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Endogenous Gibberellins in the Shoots of Normal- and Bush-Type *Cucumis sativus* L.

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Abstract. Endogenous gibberellins (GAs) in the shoots of normal- (cv. Yomaki, YO) and bush-type (cv. Spacemaster, SP) cultivars of cucumber (*Cucumis sativus* L.) grown under natural conditions were analyzed. From both YO and SP grown for 40 days, after sowing, a series of C-13-H GAs including GA_4, GA_9, GA_{15}, GA_{24}, GA_{25}, GA_{34}, and GA_{51} were identified by gas chromatography-mass spectrometry (GC-MS; full scan). In addition to the above GAs, GA_{12} and GA_{70} were similarly identified from both YO and SP grown for 61 days after sowing. The endogenous levels of GA_4 and GA_9, which are highly active in promoting cucumber hypocotyl elongation, were quantified by GC-selected ion monitoring (GC-SIM) using $[^2H_2]GA_4$ and $[^2H_4]GA_9$ as internal standards. No remarkable difference in terms of endogenous levels of $GA_{4/9}$ was observed between YO and SP in both growth stages (40 and 61 days after sowing).

There is strong evidence that gibberellin A_1 (GA_1) is the only endogenous GA active per se in the control of shoot elongation in *Zea mays* L. (maize), *Oryza sativa* L. (rice), and *Pisum sativum* L. (pea) (Phinney 1985). Indeed, GA_1 is highly active in bioassays using GA-deficient dwarf mutants of the above three species (Takahashi et al. 1986).

On the other hand, GA_1 shows low activity in the cucumber hypocotyl assay, in which C-13-H GAs such as GA_4, GA_9, and GA_{24} are highly active (Takahashi et al. 1986). This suggests that the active GA which controls cucumber shoot elongation is not GA_1, but one of the C-13-H GAs. However, no information on endogenous GAs in cucumber vegetative shoots has been reported.

We report here on the identification of endogenous GAs from the shoots of

Table 1. Shoot segment weights of *Cucumis sativus* L. used in extraction procedures (g fresh wt).

Plant materials	Qualitative analysis	Quantitative analysis
cv. Yomaki		
40-day-old	496	124
61-day-old	2590	590
cv. Spacemaster		
40-day-old	2360	1230
61-day-old	2330	1120

normal- (cv. Yomaki, YO) and bush-type (cv. Spacemaster, SP) cultivars of cucumber. The endogenous levels of identified GAs and the response of SP and YO to exogenously applied GAs are also noted.

Materials and Methods

Plant Materials

Two cultivars of *Cucumis sativus* L. cv. YO (normal-type) and cv. SP (bush-type) were used. The seeds were imbibed in running water overnight and then sowed in a field in Wako-shi, Saitama, Japan, on April 26, 1986, and grown under natural conditions. Forty and 61 days after sowing, the plants were harvested, and the hypocotyls, leaves, and flower buds were cut off to obtain shoot segments. The weight of the shoot segments is shown in Table 1. The shoot segments were stored in methanol (MeOH) at 4°C for several days before extraction.

Qualitative Analysis

Extraction and Purification

Plant material of both 40-day-old YO and SP was homogenized and extracted three times with MeOH (3 L) and fractionated by the usual method (Yamane et al. 1979) to give acidic ethyl acetate (AE) fractions. Each AE fraction was purified by silica gel partition chromatography. The AE fraction adsorbed on Celite (for YO, 1.5 g; for SP, 3.0 g) was placed on a column of silica gel (100 mesh, Kanto chemicals, Japan; for YO, 12 g; for SP, 24 g) impregnated with 0.5 N aqueous formic acid (for YO, 7.5 ml; for SP, 15 ml). The column was eluted in stepwise fractions (for YO, 30 ml; for SP, 60 ml) using an increasing percentage of ethyl acetate (EtOAc) in *n*-hexane by either 10% (0%–30% and 70%–100% EtOAc) or 5% (30%–70% EtOAc). The eluates with 10%–50% EtOAc were combined and labeled as the lower polarity (LP) fraction, and the eluates with 55%-100% EtOAc were combined and labeled as the higher polarity (HP) fraction. Each fraction from the silica gel column was dissolved in 1 ml of 80% aqueous MeOH, loaded onto a Sep-Pak (ODS) cartridge, and eluted three times with 2 ml of 80% aqueous MeOH. The eluates were combined,

evaporated to dryness in vacuo, and subjected to high-performance liquid chromatography (HPLC).

