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Gibberellin Concentration and Transport in Genetic Lines of Pea[1]

Effects of Grafting

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ABSTRACT

Effects of the *Na* and *Le* loci on gibberellin (GA) content and transport in pea (*Pisum sativum* L.) shoots were studied. GA_1, GA_8, GA_{17}, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_8 catabolite, and GA_{29} catabolite were identified by full-scan gas chromatography-mass spectrometry in extracts of expanding and fully expanded tissues of line C79–338 (*Na Le*). Quantification of GAs by gas chromatography-single-ion monitoring using deuterated internal standards in lines differing at the *Na* and *Le* alleles showed that *na* reduced the contents of GA_{19}, GA_{20}, and GA_{29} on average to <3% and of GA_1 and GA_8 to <30% of those in corresponding *Na* lines. In expanding tissues from *Na le* lines, GA_1 and GA_8 concentrations were reduced to approximately 10 and 2%, respectively, and GA_{29} content increased 2- to 3-fold compared with those in *Na Le* plants. There was a close correlation between stem length and the concentrations of GA_1 or GA_8 in shoot apices in all six genotypes investigated. In *na*/*Na* grafts, internode length and GA_1 concentration of *nana* scions were normalized, the GA_{20} content increased slightly, but GA_{19} levels were unaffected. Movement of labeled GAs applied to leaves on *Na* rootstocks indicated that GA_{19} was transported poorly to apices of *na* scions compared with GA_{20} and GA_1. Our evidence suggests that GA_{20} is the major transported GA in peas.

Considerable evidence points to GA_1[2] as the native GA controlling internode elongation in a number of plant species, including pea (*Pisum sativum* L.) (11, 15, 24, 29, 30). GA_1 is synthesized via the 13-hydroxylation pathway, in which GA_{12} is 13-hydroxylated to GA_{53}, which is then converted successively to GA_{44}, GA_{19}, and GA_{20}. GA_{20} is either 2β-hydroxylated to GA_{29} or 3β-hydroxylated to GA_1, which in turn is metabolized to GA_8. The 2β-hydroxylated GAs, GA_8 and GA_{29}, are oxidized further at C-2 in pea to give the respective catabolites (11, 28). Of these compounds, only GA_1 is thought to be active in controlling stem elongation (18).

Mutant alleles in several species block GA biosynthesis, resulting in dwarf phenotypes (5, 24, 29, 30). In pea, 12 loci are known to influence plant stature (21) and recessive alleles at four of these reduce GA_1 biosynthesis (9, 11, 23). The *le* locus results in reduced conversion of GA_{20} to GA_1 (11, 12), the *na* allele is thought to block *ent*-7α-hydroxykaurenoic acid conversion to GA_{12}-aldehyde, and the *lh* and *ls* alleles apparently affect the conversion of geranylgeranyl Pi to *ent*-kaurene (9). Genetic evidence indicates that all of these loci are "leaky" (20). The quantitative effects of different *Le* alleles on GA_1 and GA_8 concentrations have been studied previously with differing conclusions (6, 10, 25–27). Whereas similar (6, 27) levels of GA_1 were reported in shoots of Progress No. 9 (*lele*) compared with the nonisogenic Alaska (*LeLe*), in isogenic plants differing at the *Le* locus the dwarf genotype produced 10- to 20-fold less GA_1 and GA_8 than the tall genotype (10, 25, 26).

Recognition of the importance of GA_1 in stem elongation and identification of the sites of the genetic blocks caused by the mutant alleles have aided our understanding of the response of various dwarf lines to grafting. Although GA_1 application converts *le* lines from dwarf to tall, the stature of *le* is not altered when grafted to *Le* rootstocks (16, 17, 22). This suggests that GA_1 is not transported in sufficient quantity to produce a tall phenotype. In contrast, stem elongation of *na* lines, in which GA biosynthesis is blocked early in the pathway (9), is increased both by applied GAs and by grafting to either *Na Le* or *Na le* rootstocks (1, 22), indicating that GA_{20} or earlier precursors are transported from the rootstock and metabolized to GA_1 in the *na* scion. Thus, the effect of the *Le* locus is confined largely to expanding leaves and stems.

