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A Mutant Gene That Increases Gibberellin Production in *Brassica*[1]

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ABSTRACT

A single gene mutant (*elongated internode* [*ein/ein*]) with accelerated shoot elongation was identified from a rapid cycling line of *Brassica rapa*. Relative to normal plants, mutant plants had slightly accelerated floral development, greater stem dry weights, and particularly, increased internode and inflorescence elongation. The application of the triazole plant growth retardant, paclobutrazol, inhibited shoot elongation, returning *ein* to a more normal phenotype. Conversely, exogenous gibberellin A_3 (GA_3) can convert normal genotypes to a phenotype resembling *ein*. The content of endogenous GA_1 and GA_3 were estimated by gas chromatography-selected ion monitoring using $[^2H]GA_1$ as a quantitative internal standard and at day 14 were 1.5- and 12.1-fold higher per stem, respectively, in *ein* than in normal plants, although GA concentrations were more similar. The endogenous levels of GA_{20} and GA_1, and the rate of GA_{19} metabolism were simultaneously analyzed at day 7 by feeding $[^2H_2]GA_{19}$ and measuring metabolites $[^2H_2]GA_{20}$ and $[^2H_2]GA_1$ and endogenous GA_{20} and GA_1, with $[^2H_5]GA_{20}$ and $[^2H_5]GA_1$ as quantitative internal standards. Levels of GA_1 and GA_{20} were 4.6- and 12.9-fold higher, respectively, and conversions to GA_{20} and GA_1 were 8.3 and 1.3 times faster in *ein* than normal plants. Confirming the enhanced rate of GA_1 biosynthesis in *ein*, the conversion of $[^3H]GA_{20}$ to $[^3H]GA_1$ was also faster in *ein* than in the normal genotype. Thus, the *ein* allele results in accelerated GA_1 biosynthesis and an elevated content of endogenous GAs, including the dihydroxylated GAs A_1 and A_3. The enhanced GA production probably underlies the accelerated shoot growth and development, and particularly, the increased shoot elongation.

Data from a wide range of crop plants indicate a positive correlation between the concentration of endogenous gibberellins (GAs) and the rate of shoot growth, particularly elongation (1, 9, 16, 18, 19, 22). The exogenous application of GA_3 or other GAs accelerates shoot elongation in most plants, particularly in some short genotypes (13, 16, 18, 19, 22, 26). Collectively, these results indicate that endogenous GA level is a principal factor regulating (limiting) shoot elongation growth in higher plants.

The clarification of the physiological relationship between shoot elongation and GAs has largely resulted from the study of GA-deficient single gene dwarf mutants (16, 18). It is likely that the study of mutants with elevated rates of GA biosynthesis will also contribute to the understanding of GA physiology.

A number of single gene mutants of crop plants have been identified that mimic normal genotypes to which GA_3 has been applied. These include the *slender* mutants of pea, wheat, and barley (18, 25) and the *procera* mutant of tomato (8). However, subsequent analyses did not indicate an enhancement of endogenous GA_1 or its precursors in these mutants (8, 25), although the metabolism of $[^3H]GA_{20}$ to $[^3H]GA_1$ and $[^3H]GA_8$ was faster in the overgrowth tomato mutant, *procera* (8). The ma_3^R-maturity genotype of *Sorghum bicolor* also mimics a normal genotype to which GA_3 has been exogenously applied (15), and a preliminary report (2) supports an elevated level of endogenous GA_1 in ma_3^R, suggesting that this allele may enhance GA_1 production.

Recently, we identified a mutant genotype of *Brassica rapa* with a phenotype similar to that of normal plants to which exogenous GA_3 has been applied. In the present paper, this mutant, *elongated internode* (*ein*), is introduced and the levels of some of the principal endogenous bioactive GAs as well as the rate of GA_1 biosynthesis are compared between *ein* and a normal *B. rapa* genotype.

MATERIALS AND METHODS

Plant Material

The study involved a normal, rapid-cycling line (27) of *Brassica rapa* (syn. *campestris*) (designation: CrGCl-1 [formerly CrGC No. 1] base population) and a mutant which was selected from a cross of two accessions of the U.S. Plant Introduction Service (designation: CrGC 1-11 [formerly CrGC No. 95] *elongated internode, ein/ein*). Plants of the two lines were grown either in a greenhouse at Lethbridge in February (latitude 49.6 °N) at about 26 °C/22 °C (day/night)

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[2] Abbreviations: CrGC, Crucifer Genetics Cooperative; a.m.u., atomic mass units; EtOAc, ethyl acetate; GC-SIM, gas chromatography-selected ion monitoring; MeOH, methanol; M^+, molecular ion; MeTMSi, methyl ester trimethylsilyl ether; Rt, retention time; SiO_2, silicic acid.