The shoot segments of 61-day-old YO and SP were homogenized, extracted, and fractionated as described above to give AE fractions. The AE fractions were purified with some modifications as follows. Each AE fraction was dissolved in 150 ml of 0.1 M phosphate buffer (pH 8.0), and mixed with 15 g of insoluble polyvinylpyrrolidone (PVP) (Tokyo kasei kogyo Co., Japan) while stirring. PVP was filtered and washed three times with the buffer (150 ml each wash). The filtrate and the washings were combined, adjusted to pH 3.0, and extracted three times with EtOAc (200 ml). The EtOAc fractions were combined, dried over anhydrous sodium sulfate, and evaporated to dryness in vacuo. The extract was treated with a Sep-Pak (ODS) cartridge as described above. The evaporated Sep-Pak eluate was redissolved in 1 ml of MeOH and charged onto a column of Sepralyte (diethylaminopropyl, DEA; 5–6 g; Analytichem International). The column was eluted in 100–120 ml fractions with MeOH, and 0.5% acetic acid (HOAc) in MeOH, in this order. The fraction eluted with MeOH was evaporated to dryness in vacuo, redissolved in 1 ml of MeOH, and charged onto another column of Sepralyte (DEA) (3–3.5 g). The column was eluted in 60–70 ml fractions with MeOH, and 0.5% HOAc in MeOH. Eluates with 0.5% HOAc in MeOH from the first and second runs were combined, evaporated to dryness in vacuo, and subjected to HPLC.

HPLC

A Senshu-Pak ODS 4253D column (25 cm × 10 mm in diameter) was used. The solvents used were MeOH and 1% aqueous HOAc. Each sample was dissolved in 400 μl of 30% MeOH in 1% aqueous HOAc and injected onto the HPLC column. Two minutes after injection onto the column, a 28-min linear gradient (30%–100% MeOH) was applied. Isocratic elution was continued for 20 min at the end of the program. The flow rate was 3 ml/min, and fractions were collected every 1 min.

Dwarf Rice Bioassay

The HPLC fractions were bioassayed according to the dwarf rice (*Oryza sativa* L. cv. Tan-ginbozu) micro-drop method (Murakami 1968) at 30°C under continuous light (fluorescence lamps, approximately 3000 lux). Each plant was treated with an aliquot of an HPLC fraction equivalent to an extract from 8 g fresh wt material.

Radioimmunoassay (RIA)

An aliquot of each HPLC fraction was methylated with ethereal diazomethane and used for the assay. The assay was done in duplicate at a concentration of 10 g fresh wt equivalent per assay tube for each sample using anti-GA_1- and anti-GA_{20}-antisera according to the method of Yamaguchi et al. (1987).

Gas Chromatography-Mass Spectrometry (GC-MS)

A JEOL DX 303 GC-MS system was used, fitted with a fused silica capillary column DB-1 (15 m × 0.258 mm in diameter, J & W Scientific Inc., CA, USA). Each sample was methylated with ethereal diazomethane, and trimethylsilylated with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) at 80°C for 30 min. One microliter of the MSTFA solution was injected into the column. Head pressure of He-carrier gas was 64 kPa.

Quantitative Analysis

Internal Standards

Deuterated GA_9 was prepared according to the methods reported by Beale et al. (1980) and Duri et al. (1981). Thin layer chromatography (TLC) was conducted using Silica gel 60 (Merck).

3-keto-GA_4 methyl ester (3-keto-GA_4-Me) (250 mg) was dissolved in tetrahydrofuran (4 ml). $NaBD_4$ (50 ml) was added to the solution and kept for 5 h at room temperature with stirring. After acidification with D_2SO_4, H_2O (10 ml) was added to the solution, and then extracted with EtOAc. The extract was concentrated and subjected to preparative TLC ($CHCl_3$-EtOAc-*n*-hexane-HOAc, 6:6:9:1, vol/vol/vol/vol) to give 3-[^2H]GA_4-Me (19 mg) and 3-[^2H]3-*epi*-GA_4-Me (104 mg). Both products were combined (total weight, 123 mg) and refluxed with MeONa in MeOD (1.3 N, 13 ml) for 3 h to introduce two deuteriums to C-2, and one to C-6. The solution was acidified with D_2SO_4 and diluted with water. After evaporating MeOH, the solution was extracted with EtOAc. Evaporation of the solvent and separation of the residual gum (118 mg) by TLC afforded 2,2,3,6-[$^2H_4$]GA_4-Me (12 mg) and its 3-epimer (51 mg).