Klee and Estelle (14) concluded that there is still some uncertainty about the relationship between GA concentration and response and the role of GA transport in growth and development. Recently, Ross et al. (25) found a good correlation between log of the GA_1 concentration in pea apices and the final length of internodes elongating within the apical region for both *Le* and *le* genotypes. Although GA_1 content clearly limited the growth of the internodes, their capacity for growth varied during ontogeny.

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[2] Abbreviations: GA, gibberellin; MeOH, methanol; EtOAc, ethyl acetate; SIM, single-ion monitoring.

We have addressed these problems by (a) quantifying the effects of the *na* and *le* loci on GAs of the later stages of the 13-hydroxylation pathway, (b) determining the effect of grafted *Na* donors (rootstocks) on the growth and GA content of *na* receptors (scions), and (c) studying transport and metabolism of GAs in *na/Na* grafts.

MATERIALS AND METHODS

Plant Material

Seedlings of pea (*Pisum sativum* L.) lines were grown singly in peat:perlite:vermiculite (1:1:1, v/v/v) in 15-cm diameter (1500 cm^3) plastic pots. The plants were grown in a greenhouse under natural photoperiods between February 1 and November 1, with temperatures averaging 25/15°C (day/night). Plants were fertilized by pre-plant incorporation of controlled release fertilizer (Osmocote, 14–14–14; SierraBlen Chemicals, Milpitas, CA). The pea lines (genotype) used in this study were I_3, a selection from cv Alaska (*Na, Le*), C79–338 (*Na, Le*), B780–687-(2) (*na, le*), and B686–67-(3) (*na, Le*), all of which were provided by the late Professor G.A. Marx, New York Agricultural Experiment Station, Geneva, NY; line 58 (*Na, le*), provided by Dr. I.C. Murfet, Department of Plant Science, University of Tasmania, Hobart, Australia; and Progress No. 9 (*Na, le*), from the Burpee Seed Co., Warminster, PA. Leaves, stems, and stipules comprising either expanding or fully expanded tissue were harvested when the plants had grown to node 6 (cotyledonary node = 0). A random sample of eight plants was grown for measurement of stem length from nodes 3 to 8.

Cleft-grafted plants consisting of *na* scions (6 d old) as receptors and *Na* or *na* stocks (4 weeks old, three true leaves left below the graft) as donors were covered with a plastic bag for 10 d. After another 10 to 14 d when the scions were well established, they were cut back to their lowest bud. This practice resulted in more uniform scion growth. Lateral branches on the stocks were removed routinely. In feeding experiments, GAs were applied to the uppermost leaf of the stock as the first leaf of the scion unfolded. Growth response of the scions was evaluated by measuring the length of the first five internodes on eight randomly selected plants.

Extraction and Purification of GAs

GAs were extracted and purified essentially following the procedure described in ref. 6. To determine recoveries during purification, $[^3H_2]GA_1$ (1.4 TBq/mmol; Amersham International, Amersham, UK), $[^3H]GA_8$ (prepared from $[^3H_2]GA_1$ using a 2β-hydroxylase from *Phaseolus vulgaris* seed), $[^3H_4]$-16,17-dihydroGA_{19} (2.4 TBq/mmol, prepared from GA_{19} by catalytic reduction by Amersham International), and $[^3H_3]$-GA_{20} (1.5 TBq/mmol, from Professor J. MacMillan, University of Bristol, UK) were added to the plant tissue before homogenization. After homogenization in cold 80% MeOH, the extract was stirred at 4°C overnight, filtered, and concentrated at 40°C in vacuo to the aqueous phase, which was then acidified to pH 2.5–3.0 and partitioned three times against equal volumes of EtOAc. The combined EtOAc fractions were partitioned three times against one-fifth volume of 5% $NaHCO_3$, which was acidified to pH 2.5 to 3.0 and partitioned three times against equal volumes of EtOAc. This EtOAc phase was dried at 40°C in vacuo, redissolved in water, pH 8, and applied to a preequilibrated QAE-Sephadex A-25 (Sigma Chemical Co.) column (5-mL bed volume). The column was washed with 15 mL of water, pH 8, and GAs were eluted with 0.2 M formic acid (20 mL), which was applied to an ODS Sep-Pak cartridge (Waters, Milford, MA). After the cartridge was washed with water at pH 3 (5 mL), the GAs were eluted in 5 mL of 80% MeOH, and the eluate was dried in vacuo.