or in a controlled environment chamber (Conviron 123L, Winnipeg, Manitoba) at 25 °C/15 °C and a RH of 40%. Under both conditions, a 16 h photoperiod was provided by cool-white and warm white fluorescent tubes, which provided 87 and 163 μmol m^{-2} s^{-1} PPFD as determined with a Li-Cor quantum sensor (Li-Cor Inc., Lincoln, NE) for the greenhouse and growth chamber, respectively. Seeds were planted in 4 × 4 × 11 cm root trainers filled with Metro-mix, a soilless, peatlite medium (W. R. Grace & Co., Ajax, Ontario), and were fertilized weekly with 0.1 g 28-14-14 (N-P-K) with added micronutrients (Plant Prod 28-14-14, Plant Products Co. Ltd., Bramalea, Ontario). Containers were watered daily to soil saturation.

Response to Paclobutrazol (PP333)

Seedlings were grown in the growth chamber described above but were watered with solutions containing 0, 10^{-5}, 10^{-4}, or 10^{-3} M paclobutrazol [PP333; 1-(4-chloroethyl) 4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol; Imperial Chemical Industries, UK], a plant growth retardant which blocks kaurene oxidation and, hence, GA biosynthesis (17). However, in common with other triazole plant growth retardants, PP333 alters other aspects of terpenoid metabolism, including sterol biosynthesis (17).

Gibberellins

[1,2-^{3}H]GA_1 (about 1.2 TBq per mmol) was purchased from Amersham while [2,3-^{3}H]GA_{20} (62 GBq per mmol) had been previously prepared (14). The synthesis and enrichment of [^{2}H]GAs has previously been described for [17,17-$^2H_2$]GA_1 ([$^2H_2$]GA_1), [17,17-$^2H_2$]GA_{19}, and [17,17-$^2H_2$]GA_{20} (20), and for [1,2,2,3,6-$^2H_5$]GA_1 ([$^2H_5$]GA_1) and [1,2,2,3,6-$^2H_5$]GA_{20} (4). GC-SIM analyses were performed for all [^{2}H]GAs to determine contributions from the internal standards to all ions monitored during GC-SIM analyses. GC-SIM analyses were also performed on authentic standards of GAs A_1, A_3, A_{19}, and A_{20} to determine ion abundances and contributions to the ions monitored for the [^{2}H]GA internal standards.

Quantitative Analysis of GA_1 and GA_3 by GC-SIM

In experiment 1, plants were grown in a growth chamber and harvested 14 d after planting. For each extract, stems (including apices, leaves were removed) from two plants were ground in cold 80% aqueous MeOH before vacuum filtration. Phosphate buffer at pH8 and 5 ng [$^2H_2$]GA_1 were added to each extract, and the MeOH was then removed *in vacuo* at 35 °C. The pH of the aqueous residue was reduced to 3.0 with HCl and GAs were extracted three times with water-saturated EtOAc. Water was removed from the EtOAc by freezing and filtration of the ice and the EtOAc was then removed *in vacuo* at 35 °C.

The acidic EtOAc-soluble residue was dissolved in 1:1 (v:v) EtOAc:MeOH and loaded onto glass fiber filter discs with 167 Bq [^{3}H]GA_1 added as a chromatographic internal standard. After step-elution SiO_2 partition chromatography (3, 24) the region containing the [^{3}H] was collected and further purified on reversed-phase C_{18} HPLC (9). Again, the region containing [^{3}H] was collected and dried. Finally, this purified extract was derivatized to the MeTMSi, and analyzed by GC-SIM (20).

Endogenous levels of GA_1 were determined by comparing abundances of the M^+ ion (506 a.m.u.) for GA_1-MeTMSi with that from the M^+ ion (508 a.m.u.) for [$^2H_2$]GA_1-MeTMSi. This method did not involve a calibration regression and, hence, may have introduced a slight but systematic error in the GA_1 estimation (6). Intensities were corrected for contributions to the 506 a.m.u. ion from the minor amount of protio GA_1 accompanying the [$^2H_2$]GA_1 and [^{3}H]GA_1 internal standards (20) and for the contributions to the 508 a.m.u. ion from the endogenous GA_1. Two other characteristic ion pairs (491, 493 a.m.u.; 448, 450 a.m.u.) were also monitored to confirm the identity of GA_1. Endogenous levels of GA_3 were estimated by comparing abundances of the M^+ ion (504 a.m.u. ion) for GA_3-MeTMSi with the M^+ ion (508 a.m.u.) for [$^2H_2$]GA_1-MeTMSi. Two other ions (489 and 370 a.m.u.) and Rts were also monitored to confirm the identity of GA_3.