The latter product was dissolved in CCl_4 (2 ml) with triphenylphosphin (66 mg), and the solution was refluxed for 3 h. The product was purified by TLC ($CHCl_3$) to give 2,2,3,6-[$^2H_4$]3-chloro-GA_9-Me (12 mg). The product was dissolved in benzene (8 ml), to which *n*-Bu_3SnH (110 μl) and α,α-azobisisobutylonitrile (small quantity) were added. The solution was refluxed under an N_2 stream for 1 h and then diluted with EtOAc, washed with H_2O, and concentrated. Purification by TLC (*n*-hexane-EtOAc, 4:1, vol/vol) and recrystallization from an acetone-H_2O solution afforded 2,2,3,6-[$^2H_4$]GA_9-Me (8 mg).

2,2,3,6-[$^2H_4$]GA_9-Me (8 mg) was dissolved in 1 ml of 2 N NaOD (in D_2O-MeOD, 1:1, vol/vol). The solution was heated in a reaction vial at 70°C for 4 h. MeOD was evaporated under an N_2 stream, and the residual solution was acidified with D_2SO_4. Extraction with EtOAc and purification by TLC (*n*-hexane-EtOAc-HOAc, 10:4:1, vol/vol/vol) followed by crystallization gave 2 mg of 2,2,3,6-[$^2H_4$]GA_9.

The [$^2H_4$]GA_9 was methylated with diazomethane and its mass spectrum was determined. The relative intensity of the molecular ions was as follows: m/z (%) 334 ([$^2H_4$], 100); 333 ([$^2H_3$], 12); 332 ([$^2H_2$], 3); 331 ([$^2H_1$], 3); 330 ([$^2H_0$], <1).

The deuterated GA_9 was stable enough to be used as an internal standard in

the GC-SIM analysis as there was no deuterium exchange throughout the purification procedures performed by us.

$[^2H_2]GA_4$ was supplied by Dr. L. N. Mander (Australian National University).

Extraction and Purification

After the addition of the internal standards (250 ng $[^2H_2]GA_4$ to 40-day-old YO, 500 ng to 40-day-old SP, 50 ng to 61-day-old YO and SP; 250 ng $[^2H_4]GA_9$ to 40-day-old YO, 300 ng to 40-day-old SP, 200 ng to 61-day-old YO and SP), each sample of plant material was extracted and purified by the method applied to 61-day-old YO and SP in the qualitative analysis section.

GC-Selected Ion Monitoring (GC-SIM)

The pooled HPLC fractions with retention times of 26–27 min and 28–29 min were analyzed by GC-SIM for quantitation of GA_4 and GA_9, respectively. A Hewlett-Packard (HP)-5890 gas chromatograph connected with a HP-5970 mass selective detector, fitted with a fused-silica chemically bonded capillary column DB-1 (15 m $\times$ 0.258 mm in diameter) was used. Each sample was methylated and trimethylsilylated as described in the section on GC-MS. One microliter of the MSTFA solution of the derivatized sample was injected onto the column at 120°C in splitless mode. Two minutes after injection, the column temperature was programmed at 16°C/min to 280°C/min with a 10-min isothermal hold at 280°C. Head pressure of the He-carrier gas was 40 kPa. The following ions were selected: m/z 418, 403, and 328 for GA_4-Me-TMS; m/z 420, 405, and 330 for $[^2H_2]GA_4$-Me-TMS; m/z 330 and 298 for GA_9-Me; and m/z 334 and 302 for $[^2H_4]GA_9$-Me. For quantitation, the ions at m/z 420, 418, 302, and 298 for $[^2H_2]GA_4$-Me-TMS, GA_4-Me-TMS, $[^2H_4]GA_9$-Me, and GA_9-Me, respectively, were used.

Cucumber Hypocotyl Assay

Cucumis sativus L. cv. YO and cv. SP were used. The assay was carried out under continuous white light (approximately 20,000 lux) at 25°C, according to the method described by Katsumi et al. (1965) with some modifications. Instead of measuring the 20-mm long hypocotyl unit, the 10-mm long hypocotyl unit was measured. GAs were applied in 10 μl of 50% aqueous acetone solution.