HPLC

Dried samples were redissolved in 30% MeOH and fractionated by reverse-phase HPLC, using a 5-μm ODS column (0.46 cm i.d. × 25 cm) (Beckman Instruments) and a linear gradient of 30 to 100% MeOH in aqueous acetic acid (100 $\mu L \cdot L^{-1}$). Fractions coeluting with the radioactive internal standards were combined and dried in vacuo.

Qualitative Analysis of GAs by Combined GC-MS

Fresh samples consisting of 40 g of expanding tissue, 127 g of expanded leaves, or 131 g of expanded stems of line C79–338 were extracted and purified as described above. Dried, combined fractions from HPLC were methylated with ethereal diazomethane and then trimethylsilylated using *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (Aldrich Chemical Co., Milwaukee, WI) at 90°C for 30 min. The derivatized samples were analyzed using a Kratos MS80 RFA GC-MS system (Kratos Analytical, Manchester, UK) as described in ref. 8. Identifications were based on comparisons with mass spectra and Kovats retention indices in a spectral library (7).

Quantitative Analysis of GAs by GC-SIM

Fresh samples of approximately 5 g of expanding tissues or 15 g of expanded tissues (leaves and stems) were collected, immediately frozen in liquid N_2, and stored at −20°C. Before extraction, aliquots of $[17\text{-}^2H_2]GA_{20}$, $[17\text{-}^2H_2]GA_{19}$, $[17\text{-}^2H_2]$-GA_1 (from Professor L. Mander, Australian National University, Canberra, Australia), and $[17\text{-}^{13}C_1{}^3H_2]GA_8$ and $[17\text{-}^{13}C_1,{}^3H_2]GA_{29}$ (gifts from Professor B.O. Phinney, University of California, Los Angeles, CA) were added in amounts based on the estimated levels of endogenous GAs. Combined HPLC fractions were converted to methyl ester trimethylsilyl ethers as described above and analyzed using a Hewlett-Packard 5890 gas chromatograph coupled to a 5970 Mass Selective Detector (Hewlett Packard, Winnersh, UK), as described in ref. 8. The concentrations of GA_1, GA_8, GA_{19}, GA_{20}, and GA_{29} were determined by reference to calibration curves. All experiments were repeated at least three times, with at least one repetition in either Long Ashton, Bristol, UK, or Corvallis, OR.

Transport of GAs

$[^2H,{}^3H]GA_1$, -GA_{19}, or -GA_{20} (1 μg of $[^2H]$GA, 2.9, 2.8, and 3.0 nmol, respectively, plus 10.6 kBq of GA_{19}, 18.0 kBq of GA_{20}, and 28.8 kBq of GA_1) were applied as drops in ethanol:water (3:7 v/v; 160 μL per plant) to *Na* donor leaves

on six *na/Na* grafts. After 3 d, the donor (*Na*) and receptor (*na*) were harvested, extracted, and purified as described above. The presence of the applied GAs and their metabolites were confirmed by GC-SIM. This experiment was done twice.