This procedure enabled accurate measurement of GA_1 but the estimate of GA_3 level was less precise. Differences in recovery, derivatization, and subsequent quantitation between the endogenous GA_3 and the internal standard [$^2H_2$] GA_1 will introduce slight errors in the GA_3 quantitation.

Simultaneous Analyses of GA_{19} Turnover and Endogenous GA_{20} and GA_1

In a second experiment, plants were grown in the greenhouse for 5 d and then 1 μg [$^2H_2$]GA_{19} in 10 μL 95% EtOH was applied by syringe to the shoot tip of each of five plants of each genotype. After 48 h (d 7 from planting), plants were harvested and the stems plus shoot apices (leaves removed) from the five plants of a genotype were ground in 80% MeOH; 100 ng each of [$^2H_5$]GA_{20} and [$^2H_5$]GA_1 were added to each extract. Samples were evaporated to dryness with MeOH added occasionally to remove H_2O. Dry samples were dissolved in 1:1 (v:v) EtOAc:MeOH and loaded onto glass fiber discs for step-elution SiO_2 partition chromatography (3, 24) along with 1.67 KBq [^{3}H]GA_1 and 0.83 KBq [^{3}H]GA_{20} as chromatographic standards.

Radioactive fractions from the SiO_2 columns were further purified by reversed-phase C_{18} HPLC (10) and then analyzed by GC-SIM (20). Thirteen ions were monitored for [$^2H_2$], [$^2H_5$], and protio (endogenous) GA_1 (511, 508, 506, 504, 496,

Table I. *Development of Plants of Normal Rapid-Cycling B. rapa and the Mutant Elongated Internode (ein) in Greenhouse Conditions*

Developmental Stage (Growth Stage[a])	*B. rapa*	
	Normal	*ein*
	days after planting	
Seedling emergence (50%)	2	1
Bud (3.1) floral buds (inflorescence) visible	10	10
Inflorescence elongation (3.2)	13	13
Flowers open (4.1) anthesis	16	15
Lower pods elongating (4.2)	18	16

[a] As outlined by Harper and Berkenkamp (5).

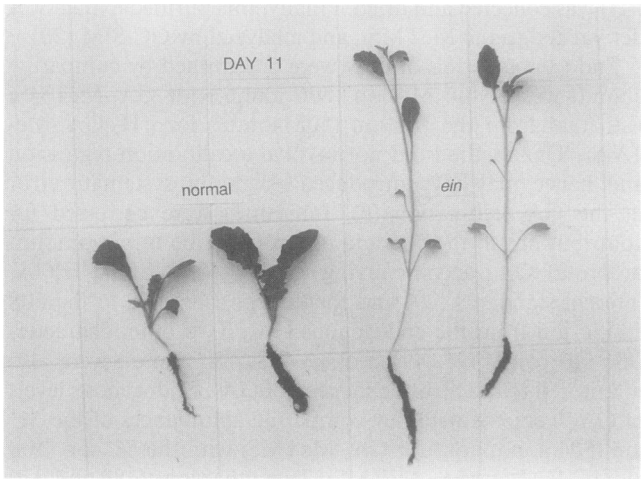

Figure 1. Two typical plants of each of a normal rapid cycling line of *B. rapa* (left), and the mutant, *elongated internode* (right), 11 d after planting in greenhouse conditions. The background grid is 10 × 10 cm.

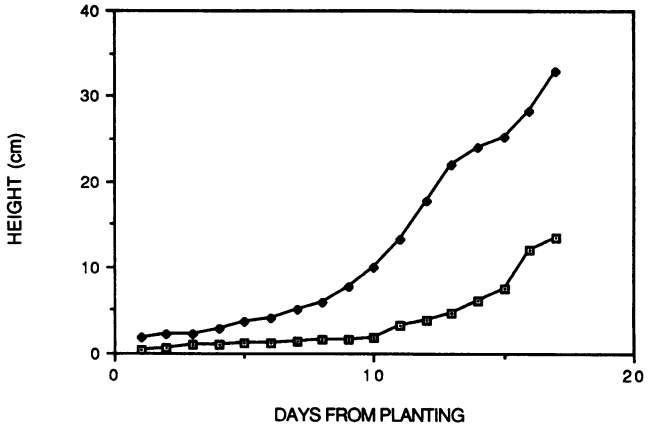

Figure 2. Height to the shoot apex of normal rapid cycling *B. rapa* (□) and the mutant, *elongated internode* (*ein*) (♦) in greenhouse conditions.