Results and Discussion

The AE fraction from 40-day-old YO was purified with silica gel partition chromatography to give LP and HP fractions. The LP fraction, treated with a Sep-

Table 2. GAs identified by GC-MS analyses of their Me or MeTMSi derivatives in 40-day-old shoots of *Cucumis sativus* L.

Origin	Rt on HPLC (min)	Identified GAs	Derivatization in GC-MS	Rt on GC (min and s)	Principal ions and relative abundance (% base peak)
GA_4	—	—	Me	11 25[a]	346 (M^+,5), 314 (99), 284 (83), 268 (26), 224 (100)
GA_9	—	—	Me	9 49[b]	330 (M^+,20), 298 (100), 270 (80), 243 (50), 226 (39)
				6 45[c]	
GA_{15}	—	—	Me	14 17[b]	344 (M^+,25), 312 (20), 298 (11), 284 (65), 239 (100)
				10 44[c]	
GA_{24}	—	—	Me	8 36[c]	374 (M^+,5), 342 (35), 314 (100), 286 (54), 225 (84)
				8 50[d]	
GA_{25}	—	—	Me	8 35[c]	404 (M^+,12), 372 (40), 312 (86), 284 (100), 225 (84)
				8 48[d]	
GA_{34}	—	—	MeTMSi	14 48[b]	506 (M^+,100), 431 (22), 416 (5), 313 (7), 288 (9)
GA_{51}	—	—	MeTMSi	12 21[b]	418 (M^+,8), 386 (28), 328 (32), 284 (100), 268 (65)
Yomaki	(24–25)	GA_{34}	MeTMSi	14 50[b]	506 (M^+,100), 431 (1), 416 (6), 313 (7), 288 (9)
		GA_{51}	MeTMSi	12 20[b]	418 (M^+,10), 386 (42), 328 (35), 284 (100), 268 (73)
	(26–27)	GA_4	Me	11 22[a]	346 (M^+,8), 314 (84), 284 (70), 268 (58), 224 (100)
	(28–29)	GA_9	Me	6 40[c]	330 (M^+,trace) 298 (100), 270 (65), 243 (42), 226 (23)
		GA_{15}	Me	10 42[c]	344 (M^+,35), 312 (29), 298 (17), 284 (50), 239 (100)
		GA_{24}	Me	8 40[c]	374 (M^+,9), 342 (58), 314 (100), 286 (91), 225 (48)
		GA_{25}	Me	8 37[c]	404 (M^+,3), 372 (54), 312 (74), 284 (100), 225 (81)
Spacemaster	(24–25)	GA_{34}	MeTMSi	14 54[b]	506 (M^+,100), 431 (4), 416 (6), 313 (8), 288 (5)
		GA_{51}	MeTMSi	12 23[b]	418 (M^+,13), 386 (35), 328 (30), 284 (100), 268 (70)
	(26–27)	GA_4	Me	11 25[a]	346 (M^+,9), 314 (77), 284 (57), 268 (22), 224 (100)
	(28–29)	GA_9	Me	9 49[b]	330 (M^+,12), 298 (100), 270 (81), 243 (55), 226 (60)
		GA_{15}	Me	14 17[b]	344 (M^+,33), 312 (24), 298 (17), 284 (66), 239 (100)
		GA_{24}	Me	8 50[d]	374 (M^+,21), 342 (37), 314 (100), 286 (75), 225 (72)
		GA_{25}	Me	8 47[d]	404 (M^+,8), 372 (51), 312 (68), 284 (100), 225 (51)

The programs of column temperature after injectin were as follows:
[a] 120°C (2 min), to 210°C at 16°C/min, to 280°C at 8°C/min and 280°C/min (10 min).
[b] 120°C (2 min), to 216°C at 16°C/min, 216°C (5 min), to 280°C at 8°C/min and 280°C/min (10 min).
[c] 120°C (2 min), to 208°C at 8°C/min, 208°C (2 min), to 280°C at 8°C/min and 280°C/min (10 min).
[d] 190°C (2 min), to 210°C at 4°C/min, to 280°C at 4°C/min and 280°C/min (10 min).