RESULTS AND DISCUSSION

GA Analysis in Ungrafted Plants

GA_1, GA_8, GA_{17}, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_8 catabolite, and GA_{29} catabolite were identified in both expanding and fully expanded tissues of C79-338 (*Na, Le*) from a comparison of their mass spectra and Kovats retention indices with those in a spectral library (7). Data for shoot apices are presented in Table I. These identifications, which support earlier work (4, 6, 19), confirm the importance of the 13-hydroxylation pathway in the biosynthesis of GA_1 in pea shoots.

Quantitative analysis of GAs in six nonisogenic lines varying at *Na* and *Le* showed that GA_1 content in expanding tissues of the *Na Le* lines was 4- to 20-fold greater than in the corresponding tissue from *Na le* lines (Table II). Ross et al. (26) also found 20-fold differences in GA_1 concentration in isogenic *le* and *Le* lines. Within each pair of nonisogenic *Na Le* or *Na le* lines, however, we found approximately 2-fold differences in GA_1 content, which correlated closely with plant height. For all genotypes, the correlation coefficient between log[GA_1] and plant height was 0.983 (Fig. 1).

The amount of GA_8 in expanding tissues was directly related to GA_1 content and height (r_{height} = 0.978), although differences in GA_8 levels between *Na Le* and *Na le* lines were much larger than for GA_1, 85-fold in the extreme case (Table II). The concentrations of the precursors, GA_{19} and GA_{20}, and of GA_{29} were very weakly correlated with shoot height. The correlation coefficients between log[GA] and height for these GAs were 0.636, 0.727, and 0.603, respectively. As expected from the position of the genetic block in the *le* genotypes, the amounts of GA_{20} and GA_{29} were higher in *Na le* than those in *Na Le*.

Table II. *GA Concentration in Expanding Tissues at Node 6 of Selected Pisum Genotypes*

GAs were quantified by GC-SIM using internal standards.

Line	GA_{19}	GA_{20}	GA_1	GA_8	GA_{29}
	ng·g⁻¹ fresh wt				
Na Le					
C79-338	2.9	37.0	10.5	57.8	21.0
I_3	0.4	20.9	4.2	29.7	15.1
Na le					
58	0.4	59.9	1.0	1.9	65.2
Progress No. 9	2.2	22.2	0.5	0.7	35.9
na Le					
B686-67-(3)	0.06	0.3	0.2	0.9	1.5
na le					
B780-687-(2)	0.04	0.2	0.1	0.2	0.4

GA_{20} was the most abundant precursor of GA_1 in expanding tissue, present in at least 10-fold higher amounts than GA_{19}. Although not quantified in absolute terms, earlier precursors in the 13-hydroxylation pathway were also present in considerably smaller concentrations than was GA_{20}. This contrasts with the case in monocotyledonous species in which levels of C_{20}-GAs, including GA_{19}, are substantially higher than those of GA_{20} (2, 3, 5). The effects of the *dwarf-1* allele of maize and *le* of pea on the content of C_{19}-GAs are similar, both resulting in the accumulation of GA_{20}, although the relative increase in *dwarf-1* (>10-fold) (5) is much greater than in *le*.

The *na* allele reduced markedly the contents of all GAs analyzed. In expanding tissues, GA_1 and GA_8 levels were reduced on average to approximately 2% in *Le* lines and approximately 15% in *le* genotypes. The concentrations of GA_{19}, GA_{20}, and GA_{29} were affected even more, averaging

Table I. *Identification of GAs in Shoot Apices of 20-d-Old Pea Seedlings of Line C79-338 by GC-MS*