495, 493, 491, 453, 450, 448, 379, 315 a.m.u.) or GA_{20} (423, 420, 418, 408, 405, 403, 389, 380, 377, 375, 361, 209, 207 a.m.u.) and as was the case for the analysis of endogenous GA_1 and GA_3, contributions of the [^{2}H] and endogenous GAs to the various ions were corrected for in the numerical analyses to determine GA contents (20). This technique enabled the simultaneous quantitation of endogenous GA_1 and GA_{20} and analyses of turnover from GA_{19} to GA_{20} and GA_1.

Table II. *Growth and Endogenous Gibberellin A_1, A_3, and A_{20} Concentration (ng/g dry wt.) and Amount per Stem in 7 and 14-d-old Seedlings of Rapid-Cycling B. rapa and the Mutant Elongated Internode (ein)*

	Normal	*ein*	Ratio *ein*/normal
Greenhouse—d 7			
Dry weight per stem (mg)	11	15	1.4
Height (mm)	17	43	2.5
GA_1 concentration (ng/g dry wt.)	27.3	92.7	3.4
Amount GA_1 per stem (ng)	0.30	1.39	4.6
GA_{20} concentration (ng/g dry wt.)	28.2	266.0	9.4
Amount GA_{20} per stem (ng)	0.31	3.89	12.9
Growth chamber—d 14			
Dry weight per stem (mg)	22	31	1.5
Height	62	239	3.9
GA_1 concentration (ng/g dry wt.)	107.7	115.2	1.1
Amount GA_1 per stem (ng)	2.37	3.57	1.5
GA_3 concentration (ng/g dry wt.)[a]	40.8	343.2	8.4
Amount GA_3 per stem (ng)	0.88	10.64	12.1

[a] Quantities of GA_3 were estimated using [$^2H_2$]GA_1 as an internal standard and hence, are not precise.

Metabolism of [^{3}H]GA_{20} in *ein* and Normal Plants

Five, 5-d-old greenhouse plants of *ein* and five of the normal genotype were each fed 333 Bq [^{3}H]GA_{20} by syringe application to the shoot tip in 5 μL 95% EtOH. After 48 h, leaves were removed and remaining stems (including apices) were ground in cold 80% MeOH. The extracts were dried *in vacuo* with MeOH being added to remove all traces of H_2O. The dried extracts were dissolved in 1:1 (v:v) EtOAC:MeOH and loaded on glass fiber discs for step-elution SiO_2 partition chromatography (3, 24) followed by gradient-elution reversed-phase C_{18} HPLC (10), and then liquid scintillation counting. This experiment was performed three times with generally similar patterns.

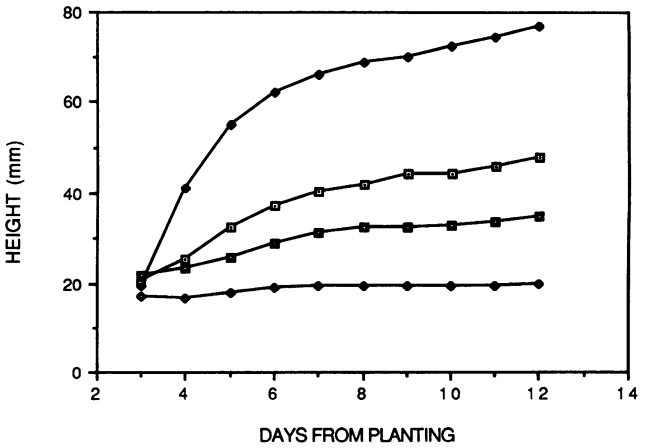

Figure 3. Influence of exogenous application of the triazole growth retardant paclobutrazol on height of the *B. rapa* mutant, *ein*. (♦), Control; (□), 10^{-5} M; (⊡), 10^{-4} M; (◇) 10^{-3} M paclobutrazol in greenhouse conditions.