Table 3. *GAs identified by GC-MS analyses of their Me or MeTMSi derivatives in 61-day-old shoots of Cucumis sativus L.*

Origin	Rt on HPLC (min)	Identified GAs	Derivatization in GC-MS	Rt on GC (min and s)	Principal ions and relative abundance (% base peak)
GA_4	—	—	MeTMSi	14 10	418 (M^+,40), 400 (9), 386 (15), 328 (32), 294 (100)
				14 44[a]	418 (M^+,13), 400 (11), 386 (18), 328 (37), 284 (100)
GA_9	—	—	Me	10 55	(see *Table 1*)
GA_{12}	—	—	Me	11 16	360 (M^+,5), 328 (40), 300 (100), 285 (31), 240 (17)
GA_{15}	—	—	Me	15 46	(see *Table 1*)
GA_{24}	—	—	Me	13 10	(see *Table 1*)
GA_{25}	—	—	Me	13 07	(see *Table 1*)
GA_{34}	—	—	MeTMSi	16 35	(see *Table 1*)
GA_{51}	—	—	MeTMSi	14 34	(see *Table 1*)
GA_{70}	—	—	MeTMSi	14 55	418 (M^+,40), 386 (22), 328 (63), 298 (90), 268 (100)
Yomaki	(19–20)	GA_{70}	MeTMSi	14 54	418 (M^+,36), 386 (11), 328 (40), 296 (70), 268 (100)
	(23–24)	GA_{34}	MeTMSi	16 36	506 (M^+,100), 431 (2), 416 (3), 313 (13), 288 (11)
		GA_{51}	MeTMSi	14 32	418 (M^+,3), 386 (20), 328 (20), 284 (100), 268 (54)
	(25–26)	GA_4	MeTMSi	14 44[a]	418 (M^+,43), 400 (13), 386 (29), 328 (43), 284 (100)
	(27–28)	GA_{24}	Me	13 12	374 (M^+,5), 342 (44), 314 (100), 286 (100), 225 (74)
	(28–29)	GA_9	Me	10 57	330 (M^+,15), 298 (100), 270 (100), 243 (65), 226 (83)
		GA_{15}	Me	15 46	344 (M^+,25), 312 (20), 298 (13), 284 (50), 239 (100)
		GA_{25}	Me	13 07	404 (M^+,trace), 372 (40), 312 (70), 284 (100), 225 (74)
	(29–32)	GA_{12}	Me	11 19	360 (M^+,2), 328 (27), 300 (100), 285 (20), 240 (26)
Spacemaster	(17–18)	GA_{70}	MeTMSi	14 58	418 (M^+,30), 386 (20), 328 (50), 296 (100), 268 (100)
	(21–22)	GA_{34}	MeTMSi	16 38	506 (M^+,100), 431 (1), 416 (5), 313 (5), 288 (13)
		GA_{51}	MeTMSi	14 33	418 (M^+,10), 386 (30), 328 (25), 284 (100), 268 (65)
	(24–25)	GA_4	MeTMSi	14 11	418 (M^+,33), 400 (20), 386 (22), 328 (28), 284 (100)
	(26–27)	GA_{24}	Me	13 10	374 (M^+,10), 342 (57), 314 (100), 286 (88), 225 (100)
	(27–28)	GA_9	Me	10 57	330 (M^+,10), 298 (100), 270 (80), 243 (46), 226 (61)
		GA_{15}	Me	15 46	344 (M^+,37), 312 (25), 298 (11), 284 (53), 239 (100)
		GA_{25}	Me	13 08	404 (M^+,2), 372 (40), 312 (70), 284 (100), 225 (43)
	(30–31)	GA_{12}	Me	11 15	360 (M^+,3), 328 (30), 300 (100), 285 (20), 240 (24)

The programs of column temperature used above were ([b]) from *Table 1*.
[a] The derivatized sample was analyzed by GC-SIM.

Pak (ODS) cartridge, and then purified by ODS-HPLC, was estimated by the dwarf rice bioassay and RIA. The GA active fractions were analyzed by capillary GC-MS after derivatization. Gibberellin A_4, GA_9, GA_{15}, GA_{24}, GA_{25}, GA_{34}, and GA_{51} were identified by the comparison of their retention times (Rt) and mass spectra (full scan) of their Me-TMS or Me derivatives with those of authentic specimens (Table 2). The HP fraction was purified as above and assayed. However, no significant GA activities were detected.

The AE fraction from 40-day-old SP was purified and analyzed by capillary GC-MS, as in the case of 40-day-old YO, resulting in identification of the same GAs as those found in 40-day-old YO (Table 2).

