyd (19) "have not found any changes in GA₃ responsiveness of three-leaf seedlings after pretreatment at 5°C for up to 10 d." By contrast, aleurone layers of mature grains of wheat cultivars carrying, *Rht1*, *Rht2*, or *Rht3* alleles do become responsive to applied GA₃ after preincubation at 5°C for 20 h (16, 17). Effects of temperature on leaf elongation and on its response to GA application of wheat genotypes other than *rht* and *Rht3*

have not yet been reported. Decreasing effects of low temperature on the content of endogenous GA-like substances have been found in maize (15) and in wheat (13, 14).

The objective of the present study was to examine the effect of temperature on leaf elongation, responsiveness to GA₃, and endogenous GA₁ content in the expansion zone of near-isogenic wheat lines carrying all possible homozygous combinations of the *rht*, *Rht1*, *Rht2*, and *Rht3* alleles.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) seedlings were grown in controlled environments at 85% RH and under 12 h photoperiod, 214 $\mu\text{Em}^{-2}\text{s}^{-1}$ PAR at plant height. Three temperature regimes, constant 11, 18, and 25°C, were used. Grains of similar size for all genotypes, obtained by selecting only those retained on a 2.5 mm sieve, were sown in 32 × 24 × 5 cm trays filled with vermiculite that had been previously saturated with 1.2 L of 7.2 × 10⁻⁵ M (25 ppm) aqueous GA₃ solution or 1.2 L H₂O for the control treatment. After sowing, the trays were kept for 3 d at 3 to 4°C to ensure uniform germination before being moved to controlled environment cabinets, where they were watered with tap water as needed.

Growth Measurements

Six near-isogenic genotypes, (*rht*, *Rht1*, *Rht2*, *Rht3*, *Rht1+2*, *Rht2+3*) in each of two varietal backgrounds (the winter cv Maris Huntsman and the spring cv April Bearded) were grown. Each tray contained six rows of 12 to 15 seedlings, one row of each of the six genotypes. Five replicate trays were sown of each varietal background for each of the six treatments (GA and control at 11, 18, and 25°C). Seedlings were harvested at the time of emergence of the tip of the third leaf, which occurred at the age of 29, 18, and 14 d for 11, 18, and 25°C, respectively. The lengths of the sheath and lamina of the first leaf were recorded.

GA₁ Determinations

The Maris Huntsman series of six near-isogenic lines were grown to the same stage (emergence of the tip of the third leaf). Three trays of each genotype, 160 to 170 seedlings per tray, were grown at 10 and 25°C and watered with tap water. Analyses of endogenous GA₁ were performed on two replicates each of about 150 seedlings per genotype.

Lower segments, cut from the point of emergence from the caryopsis to the ligule of the first leaf, were frozen in liquid N₂, crushed in a mortar, and freeze dried. These segments included the coleoptile, the first leaf sheath, the shoot apex, and parts of young leaves enclosed by the sheath.

GA Extraction and Analysis

Qualitative Analysis

Lower leaf segments (150, 12 g fresh weight) cut from *Rht3* wheat seedlings grown at 20°C for 14 d were extracted twice with 80% MeOH² at 4°C and 833 Bq [1,2-³H₂]GA₁ (1.21 T