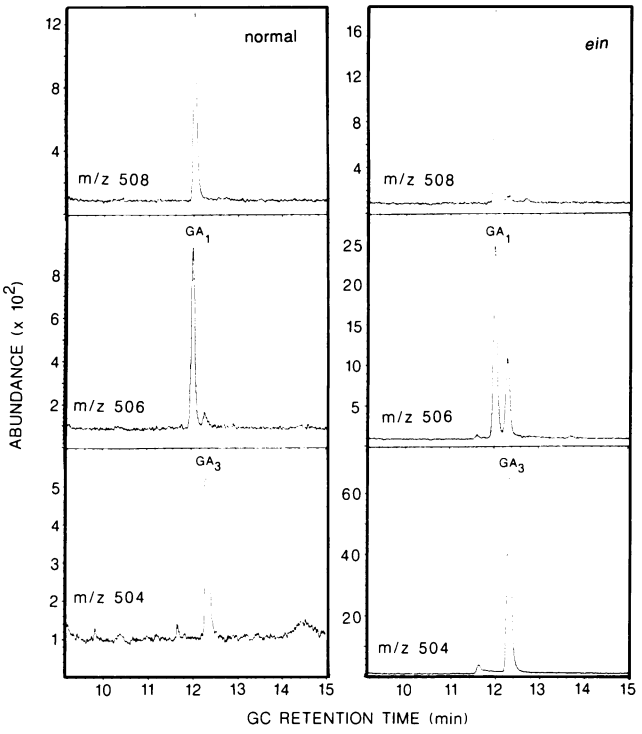

Figure 4. Gas chromatography-selected ion monitering profiles enlarged for the 9 to 15 min region for the M^+ ions characteristic of $[^2H_2]GA_1$-MeTMSi (quantitative internal standard—508 a.m.u.), GA_1-MeTMSi (506 a.m.u.), and GA_3-MeTMSi (504 a.m.u.) from which endogenous GA_1 and GA_3 concentrations were calculated from normal, rapid cycling *B. rapa* (left) and the mutant *ein* (right).

Identification of Principal Metabolites from $[^2H_2]GA_{19}$ and $[^2H_2]GA_{20}$

To identify metabolites from a $[^2H_2]GA_{19}$ feed, normal plants were grown and fed $[^2H_2]GA_{19}$ as described previously. Subsequent analysis by SiO_2 partition chromatography and C_{18} HPLC along with $[^3H]GA_1$ and $[^3H]GA_{20}$ standards was performed as previously described. However, no $[^2H_5]GA$ standard was added to the extract. Regions co-eluting with the $[^3H]GA_1$ and $[^3H]GA_{20}$ were collected and analyzed by GC-SIM for $[^2H_2]GA_1$ and $[^2H_2]GA_{20}$, respectively (20).

To identify the principal metabolite from a GA_{20} feed, normal plants were fed 1.67 KBq $[^3H]GA_{20}$ plus 1 μg $[^2H_2]GA_{20}$ by syringe application to the shoot tip as previously described. Purification of metabolites was as described for the metabolism of $[^3H]GA_{20}$ study. After HPLC, the major radioactive region (Rt of GA_1) was analyzed by GC-MS and GC-SIM (20).

RESULTS AND DISCUSSION

Plants of the mutant line *elongated internode* (*ein*) were readily distinguishable from normal plants, the most conspicuous difference being longer internodes and taller inflorescences (Fig. 1). By the time of seedling emergence (Table I) differences in shoot elongation between normal and *ein* plants were significant and the differences persisted throughout the life cycle of the plants (Fig. 2). The *ein* phenotype partially resembles that of dark-grown etiolated normal plants. However, *ein* leaves are darker green than the chlorotic leaves of etiolated normal seedlings.

The mutant *ein* plants had heavier stems than those of the normal genotype (Table II). However, leaves of *ein* were smaller than those of normal plants and hence, the observed stem growth was at the expense of leaf growth (Fig. 1). The time of the appearance of the floral bud was similar in *ein* and the normal plants, but subsequent inflorescence elongation, anthesis, and the onset of pod elongation were slightly accelerated in *ein* (Table I).

Application by root drench of a triazole-type plant growth retardant, paclobutrazol, which inhibits GA biosynthesis (17), inhibited shoot elongation in *ein* plants (Fig. 3). At higher paclobutrazol dosages, *ein* remained as a rosette without stem elongation (Fig. 3), a response also shown by normal *Brassica* plants treated with 'anti-GA' plant growth retardants (21). Thus, the presumed blockage of GA biosynthesis with paclobutrazol eliminated the *elongated internode* phenotype, returning *ein* plants to a more normal growth form.

The principal bioactive endogenous GAs of *Brassica* shoots have recently been characterized as those typical of the early C-13-hydroxylation biosynthetic pathway, GA_{19}, GA_{20}, GA_1