The AE fractions from 61-day-old YO and SP were purified by PVP, Sep-Pak (ODS), and Sepralyte (DEA) columns prior to ODS-HPLC. The three prepurification steps for HPLC were quite effective in purifying nonconjugated GAs. GA activity in each of the HPLC fractions obtained from 61-day-old YO and SP was estimated by the dwarf rice bioassay and RIA, and the GA-active fractions were analyzed by capillary GC-MS after derivatization. Thus, GA_4, GA_9, GA_{12}, GA_{15}, GA_{24}, GA_{25}, GA_{34}, GA_{51}, and GA_{70} were identified by capillary GC-MS from both 61-day-old YO and SP (Table 3). Based on the current knowledge of GA biosynthesis, hypothetical biosynthetic relationships of GAs identified from YO and SP are shown in Fig. 1.

It has been thought that a precursor of GA_4 might be GA_{36} in immature seeds of a *Cucurbitaceae* (Graebe et al. 1980). However, formation of GA_4 from GA_9 is more likely in cucumber shoots because of the co-occurrence of GA_4 and GA_9 in these shoots, and the conversion of GA_9 to GA_4 observed previously in cell-free systems from seeds of *Cucurbita maxima* L. (Graebe et al. 1980) and *Phaseolus vulgaris* (Takahashi et al. 1986).

With respect to the biosynthesis of GA_{70}, one possible precursor of GA_{70} is GA_9, because GA_9-Me was metabolized to GA_{70}-Me, as well as GA_{69}-Me in the fern, *Lygodium japonicum* (Sato et al. 1985). Another possible precursor of GA_{70} is 12α-hydroxy-GA_{12}-aldehyde on the basis of the fact that GA_{12}-aldehyde was metabolized to 12α-hydroxy-GAs via 12α-hydroxy-GA_{12}-aldehyde in a cell-free system from seeds of *C. maxima* (Graebe et al. 1980), although GA_{70} has not been identified as a metabolite of 12α-hydroxy-GA_{12}-aldehyde.

In vegetative shoots of maize, a series of C-13-OH GAs have been identified, showing the existence of the early 13-hydroxylation GA biosynthetic pathway leading to the active GA, GA_1 (Phinney 1985). The structures of the GAs identified from *C. sativus* shoots showed a quite good correspondence to those of the GAs from maize shoots except for the lack of the 13-hydroxyl. Considering that introduction of 3β-hydroxyl into GA_{20} is the final step in the production of active GA (GA_1) in maize, introduction of 3β-hydroxyl into GA_9 may be the final step in the production of active GA in *C. sativus*. Accordingly, GA_4 might be the active GA per se for the control of shoot elongation in cucumber. The high activity of GA_9 and GA_4 and rather low activity of GA_1 on hypocotyl elongation (as shown in Fig. 2) support the above hypothesis.

SP is a bush-type of cucumber, while YO is an ordinary vine-type. Although the shoots of SP were longer than those of YO 40 days after sowing; the bush character of SP was observed 61 days after sowing, when the shoot lengths of 61-day-old YO were almost twice as long as those of 61-day-old SP. According