GAs	HPLC Fractions	Kovats Retention Index	Characteristic Ions *m/z* (% Base Peak)[a]
GA_{29}	6-9	2694	506(100), 491(11), 447(5), 389(8), 375(9), 303(17), 207(23)
GA_8 catabolite	6-9	2710	606(84)[b], 591(26), 565(22), 487(44), 457(100), 397(50)
GA_8	6-9	2824	594(100), 579(7), 535(4), 448(11), 379(7), 375(7), 207(18)
GA_{29} catabolite	10-13	2634	518(100), 503(10), 477(68), 429(8), 399(10), 397(12), 309(10)
GA_1	10-13	2686	506(100), 491(10), 448(12), 377(11), 376(10), 313(5), 207(17)
GA_{20}	18-20	2516	418(100), 403(16), 375(40), 359(11), 301(13), 235(5), 207(19)
GA_{17}	21-24	2598	492(61), 460(12), 433(29), 432(24), 401(11), 373(12), 208(100)
GA_{19}	21-24	2624	462(6)[b], 434(100), 405(11), 402(18), 375(31), 374(53), 345(25)
GA_{44}	21-24	2821	432(100), 417(10), 373(15), 238(32), 207(58), 193(11)

[a] Based on ions above *m/z* 100. [b] Spectrum contaminated with extraneous ions, relative ion intensities refer only to relevant ions.

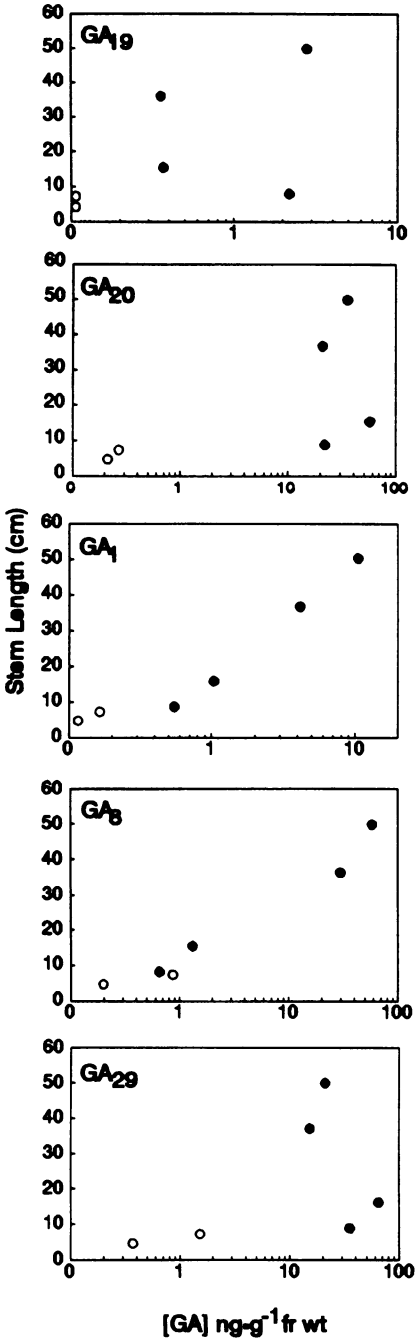

Figure 1. Relationship between GA concentration and stem length from nodes three to eight in six genetic lines of pea. ●, *Na*; O, *na*. fr wt, Fresh weight.

<4% of the corresponding levels in the *Na* genotypes. These effects of *na* in pea were similar to those reported for *dwarf-5* in maize (5). Clearly, these mutations are leaky and allow measurable GA production. Homeostasis of GA_1 levels is nevertheless insufficient to compensate for the general reduction of GA content.

GA concentrations in fully expanded leaves and stems were lower than those in expanding tissues (Table III). Levels of GA_{19} in *Na* were least affected, whereas GA_1 content declined nearly 20-fold. The amount of GA_1 was comparable in both *Na* and *na* lines, except in the tallest line, C79–338. As a result, GA_1 content of expanded tissue was not significantly correlated with plant height ($r = 0.772$). The concentration of GA_8, although reduced 5- to 10-fold compared with expanding tissues, was significantly correlated with plant height ($r = 0.983$). Amounts of GA_{20} and GA_{29} in expanded tissues were higher in *Na le* than in *Na Le* lines, whereas GA_{19} content was about equal in each genotype. In expanded tissues of the *na* lines, GA_{19} was not detected, whereas the other GAs were reduced to the same extent relative to expanding tissues as in *Na* genotypes. Reduced concentration of GAs in older nongrowing organs has been noted in other systems, such as in oilseed rape (8).