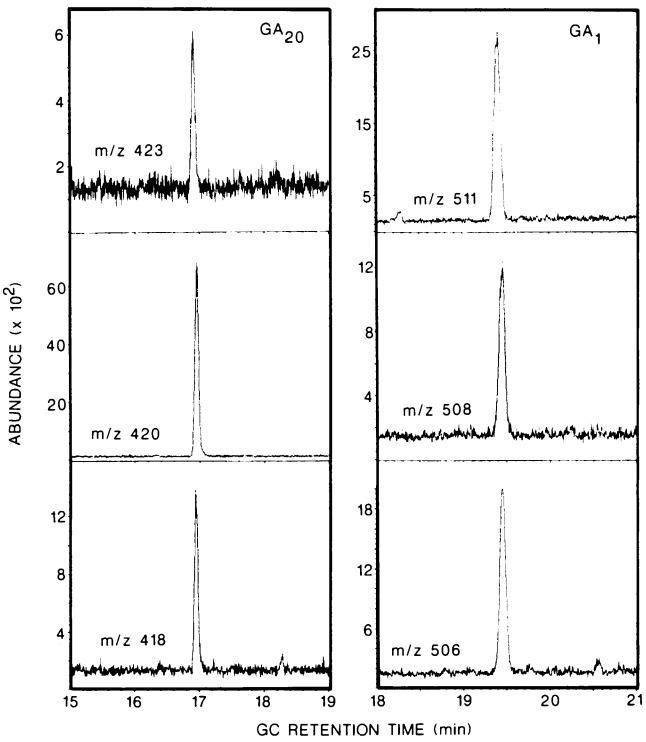

Figure 5. Gas chromatography-selected ion monitering profiles for the M^+ ions characteristic of $[^2H_5]GA$ (quantitative internal standard) (top), $[^2H_2]GA$ (metabolite from $[^2H_2]GA_{19}$) (middle), and endogenous GA (bottom) for GA_{20} (left column of three figures), and GA_1 (right) from the rapid cycling *B. rapa* mutant *ein*. Note that the $[^2H_5]GA$ eluted 0.04 min earlier than the $[^2H_2]GA$, which eluted 0.01 min earlier than the endogenous (protio) GA.

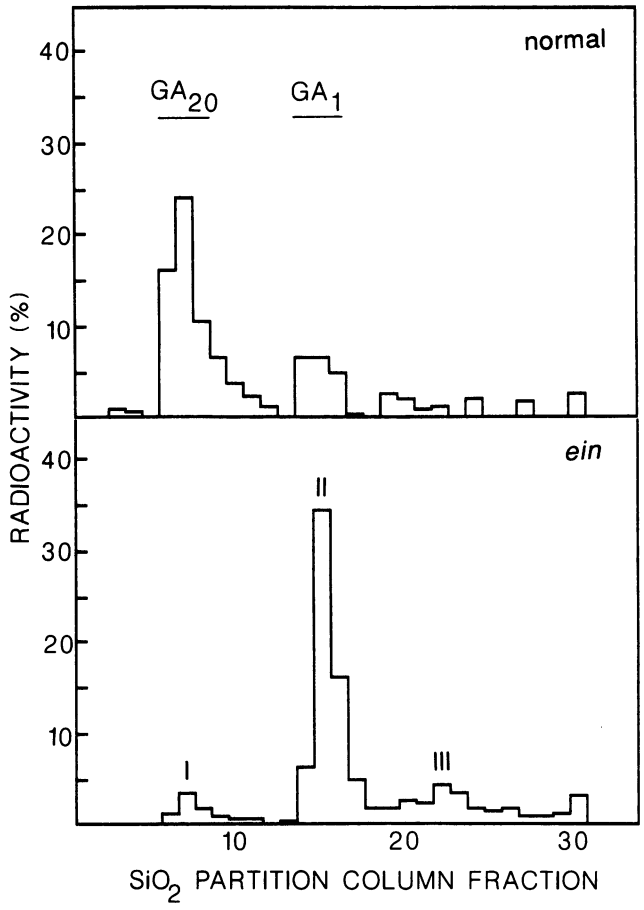

Figure 6. Elution of radioactivity from step-elution SiO_2 partition columns loaded with extracts from normal *B. rapa* (top) and *ein* plants (bottom) fed [^{3}H]GA_{20}. Elution regions of authentic [^{3}H]GAs are included in the top figure.

and, additionally, GA_3, a 1,2-dehydro analog of GA_1 (23). The level of endogenous GA_1 and GA_3 were determined in growth chamber-grown plants of *ein* and a normal genotype (Fig. 4). Concentrations of GA_1 were only slightly different between 14-d-old growth chamber-growth *ein* and normal plants, but the levels per shoot were about 1.5-fold higher in *ein* than in normal plants (Table II).

Differences in the endogenous levels of GA_3 between the two genotypes were considerably greater than the differences observed for GA_1 (Table II). This increased variation of GA_3 *versus* GA_1 is similar to the pattern recently observed for the GA-deficient *Brassica* dwarf mutant *rosette* (22). Stems of *rosette* contained about one-quarter the amount of GA_1 and one-tenth the amount of GA_3 of normal plants (22).

