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ENDOGENOUS GIBBERELLINS IN FOLIAGE OF TOMATO (*LYCOPERSICON ESCULENTUM*)

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; gibberellins; identification; isotope-dilution analysis; soil solarisation.

Abstract—Endogenous gibberellins (GAs) from the leaves and shoot tips of 13–14 days old tomato transplants (*Lycopersicon esculentum* Mill.) grown in solarised or non-solarised soil were analysed by GC-mass spectrometry. Full-scan mass spectra and Kovats retention indices, revealed in plants from both soils the presence of GA_1, GA_3, GA_3-isolactone, GA_4, GA_8,GA_{19}, GA_{20}, GA_{29}, GA_{34} and GA_{51}. Further analyses by GC-SIM indicated the presence of GA_{44} and GA_{53} in the tomato foliage. An isotope-dilution analysis revealed that most of the GA_3-isolactone was a methodological artefact. Our results provide the first conclusive identification of most members of the early-13-hydroxylation biosynthetic pathway in vegetative tomato tissues. In addition, the presence of GA_4, GA_{34} and GA_{51} which were not reported previously in the tomato, indicates that the early-non-3,13-hydroxylation biosynthetic pathway is also active. Three potentially active GAs: GA_1, GA_3 and GA_4, were identified in tomato foliage, all of which may play an important role in regulating growth in this species. © 1997 Elsevier Science Ltd

INTRODUCTION

Over 100 gibberellins (GAs) are known, but only the 3β-hydroxylated C_{19}-GAs, GA_1, GA_3, GA_4 and GA_7, are potentially active *per se* in plant tissues [1]. Shoot elongation is controlled by GA_1 in a number of plant species [1, 2], and by GA_4 in *Arabidopsis* and cucumber [3, 4].

The following GAs have been identified in developing fruits and seeds of the tomato (*Lycopersicon esculentum* Mill.): GA_1, GA_8, GA_{17}, GA_{19}, GA_{20}, GA_{29} and GA_{44}, which evolve in the early-13-hydroxylation biosynthetic pathway, and GA_9, GA_{15}, GA_{24} and GA_{25}, which evolve in the early-non-3,13-hydroxylation pathway [5, 6]. The presence of GA_{12} and GA_{53} has also been suggested (unpublished cited in [7]). GA_1 has been proposed to control tomato fruit growth [6], possibly in combination with other hormonal factors [5].

Little is known about the GA biosynthetic pathways in vegetative tissues of the tomato [8], probably because of their low concentrations [7]. To date, only GA_1, GA_{19} and GA_{20} have been identified in tomato shoots [9–11], and GA_1 and GA_3 in tomato root cultures [12].

Soil solarisation is a disinfestation technique that frequently enhances plant growth, even in the absence of known pathogens. This effect is attributed to both biotic and abiotic soil factors [13, 14]. It was suggested that environmental factors can induce the production of GAs, which are not commonly present in untreated plants [15]. Hence, the increased growth in solarised *vs.* non-solarised soils may be promoted by qualitative differences in active GAs. We therefore studied the endogenous GAs in young leaves of tomato plants in solarised and non-solarised soils.

RESULTS AND DISCUSSION

Vegetative tissues contain low concentrations of endogenous GAs, sometimes 10^3 times less than seeds [16]. We studied GAs in tomato seedlings at their fast-developing stage, grown in either solarised or non-solarised soil. The purified extracts of pooled true leaves and shoot tips from 13–14 days old transplants were fractionated by C_{18} reverse-phase HPLC and the fractions were grouped according to their activity as

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revealed by the dwarf-rice bioassay (data not shown). GAs and related compounds were then identified by comparing their characteristic full-scan mass spectra and Kovats retention indices (KRIs) with those of standards and with published data [17]. In foliage extracts of both soil treatments, the following GAs were identified: GA_1, GA_3, GA_3-isolactone (iso-GA_3), GA_4, GA_8, GA_{19}, GA_{20}, GA_{29}, GA_{34} and GA_{51} (Table 1; shown for the solarisation treatment only). Additionally, GC-SIM analyses provided evidence for GA_{44} and GA_{53} in all plants. These findings provided the first conclusive identification of most members of the early-13-hydroxylation pathway in vegetative tomato tissues (Scheme 1). These results are in agreement with reports on GAs in tomato fruits and seeds [5, 6]. Moreover, GA_3 and iso-GA_3 which may derive from GA_{20}, were also identified. However, we did not detect GA_5, which is known as the intermediate between GA_{20} and GA_3 in maize [18].