GA Content of *na*/*Na* Grafts

When grafted to *Na Le* or *Na le* rootstocks, both GA content of expanding tissue and stem length of the *na* scions increased compared with the ungrafted and self-grafted controls (Table IV, Fig. 2). In *na le* scions grafted to *Na* donors, the concentrations of GA_{20}, GA_1, GA_8, and GA_{29} were 9-, 13-, 15- and 7-fold higher, respectively, compared with the controls and were similar irrespective of whether line I_3 (*Na Le*) or 58 (*Na le*) was the donor. GA_{19} could not be detected in *na*, even after grafting. The concentrations of GA_1 and GA_8 were restored to within the range measured in ungrafted *Na le* lines (Table II). In contrast, GA_{20} and GA_{29} levels in grafted *na le* averaged about 7 and 13%, respectively, of the levels in *Na* compared with 0.7 and 1.7%, respectively, in ungrafted or self-grafted *na*. Thus, despite only partial restoration of the GA_{20} level, GA_1 concentration in grafted *na* was similar to that in *Na le*.

Unexpectedly, GA_1 levels in *na Le* receptors grafted to *Na* donors were not higher than in *na le* receptors. In several experiments, we measured no more than small differences in GA_1 content or scion growth between the two grafted genotypes. Although the amount of GA_1 in *na Le* receptors was less than expected, the proportion of GA_1 to GA_{20} in the grafted receptors of both *na Le* and *na le* equaled or was

Table III. *GA Concentration in Fully Expanded Leaves and Stems between Nodes 3 to 5 of Selected Pisum Genotypes*

GAs were quantified by GC-SIM using internal standards.

Line	GA_{19}	GA_{20}	GA_1	GA_8	GA_{29}
			$ng \cdot g^{-1}$ *fresh wt*		
Na Le					
C79-338	0.8	2.1	0.6	11.6	2.9
I_3	1.1	1.1	0.04	3.1	2.0
Na le					
58	0.3	7.0	0.06	0.5	15.1
Progress No. 9	0.3	2.8	0.02	0.3	12.9
na Le					
B686-67-(3)	—[a]	0.1	0.04	0.1	0.3
na le					
B780-687-(2)	—	0.1	0.02	0.06	0.2

[a] —, Not detected.

greater than in expanding tissues of *Na Le*. Thus, metabolic regulation of GAs in grafted *nana* appears to be altered. The relatively high GA_1 content of self-grafted *na Le* in this experiment was almost certainly due to laboratory contamination because GA_3 was also present in this sample. In other analyses of these tissues, GA_1 levels, in common with those of other GAs, were slightly lower than those in ungrafted *na*.

Stem lengths of *na* scions grafted to *Na* stocks fell between the values for *Na Le* and *Na le* (Table II), although GA_1 contents of these scions were characteristic of *Na le*. We were unable to distinguish the responses of *na Le* and *na le* grafted to *Na* as reported by Reid et al. (22).

Transport of [^{2}H,^{3}H]GAs

Application of labeled GA_{19}, GA_{20}, and GA_1 to the *Na* donor of *na/Na* grafts resulted in the expected metabolites in the donor, as identified on the basis of HPLC retention times and GC-SIM (Fig. 3). Furthermore, labeled GAs were detected in the *na* receptor, although translocation depended on the GA applied. Based on recovery of radioactivity before partitioning, about twice as much of the recovered ^{3}H was present in the receptor in GA_{20} feedings (7.8%) as in feedings of GA_{19} or GA_1 (3.7 and 4.8%, respectively). We estimated that about 54 pmol of GA_{19}, 87 pmol of GA_{20}, and 70 pmol of GA_1 equivalents were transported in the respective experiments. Furthermore, neither [^{3}H]- nor [^{2}H]GA_{19} were detected in receptors of plants to which labeled GA_{19} was applied. Instead, GA_{20} was the major product in these receptors, with lesser amounts of GA_1, GA_8, and GA_{29}. Likewise, when GA_{20} was applied to the donor, GA_{20} was the major transported metabolite, with lesser amounts of labeled GA_1, GA_8, and GA_{29} present in the receptor. Although transported somewhat less efficiently than GA_{20}, GA_1 was identified in receptors after application to the donor.