Bq mmol⁻¹, Amersham plc) added to the combined methanolic extracts. After removal of the MeOH under reduced pressure at 35°C, the aqueous phase was adjusted to pH 3.0 (2 N HCl) and partitioned against ethyl acetate (4 × 1/2 volume). The combined organic phases were partitioned against 0.1 M sodium bicarbonate (3 × 1/2 volume), acidified to pH 3.0, and reextracted into ethyl acetate (4 × 1/2 volume). The ethyl acetate was reduced to dryness *in vacuo* at <35°C, dissolved in water (pH 5–6), adjusted to pH 7.5 (1 M KOH), and loaded onto a QAE Sephadex A-25 (Pharmacia) anion exchange column (5 mL bed volume) that had been preequilibrated with 0.5 M sodium formate then washed with 1% formic acid and water (pH 8). After loading, the column was washed with three volumes of water (pH 8) and GAs were eluted with four volumes of 0.2 M formic acid. The formic acid solution was applied directly to a preequilibrated C₁₈ Sep-Pak cartridge (Waters Assoc.) which was washed with 2 mM acetic acid (pH 4); GAs were eluted with 80% MeOH and taken to dryness *in vacuo*. GAs were resolved by reverse-phase HPLC (LDC/Milton Roy Gradient system 3, Stone, Staffs) using a 4.9 mm i.d. × 250 mm column containing Partisil 5 ODS 3 (Whatman) and a linear gradient of increasing MeOH in 2 mM acetic acid (28% MeOH to 100% MeOH over 40 min) at a flow rate of 1 mL min⁻¹. Samples were dissolved in 100 μL MeOH, then 400 μL 2 mM acetic acid was added, and the solution was injected onto the column (Hichrom Ltd, Reading) using a Rheodyne 7125 valve fitted with a 500 μL loop. Forty 1-mL fractions were collected and aliquots (1/20) removed for scintillation counting to locate GA₁ (usually fractions 15, 16). The dried fractions containing GA₁ were methylated with ethereal diazomethane, transferred to glass ampoules and trimethylsilylated with MSTFA (5 μL) at 90°C for 30 min. Derivatized samples were analyzed using a Kratos MS80 RFA GC-MS system. Samples (1 μL) were coinjected with Parafilm (to determine Kovats retention indices) into a fused silica wall-coated open tubular (WCOT) BP-1 capillary column (SGE) (0.32 mm × 25 m × 0.33 μm film thickness) at an oven temperature of 50°C with the injector split valve closed. After 0.5 min the split (50:1) was opened and after 1 min the oven temperature was increased at 15°C min⁻¹ to 240°C and then at 4°C to 300°C. The He inlet pressure was 2.94 × 10⁴ Pa and the injector and interface temperature was 250°C. After 12 min, positive ion electron impact mass spectra were acquired, scanning from 700 to 50 atomic mass units at 1 s per mass decade. The electron energy was 70 electron volts and the source temperature 200°C.

Quantitative Analysis

Freeze-dried samples were extracted as above and, in addition to the tritiated GA₁, [1,2-³H₂]-GA₁ (gift from Prof. J. MacMillan, University of Bristol) was added as an internal standard. The deuterium isotope enrichment, determined by mass spectrometry (P. Gaskin, Univ. of Bristol) was no deuterium atoms 9.1%, 1 deuterium atom 29.0%, 2 deuterium atoms 59.2%, and 3 deuterium atoms 2.5% (2). The amounts of deuterated GA₁ added to extracts varied between 3 and 80 ng depending on the weight of tissue (3–9 g), genotype and growing temperature. After purification, as above, samples were analyzed using a Hewlett-Packard 5890 gas chromato-

² Abbreviations: MeOH, methanol; MSTFA, *N*-methyl-*O*-trimethylsilyltrifluoroacetamide; GC-SIM, gas chromatography mass spectrometry/selected ion monitoring.

graph coupled to an HP 5970 mass selective detector. Samples were injected into a fused silica WCOT BP-1 capillary column (SGE) (0.2 mm \times 25 m \times 0.3 μ m film thickness) at an oven temperature of 60°C for 1 min, then heated at 20°C min⁻¹ to 240°C and at 4°C min⁻¹ to 300°C. The helium inlet pressure was 8.96×10^4 Pa and the injector, interface, and source temperatures were 220, 270, and 200°C, respectively. Ions at *m/z* 508, 506, 448, and 376 were monitored with dwell times of 0.1 s and the concentration of GA₁ present in the original extract determined from a previously established calibration curve of the peak area ratio of unlabeled (*m/z* 506) and deuterated (*m/z* 508) GA₁ plotted against a varying molar ratio of the two compounds (7). The calibration curve was established using a constant amount of deuterated GA₁ and varying amounts of unlabeled GA₁. The same stock solution of deuterated GA₁ was used as the internal standard for plant extracts.