The biosynthetic origin of GA_3 in *Brassica* is presently unknown. It has recently been reported that GA_{20} can be a precursor for both GA_1 and GA_3 in maize, the conversion to GA_3 probably occurring via GA_5 (12). The conversion of GA_{20} to GA_5 also occurs in *Phaseolus vulgaris* (12) while the conversion of GA_5 to GA_3 has been demonstrated in a number of plants (12). Thus, GA_{20} could be a precursor for both GA_1 and GA_3 in *Brassica*. It will be interesting to examine the metabolic differences that apparently result in differential ratios of GA_1 and GA_3 in *ein*, normal genotypes, and the GA-deficient mutant *rosette*. Further, developmental changes in the relative biosynthesis of GA_1 and GA_3 in *ein* are possible since only trace quantities of GA_3 were detected in the extracts from these 7-d-old plants.

Consistent with the increase in GA_1 content in *ein* in the growth chamber study, levels of GA_1 and GA_{20} were elevated in *ein* at d 7 under greenhouse conditions (Table II). As was the case of GA_1 versus GA_3, the difference in levels of GA_1

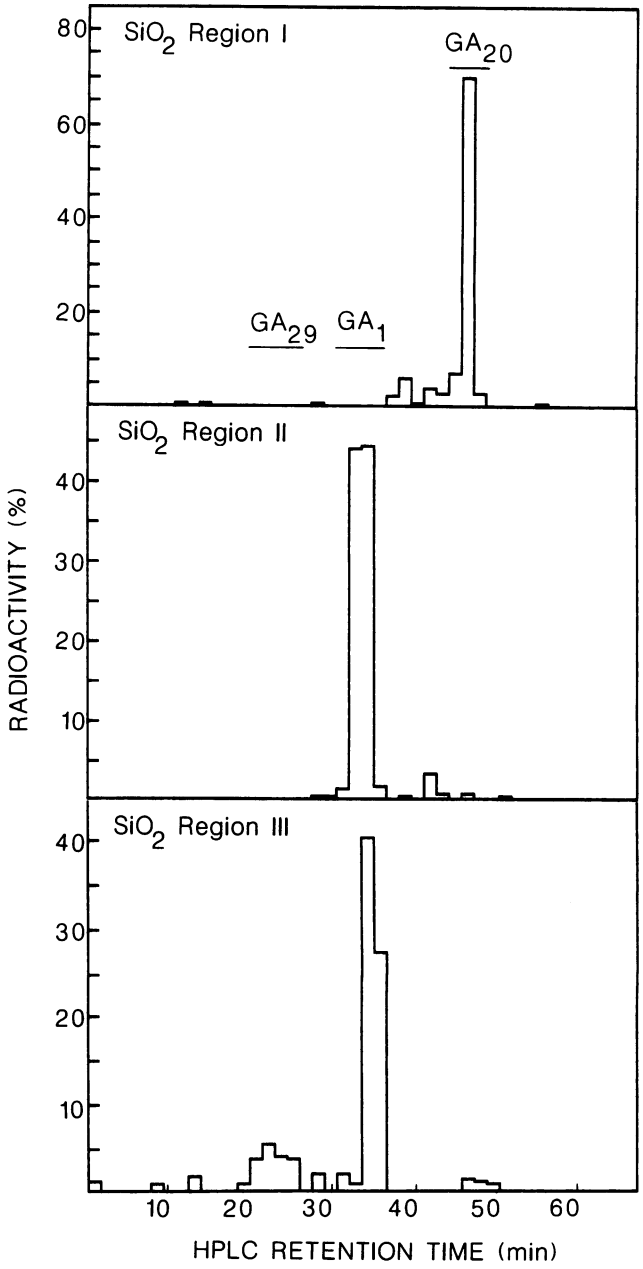

Figure 7. Elution of radioactivity from reversed-phase C_{18} HPLC columns loaded with SiO_2 peaks (Fig. 6) following a feed of [^{3}H]GA_{20} to the *B. rapa* mutant *ein*.

Table III. *Capillary GC-SIM of MeTMSi Derivatives of [$^2H_2$]GA Metabolites from Brassica Seedlings*

	SiO_2 Fraction	HPLC Rt	GC Rt	Constituent Ions		
		min		*m/z (%)*		
Authentic [$^2H_2$]GA_1	16–17	34–35	19.45	508(100)	493(16)	450(33)
				379(31)	315(17)	
Putative [$^2H_2$]GA_1 from [$^2H_2$]GA_{19}	16–17	34–35	19.44	508(100)	493 (13)	450(39)
				379(41)	315(15)	
Authentic [$^2H_2$]GA_{20}	9–10	46–47	16.95[a]	420(100)	405(15)	377(68)
				361(21)	303(31)	
Putative [$^2H_2$]GA_{20} from [$^2H_2$]GA_{19}	9–10	46–47	16.95	420(100)	405(14)	377(64)
				361(21)	303(17)	

[a] These analyses involved a different GC temperature program, and hence, retention times for GA_1 and GA_{20} are not directly comparable.

between *ein* and normal plants was less than the difference in GA_{20} level (Table II).