The results of the transport experiments were consistent with those of the GA analyses of grafted plants. Levels of endogenous GA_{20} were higher in *na* scions grafted to *Na* rootstocks than in the controls, but GA_{19} was essentially undetectable in all *na* tissues. This lack of GA_{19} in *na* grafted to *Na* could be due to the generally low content of this GA in expanded *Na* tissues, although in I_3, one of the genotypes grafted to *na*, the amounts of GA_{19} and GA_{20} were similar. If GA_{19} was similarly transported, we would have expected measurable GA_{19} in *nana*. Because peas do not accumulate GA_{19}, however, conversion to GA_{20} could reduce the amount of detectable labeled GA_{19} in the scion. Additionally, poor long-distance transport of GA_{19}, as indicated from the transport experiments, could explain, in part, the failure to detect GA_{19} in *na*/I_3 grafts.

As opposed to GA_{19}, there was detectable transport of GA_1

Figure 2. Comparison of *na Le* line (left) compared to *na Le* scion grafted onto *Na le* stock (right). Arrow indicates position of graft union.

Table IV. *GA Content and Stem Height of na Scions Grafted to Na Stocks*

All tissue above node 6, counting from the graft union, was collected for GA quantification by GC-SIM using internal standards. Growth data are the mean lengths between nodes 3 and 8 of eight randomly selected grafts.

Receptor	Donor	GA_{19}	GA_{20}	GA_1	GA_8	GA_{29}	Height ± SE[a]
				ng·g^{-1} fresh wt			*cm*
na Le	58 (*Nale*)	0.02	2.6	0.7	5.2	4.7	25.14 ± 1.93
	I_3 (*NaLe*)	—[b]	1.4	0.4	4.3	3.7	24.10 ± 1.12
	Self	—	0.1	0.2	0.2	0.3	8.16 ± 0.29
	None	—	0.5	0.07	0.5	1.1	6.04 ± 0.32
na le	58 (*Nale*)	—	2.7	1.0	6.0	4.7	24.65 ± 0.42
	I_3 (*NaLe*)	—	2.7	1.0	6.8	4.2	19.78 ± 0.39
	Self	—	0.2	0.02	—	0.5	3.71 ± 0.09
	None	—	0.3	0.09	0.4	0.5	3.89 ± 0.15

[a] Height of scion between leaves 1 and 6 and of ungrafted plants between nodes 3 and 8.
[b] —, Not detected.