RESULTS

Growth Responses

The effects of temperature and applied GA₃ on the elongation of the first leaf of six near-isogenic lines of two wheat cultivars, carrying different *Rht* alleles, are presented in Table I. The two cultivars responded similarly to the factors tested and there were no differential effects on lamina and sheath lengths.

In the untreated seedlings of the *rht* genotype, higher growth temperature resulted in a statistically significant increase in the length of the first leaf. In the *Rht1* and *Rht2* genotypes, the effect of increasing temperature on leaf elongation was in most cases less than half the effect observed in the *rht* genotype. In the *Rht1+2*, *Rht3*, and *Rht2+3* genotypes, no consistent effects of temperature on first-leaf elongation were detected. There was an indication that increasing temperature reduced the final lengths of the leaves of the *Rht2+3* genotype in the April Bearded cultivar.

At 11°C, no great difference in leaf length was observed between the *rht* genotype and the *Rht1*, *Rht2*, and *Rht1+2* genotypes. The first leaves of the *Rht3* and *Rht2+3* genotypes were significantly shorter than those of the other four genotypes at all three temperatures tested.

Application of GA₃ to the *rht* (tall) genotype increased sheath length by 110 to 170% and lamina length by 50 to 60%, although the increase in absolute length was similar in both tissues. The absolute response of these tissues to GA₃ application was similar at the three temperatures tested (Fig. 1).

In the *Rht1* and *Rht2* genotypes, the effect of GA₃ application on leaf elongation was much less than that in the tall genotype and it was temperature dependent. At high temperature (25°C), there was little response to applied GA₃. However, at lower temperatures leaf growth of these genotypes was responsive to applied GA₃, and at 11°C it amounted to almost half the response of the tall genotype.

In the *Rht1+2* genotype, a significant increase in leaf length following GA₃ application was observed only at 11°C. In the *Rht3* and *Rht2+3* genotypes, no statistically significant responses to GA were observed at any of the temperatures

tested, but the trend of decreasing response with increasing temperature was maintained (Fig. 1).

Endogenous GA Analyses

GA₁ was identified by full spectrum GC-MS in leaf segments of Maris Huntsman *Rht3* dwarf wheat. The endogenous compound had the same mass spectrum and Kovats retention index as the authentic compound run under identical conditions (data not shown). GA₃ was also present but at much lower concentration (*m/z* 506: *m/z* 504 = 8.2:1) and there was only a trace of 3 epi-GA₁. Most significantly, there were no ions above *m/z* 506 in the spectrum of GA₁ that would have caused interference when using deuterated internal standard and selected ion monitoring for quantitation.

GA₁ content was determined in the lower leaf segments of Maris Huntsman seedlings grown at 10 and 25°C and harvested when they had accumulated 300° d (after 30 and 12 d growth at 10 and 25°C, respectively). At this stage, the tip of the third leaf had just appeared in the majority of seedlings. The lengths of the first-leaf sheaths of the different genotypes grown at 10 and 25°C (Table II) were quite similar to those shown in Table I for 11 and 25°C. The fresh weights as well as the lengths of the leaf segments from the *rht*, *Rht1*, and *Rht2* genotypes were much lower at 10°C than at 25°C, whereas there was little effect of temperature on the fresh weight and the length of the segments from the *Rht1+2*, *Rht3*, and *Rht2+3* genotypes (Table II). The six genotypes did not differ in dry matter percentage which was 50% higher in each at 10°C than at 25°C.