In the same greenhouse-grown plants, the turnover of [$^2H_2$]GA_{19} to [$^2H_2$]GA_{20} and [$^2H_2$]GA_1 was also quantified by GC-SIM (Fig. 5). During the 48 h incubation, each *ein* plant produced 29.5 ng of [$^2H_2$]GA_{20} while each normal plant produced only 3.4 ng, an 8.3-fold difference. Each *ein* plant also produced 0.53 ng [$^2H_2$]GA_1, 1.3 fold greater than the 0.42 ng produced by each normal plant. Thus, in these plants, GA turnover (biosynthesis) paralleled endogenous GA level, being faster in *ein* than in the normal plants.

The use of high specific activity [^3H]GA_{20} enabled the quantitative investigation of metabolism without the need for the application of GA in amounts which considerably exceeded the endogenous pool. The principal [^3H] metabolite following the [^3H]GA_{20} feed eluted from sequential SiO_2 partition (Fig. 6) and reversed-phase HPLC (Fig. 7) coincidental with authentic [^3H]GA_1 (Table III) and the conversion of [$^2H_2$]GA_{20} to [$^2H_2$]GA_1 in separate plants was confirmed by GC-SIM (data not presented). The possible conversion of [^2H] or [^3H]GA_{20} to [^2H] or [^3H]GA_3 could not be resolved by this study and awaits further investigation.

Confirming the pattern observed for the turnover of [$^2H_2$]GA_{19}, [^3H]GA_{20} metabolism to [^3H]GA_1 was also faster in *ein* than in the normal genotype (Fig. 6; Table IV), the difference being up to threefold. GA_1 is the principal metabolite from GA_{20} in *Brassica*, although it is not the only metabolite (11). Epi-GA_1 is also produced in minor quantity (11) and GA_{29} and GA_8 are both native GAs of *Brassica* (7) and probable metabolites of GA_{20} (11, 12). In the present study, elution of radioactivity from SiO_2 and subsequent HPLC columns suggested the minor conversion to [^3H]GA_{29} and possibly [^3H]GA_8 (Fig. 7) but amounts of the corresponding [$^2H_2$]GAs following the [$^2H_2$]GA_{20} feed were inadequate for identification by GC-SIM. Finally, small amounts of EtOAc-insoluble radioactive metabolites eluted in the final MeOH wash from the SiO_2 columns (fractions 26–30, Fig. 6) and these might represent GA glucosyl conjugates. Quantities of these various unidentified metabolites were minor and their total quantities were generally similar in *ein* and the normal genotype (Table IV).

Table IV. *Distribution of [^3H] following Feeds of [^3H]GA_{20} to Normal B. rapa Seedlings and the Mutant ein*

	Normal	*ein*	Ratio *ein*/Normal
Radioactivity recovered in stems (KBq)	1.1	1.7	
% of applied radioactivity	65%	100%	
[^3H]GA_{20} (% of radioactivity recovered)	76.2	52.4	0.7
Putative [^3H]GA_1	13.9	37.5	2.7
Other (putative [^3H]GA_{29} and [^3H]GA_8 and putative glucosyl conjugates)	9.8	10.1	1.0

From previous work with dwarf mutants of maize, peas and rice (12, 16, 18), the level of GA_1 is expected to be particularly relevant to the regulation of shoot elongation growth in *Brassica*. GA_3 is structurally similar to GA_1 excepting the Δ^1 olefinic bond, a structural difference which may inhibit 2β-hydroxylation, thereby conferring added persistence. With the enhanced GA_3/GA_1 ratio in *ein* and reduced GA_3/GA_1 ratio in the GA-deficient dwarf, *rosette*, it is possible that GA_3 in addition to GA_1, may regulate shoot growth in *Brassica*.

While the primary biochemical effect of *ein* requires clarification, these findings indicate that *ein* results in accelerated GA_1 and GA_{20} biosynthesis and enhanced GA_1, GA_3, and GA_{20} content (at least per stem) in *B. rapa*. The enhanced GA production probably underlies the observed *elongated internode* phenotype.

The mutant gene *ein* may have potential for further elucidating the mechanism of GA action in the regulation of shoot growth. Further, incorporation of *ein* should result in enhanced stem growth which could be desirable for a few crops. Thus, as well as providing an extremely useful gene for studies of GA physiology and the regulation of GA biosynthesis, there may be other interesting or useful applications for this hormone-altering mutant gene.

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