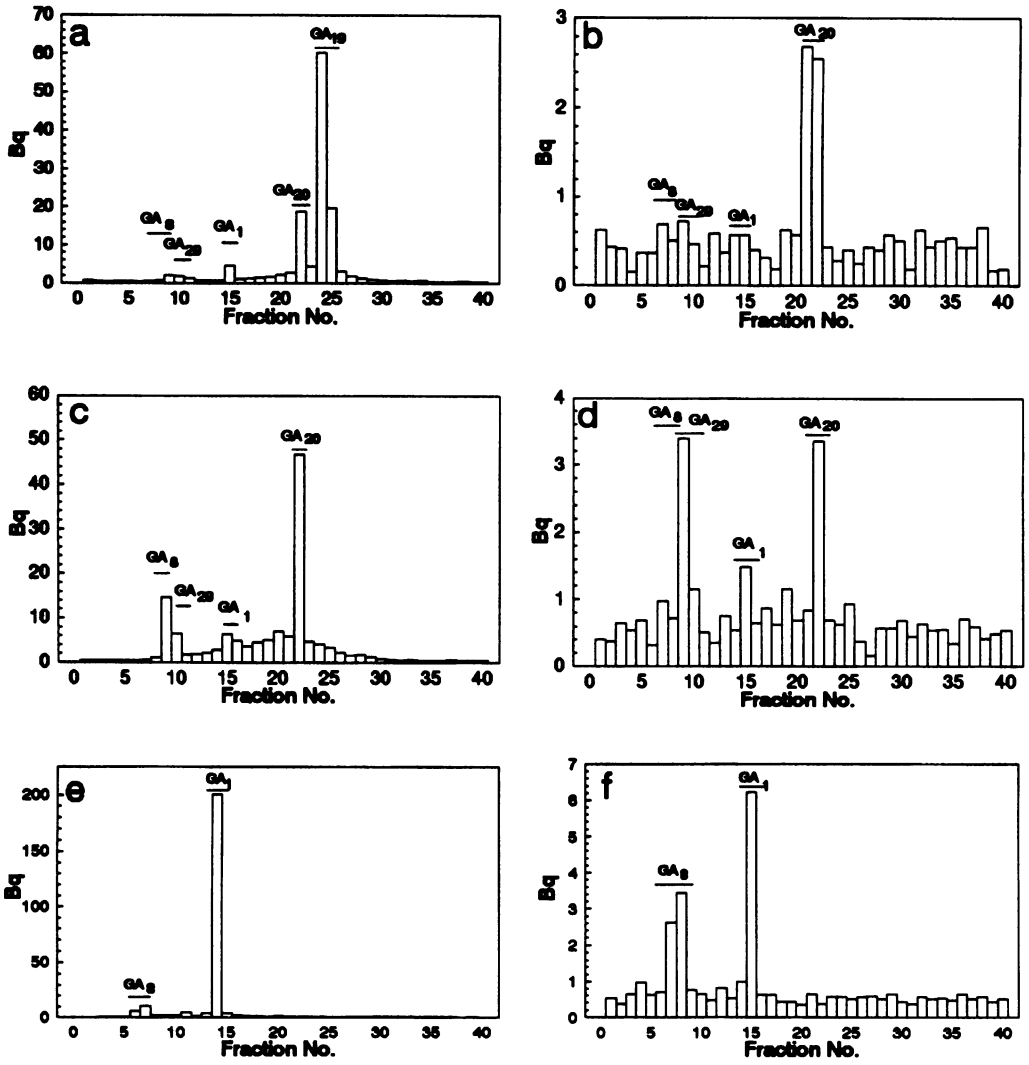

Figure 3. Analysis by HPLC of GAs recovered from *Na le* rootstocks (donor) and *na le* scions (receptor) after application of [^{2}H,^{3}H]GA_{19} (a and b), [^{2}H,^{3}H]GA_{20} (c and d), or [^{2}H,^{3}H]GA_1 (e and f) to the rootstocks. Donors (*Na*) are panels a, c, and e, and receptors (*na*) are panels b, d, and f. The presence of the applied GAs and their metabolites were confirmed by GC-SIM in the fractions indicated.

from donor to receptor. However, in view of the amounts transported, the small quantities of GA_1 in expanded tissues would be unlikely to exert a significant effect on the growth of the receptor, particularly when *Na le* was used as rootstock. Compared with other GAs in the 13-hydroxylation pathway, GA_{20} is present at relatively high abundance in mature tissues (Table III) and is transported at least as well as GA_1 and probably better than GA_{19}. It is likely, therefore, that GA_{20} is the major GA transported in pea.

On the basis of about 16% growth stimulation of *dwarf-1* maize grafted to normal plants, Katsumi et al. (13) assumed transport of GA_1 in that system. They also gave evidence for movement of applied GA_3 across the graft union. Although Klee and Estelle (14) suggested that these results imply that GA transport differs in pea and maize, responses in some *Na le/Na Le* grafts of peas, although similar to those in maize, were thought not to be significant (16, 17, 22). Therefore, there is still insufficient evidence to distinguish differences in GA transport in pea and maize.

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