In all six genotypes, the content of endogenous GA₁ was considerably lower at 10°C than at 25°C, with mean values of 12.5 pg per segment and 50.8 pg per segment, respectively (Table II). At 25°C, the amount of GA₁ was lowest in the *rht* genotype, higher in the *Rht1* and *Rht2* genotypes and highest in the *Rht1+2*, *Rht3*, and *Rht2+3* genotypes. At the lower temperature, however, the amount of endogenous GA₁ was similar in all the genotypes, except *Rht2+3* in which an increased GA₁ content was observed.

DISCUSSION

In general, those genotypes that were responsive to increasing temperature were also responsive to applied GA₃. The responses were greatest in the *rht* (tall) genotype, moderate in *Rht1* and *Rht2*, only slight in *Rht1+2* and negligible in *Rht3* and *Rht2+3* (Fig. 1). There was little difference in leaf growth between *Rht1* and *Rht2* genotypes, which is also reflected in their final plant heights and yield components (5, 11). The measurements of final leaf length for the *rht* and *Rht3* near-isogenic lines reported here are in agreement with the high-resolution growth measurements on nonisogenic *rht* and *Rht3* cultivars reported by Stoddart and Lloyd (19).

Comparison of the final leaf lengths of the untreated *rht*, *Rht1*, *Rht2*, and *Rht1+2* genotypes, growing at different temperatures, suggests that these *Rht* alleles are expressed more effectively at higher temperatures and/or in potentially faster growing tissues (Table I). In contrast, the more potent *Rht3* allele, alone or in combination with *Rht2* (*Rht2+3*), is also markedly expressed at lower temperatures. However, even

Table I. Effects of Temperature, GA₃ Application, and *Rht* Genotype on the Length of the First Leaf Measured in Two Series of Near-Isogenic Wheat Lines

The data are means of 10 seedlings in each of 5 replicates. Within each treatment values followed by the same letter do not differ significantly according to Duncan's multiple range test. Values for the GA₃ treatments accompanied by an asterisk significantly exceed those of their untreated controls ($P \leq 0.01$). The significance of the difference between these values was determined separately for each line using the SE of the respective difference.

Temperature	Genotype	cv Maris Huntsman				cv April Bearded			
		Sheath length		Total leaf length		Sheath length		Total leaf length	
		Untreated	GA ₃ Treated	Untreated	GA ₃ Treated	Untreated	GA ₃ Treated	Untreated	GA ₃ Treated
<i>mm</i>									
11°C	Tall (<i>rht</i>)	29a	79a*	126a	234a*	37a	101a*	140a	268a*
	<i>Rht1</i>	26b	47b*	109c	153bc*	37a	65b*	137a	198b*
	<i>Rht2</i>	27b	46b*	117b	162b*	36a	63b*	137a	195b*
	<i>Rht1+2</i>	24c	33c*	114bc	138c*	35a	42c*	131a	150c*
	<i>Rht3</i>	21d	24d	97d	109d*	27b	30d	102b	108d
	<i>Rht2+3</i>	18e	19e	87e	93e	23c	23e	96b	97e
	SE	0.49	1.17	2.26	3.43	0.86	0.89	2.95	2.71
18°C	Tall (<i>rht</i>)	42a	94a*	168a	286a*	46a	112a*	178a	308a*
	<i>Rht1</i>	35c	50b*	131c	167b*	40b	61b*	152b	193b*
	<i>Rht2</i>	37b	46c*	147b	171b*	40b	53c*	154b	181c*
	<i>Rht1+2</i>	29d	31d	130c	135c	33c	36d	132c	141d
	<i>Rht3</i>	22e	23e	103d	107d	23d	25e	99d	104e
	<i>Rht2+3</i>	19f	19f	90e	90e	20e	20f	90e	90f
	SE	0.48	0.64	1.69	1.84	0.64	0.70	2.57	2.00
25°C	Tall (<i>rht</i>)	49a	104a*	195a	308a*	58a	112a*	202a	316a*
	<i>Rht1</i>	38b	45b*	143c	158b*	45b	53b*	160b	180b*
	<i>Rht2</i>	37b	42c*	154b	162b*	44b	47c	158b	173b*
	<i>Rht1+2</i>	27c	28d	124d	127c	31c	31d	127c	131c
	<i>Rht3</i>	21d	22e	103e	104d	23d	24e	97d	101d
	<i>Rht2+3</i>	17e	19f	88f	88e	18d	19f	83e	86e
	SE	0.69	0.54	1.82	1.42	1.89	1.32	3.21	2.65