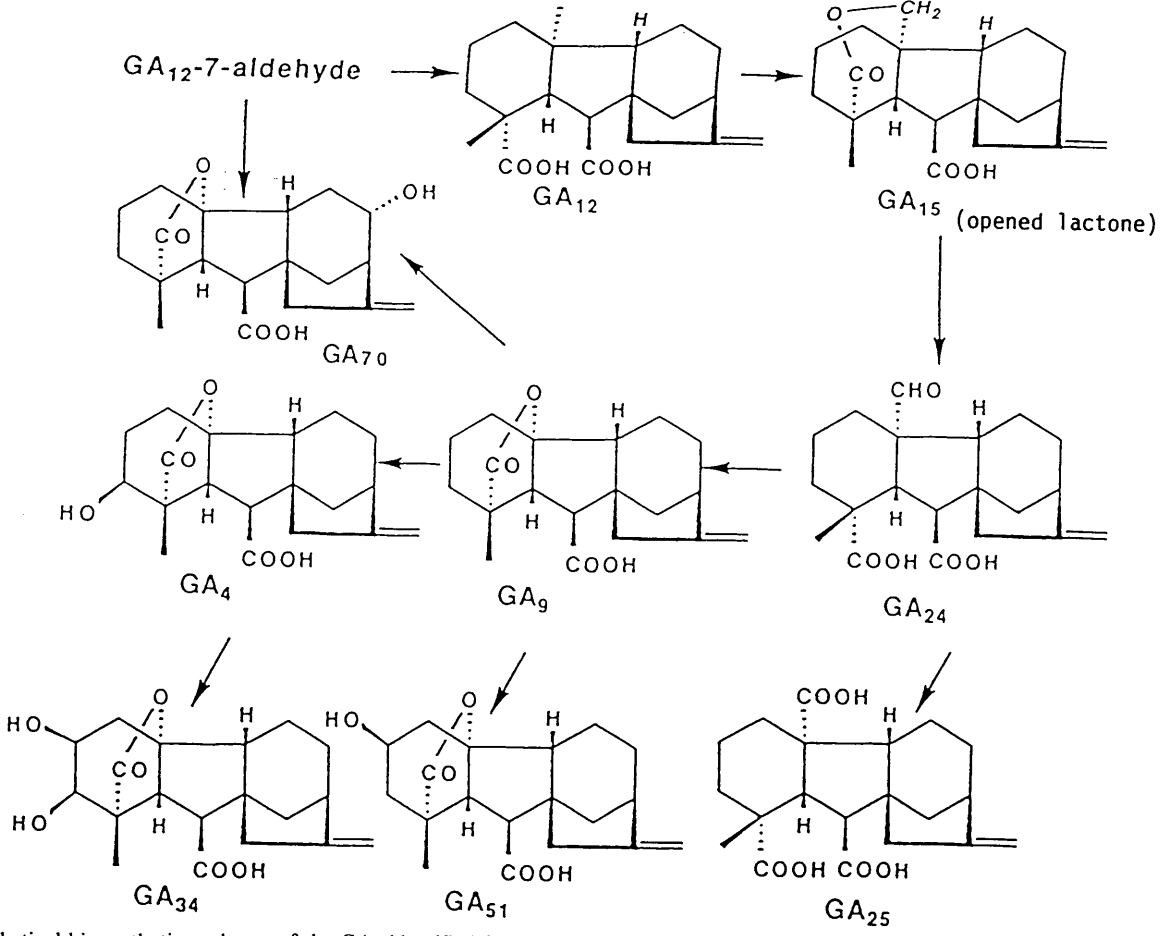
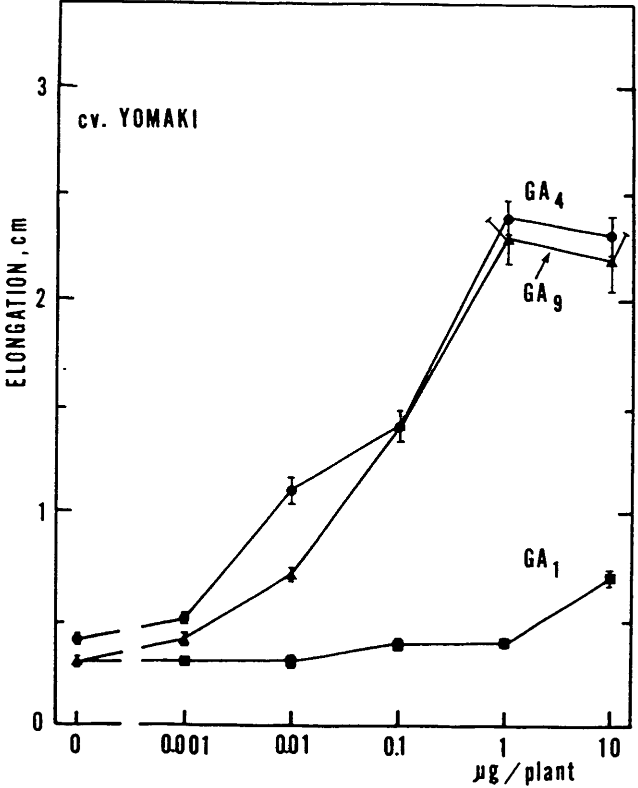


Fig. 1. Hypothetical biosynthetic pathway of the GAs identified from vegetative shoots of *Cucumis sativus* L. cv. Yomaki (YO) and cv. Spacemaster (SP).