these latter two genotypes have greater sheath lengths at 10°C than at 25°C (Table II), showing that the *Rht3* allele does not suppress the growth potential completely at the lower temperature. Perhaps the most interesting observation from the present results is that while the sensitivity to GA₃ of the *rht* genotype is not affected by temperature, the *Rht1*, *Rht2*, and *Rht1+2* genotypes are more responsive to applied GA₃ at low than at high temperatures, whereas the *Rht3* and *Rht2+3* alleles confer GA-insensitivity at all temperatures tested (Fig. 1). The *Rht* allele and the growing temperature can be viewed together, therefore, as setting the 'upper limit' for the extent of growth response to either endogenous or applied GA. Thus, the term 'GA-insensitivity,' which is usually coupled with these *Rht* alleles (5), should not be considered as being absolute but dependent on the nature of the allele under consideration and the growing temperature. Previous work (9) has shown that the responsiveness of the *Rht1* near-isogenic Maris Huntsman line saturates at a lower concentration of applied GA₃ than the corresponding *rht* line, again suggesting a reduced 'response capacity' (3).

The altered responsiveness to applied GA₃ of the *Rht* genotypes grown at different temperatures might be related to differences in endogenous GA₁ pool sizes. The concentration of biologically active GA₁ was measured, therefore, in lower

leaf segments which included the elongation zone, the presumed GA-responsive tissue (1, 8, 18). In the *rht* line growing at 10 and 25°C, the content of GA₁ increased in proportion to the length of the leaf sheath (Table II). However, *rht* leaves were equally responsive to applied GA₃ at both 11 and 25°C (Fig. 1) possibly indicating that the endogenous concentration of GA₁ was suboptimal for maximum growth at these temperatures. It is difficult to determine whether or not endogenous GA₁ is regulating leaf growth in the *rht* line under these conditions. The 38% reduction in endogenous GA₁ in *rht* tissue grown at 10°C compared with 25°C (Table II) was associated with a 35% reduction in final leaf length (Table I), which might make it a sensitive regulator of leaf extension growth. However, in previous work with the same genotype growing at 20°C, a 10-fold reduction in GA₁ concentration was associated with a 30% reduction in final leaf length following treatment with the GA-biosynthesis inhibitor, 2S,3S paclobutrazol (9). It should be emphasized that in the present experiments, the plants were grown under relatively low light intensity and without added nutrients to maximize the responsiveness to applied GA₃. These growth conditions may have caused the lower endogenous GA₁ concentration in *rht* leaf segments and much smaller differences between the genotypes than in more normally grown plants (JR Lenton, NEJ

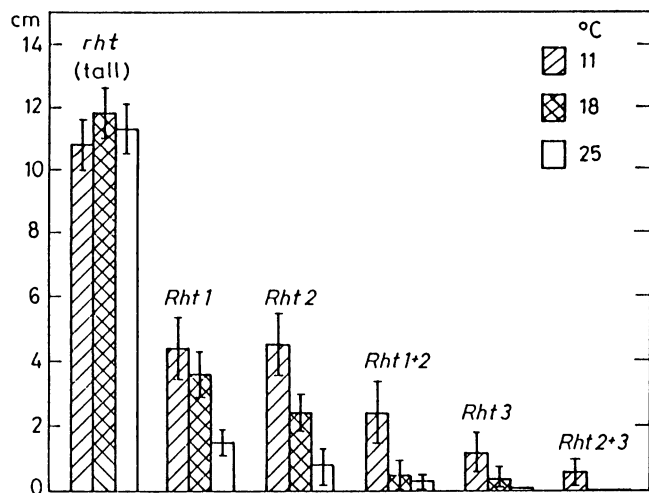


Figure 1. Effect of temperature on the response to applied GA₃ of six near-isogenic genotypes of cv Maris Huntsman wheat. The columns represent the difference in the final length of the first leaf between GA₃-treated and untreated seedlings. Error bars are \pm SE of the difference between the treated and untreated seedlings.

Table II. Growth and GA₁ Content of Lower Leaf Segments of Six Near-isogenic Genotypes of cv Maris Huntsman Wheat Grown at 10°C and 25°C

The plant data are means of three replicates, each of 15 seedlings. The GA₁ data are the means of two replicate measurements (except *Rht3*/10°C where only one was assayed) each of about 150 seedlings. Variability between the two replicates appeared more at the higher GA₁ levels but no evidence of nonhomogeneity of variance was found.

Genotype	Fresh weight		Dry matter		Length		GA ₁	
	10°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C
	mg/segment		%		mm		pg/segment	
Tall (<i>rht</i>)	47.8	58.1	19.6	13.0	30.3	50.2	13	21
<i>Rht1</i>	39.7	46.5	20.6	13.2	29.1	37.7	12	35
<i>Rht2</i>	35.4	48.6	20.6	13.1	26.5	38.1	9	30
<i>Rht1+2</i>	40.7	42.3	18.8	13.1	29.9	28.7	8	75
<i>Rht3</i>	35.6	31.8	20.2	13.6	24.9	21.4	13	82
<i>Rht2+3</i>	30.0	27.7	20.0	13.6	22.7	18.3	20	62
SE	1.98	1.39	0.25	0.39	0.54	0.61	3.99	8.43

Appleford, unpublished results). Such observations emphasize the importance of establishing hormone concentration growth-rate relationships based on measurement of endogenous levels in the responding tissue of plants growing under a range of environmental conditions (9).

In the present experiments, it seems reasonable to assume that in the *Rht* genotypes growing at 10°C (with the possible exception of *Rht2+3*) the GA₁ content was suboptimal for maximum growth, since it was similar to that of *rht* (Table II). Thus, the potential to respond to applied GA₃ was greater at lower temperatures (Fig. 1), although the magnitude of the response was determined by the nature and degree of expression of the *Rht* allele. Thus, *Rht1* and *Rht2* lines were partially responsive, *Rht1+2* less so, and *Rht3* and *Rht2+3* virtually unresponsive to applied GA₃ at 11°C (Fig. 1). In the *Rht1* and *Rht2* genotypes, the threefold increase in GA₁ content of leaf

segments grown at 25°C compared to 10°C (Table II) was associated with an increase in final leaf length and the tissues retained some responsiveness to applied GA₃ (Table I) showing that GA₁ is a potential regulator of leaf growth at this temperature under these growing conditions. In contrast, leaf segments of the *Rht1+2*, *Rht3*, and *Rht2+3* genotypes were somewhat shorter at 25°C than at 10°C, endogenous GA₁ accumulated (Table II) and there was no response to applied GA₃ (Fig. 1).

In conclusion, we suggest that the elongation growth response of these wheat seedlings to both endogenous and applied GAs is restricted by an upper limit set by the *Rht* alleles. Up to this limit, which is different for each genotype and growing temperature, GA may promote leaf extension whereas beyond this limit any increase in endogenous GA₁ or applied GA will not affect elongation growth. It becomes important, therefore, to determine the upper limit and endogenous GA₁ dose-growth response relationship for the *rht* genotype growing under different conditions before dismissing changing endogenous hormone concentration as a sensitive regulator of growth rate (20–22).

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