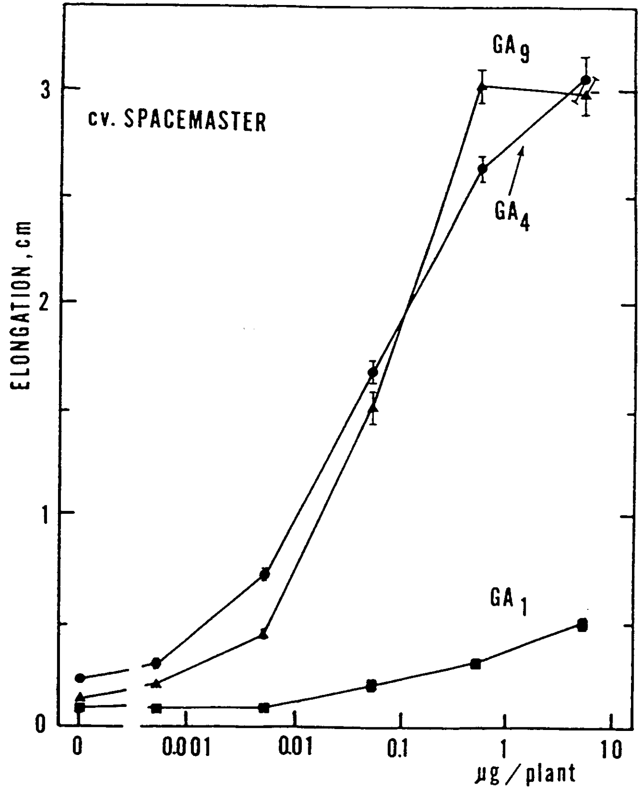


Fig. 2. Responses of YO and SP at young stage to GA_1, GA_4, and GA_9.

Table 4. Contents of endogenous GA_4 and GA_9 in the shoots of *Cucumis sativus* L. cv. Yomaki and cv. Spacemaster (ng/kg fresh wt).

Plant materials	GA_4	GA_9
cv. Yomaki		
40-day-old	270	860
61-day-old	110	160
cv. Spacemaster		
40-day-old	360	420
61-day-old	90	125

to quantitation by GC-SIM using $[^2H_2]GA_4$ and $[^2H_4]GA_9$ as internal standards, no remarkable difference in the endogenous level of GA_4 or GA_9 was observed between YO and SP at both growth stages (40 and 61 days after sowing) as shown in Table 4. It should be noted that the two cucumber cultivars showing quite different growth patterns contained the same GAs at the similar endogenous levels at two different growth stages.

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References

Beale MH, Gaskin P, Kirkwood PS, MacMillan J (1980) Partial synthesis of gibberellin A_9 and [3α- and 3β-$^2H_1$]gibberellin A_9; gibberellin A_5 and [1β,3-$^2H_2$ and $^3H_2$]gibberellin A_5; and gibberellin A_{20} and [1β,3α-$^2H_2$ and -$^3H_2$]gibberellin A_{20}. J Chem Soc Perkin Trans 1:885

Duri ZJ, Fraga BM, Hanson JR (1981) Preparation of gibberellins A_9 and A_{20} from gibberellic acid. J Chem Soc Perkin Trans 1:161

Graebe JE, Hedden P, Rademacher W (1980) Gibberellin biosynthesis. Monograph 5-1980 gibberellins. British Plant Growth Regulator Group, p 31

Katsumi M, Phinney BO, Purves WK (1965) The roles of gibberellin and auxin in cucumber. Physiol Plant 18:426

Murakami Y (1968) A new rice seedling test for gibberellins, microdrop method, and its use for testing extracts of rice and morning glory. Bot Mag 81:33

Phinney BO (1985) Gibberellin A_1 dwarfism and shoot elongation in higher plants. Biol Plant 27:172

Sato Y, Yamane H, Kobayashi M, Yamaguchi I, Takahashi N (1985) Metabolism of GA_9 methyl ester in a culture of prothallia of *Lygodium japonicum*. Agric Biol Chem 49:255

Takahashi M, Kamiya Y, Takahashi N, Graebe JE (1986) Metabolism of gibberellins in a cell-free system from immature seeds of *Phaseolus vulgaris* L. Planta 168:190

Takahashi N, Yamaguchi I, Yamane H (1986) Chemistry of plant hormones. CRC Press Inc., Florida, p 136

Yamaguchi I, Nakagawa R, Kurogochi S, Murofushi N, Takahashi N, Weiler EW (1987) Radioimmunoassay of gibberellins A_5 and A_{20}. Plant Cell Physiol 28:815

Yamane H, Takahashi N, Takeno K, Furuya M (1979) Identification of gibberellin A_9 methyl ester as a natural substance regulating formation of reproductive organs in *Lygodium japonicum*. Planta 147:251