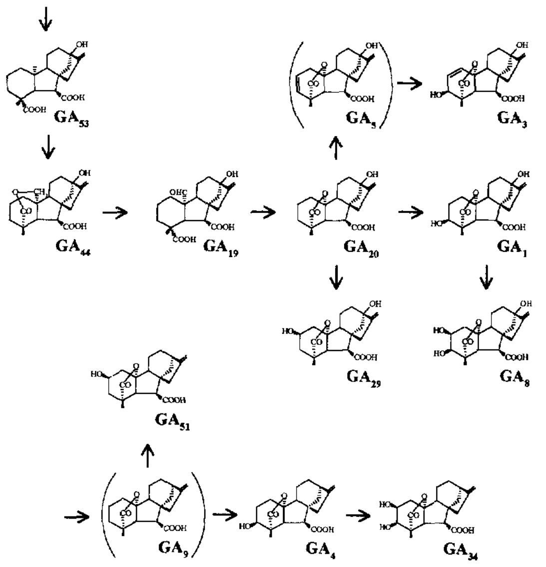
The iso-GA_3 may either occur naturally in the tested tissues or be formed artificially by isomerization of GA_3 in neutral or weak alkaline aqueous solutions [19, 20], or during derivatization and the GC-mass spectrometric run. The latter possibility was tested by GC-mass spectral analysis of pure GA_3, and resulted in partial conversion of GA_3 to iso-GA_3. In order to pursue the natural or artefactual origin of iso-GA_3 in the tested tomato tissues, an isotope-dilution analysis

Table 1. Kovats retention indices (KRIs) and relative intensities of characteristic ions for MeTMSi derivatives of GAs in tomato foliage from the solarisation treatment and of standard GAs and published data [17]

GA	Source	KRI	Ion m/z (relative intensity)								HPLC fraction
GA_1	Sample	2673	506 (100)	491 (3)	448 (13)	377 (27)	313 (52)	235 (20)	207 (86)		4–8
	Standard	2672	506 (100)	491 (9)	448 (20)	377 (22)	313 (20)	235 (25)	207 (72)		
	Literature	2669	506 (100)	491 (9)	448 (18)	377 (12)	313 (9)	235 (6)	207 (23)		
GA_3	Sample	2696	504 (100)	489 (8)	475 (5)	460 (9)	431 (8)	387 (12)	370 (20)	238 (30)	4–8
	Standard	2696	504 (100)	489 (9)	475 (2)	460 (5)	431 (6)	387 (13)	370 (16)	238 (27)	
	Literature	2692	504 (100)	489 (7)	475 (12)	460 (9)	431 (9)	387 (11)	370 (24)	238 (21)	
iso-GA_3	Sample	2638	504 (100)	475 (12)	445 (10)	431 (10)	370 (13)	355 (30)	238 (88)		4–8
	Standard	2637	504 (100)	475 (13)	445 (18)	431 (5)	370 (15)	355 (18)	238 (55)		
	Literature	2633	504 (100)	475 (13)	445 (9)	431 (6)	370 (13)	355 (7)	238 (27)		
GA_4	Sample	2507	418 (6)	386 (6)	358 (4)	328 (16)	289 (24)	284 (100)	225 (86)	224 (87)	23–26
	Standard	2509	418 (10)	386 (9)	358 (5)	328 (19)	289 (33)	284 (100)	225 (78)	224 (83)	
	Literature	2503	418 (22)	386 (17)	358 (10)	328 (25)	289 (58)	284 (100)	225 (78)	224 (77)	
GA_8	Sample	2820	594 (100)	535 (3)	448 (24)	375 (21)	311 (15)	268 (16)	238 (31)		4–8
	Standard	2819	594 (100)	535 (6)	448 (25)	375 (21)	311 (15)	268 (31)	238 (89)		
	Literature	2818	594 (100)	535 (6)	448 (14)	375 (5)	311 (4)	268 (5)	238 (13)		
GA_{19}	Sample	2601	462 (4)	434 (100)	402 (26)	374 (60)	345 (30)	315 (26)	285 (60)	239 (70)	19–22
	Standard	2597	462 (7)	434 (100)	402 (35)	374 (73)	345 (29)	315 (22)	285 (42)	239 (86)	
	Literature	2596	462 (7)	434 (100)	402 (37)	374 (64)	345 (24)	315 (15)	285 (21)	239 (33)	
GA_{20}	Sample	2487	418 (100)	403 (19)	375 (71)	359 (21)	301 (27)	235 (10)	207 (52)		15–18
	Standard	2485	418 (100)	403 (14)	375 (61)	359 (16)	301 (19)	235 (14)	207 (70)		
	Literature	2482	418 (100)	403 (16)	375 (46)	359 (12)	301 (12)	235 (8)	207 (30)		
GA_{29}	Sample	2690	506 (100)	491 (7)	477 (3)	447 (7)	389 (17)	375 (20)	235 (36)		4–8
	Standard	2686	506 (100)	491 (9)	477 (3)	447 (6)	389 (12)	375 (18)	235 (20)		
	Literature	2684	506 (100)	491 (11)	477 (3)	447 (6)	389 (8)	375 (15)	235 (10)		
GA_{34}	Sample	2671	506 (100)	459 (4)	431 (4)	416 (5)	387 (6)	372 (9)	356 (9)	288 (18)	19–22
	Standard	2666	506 (100)	459 (6)	431 (5)	416 (12)	387 (9)	372 (12)	356 (11)	288 (24)	
	Literature	2665	506 (100)	459 (4)	431 (5)	416 (9)	387 (7)	372 (8)	356 (9)	288 (24)	
GA_{44}*	Sample	2793	432 (22)	417 (5)	404 (1)	373 (8)	357 (3)	208 (33)	207 (100)		19–22
	Literature	2786	432 (46)	417 (6)	404 (3)	373 (14)	357 (2)	208 (39)	207 (100)		
GA_{51}	Sample	2528	418 (–)	386 (22)	371 (13)	343 (13)	296 (18)	284 (100)	268 (65)	225 (107)	19–22
	Standard	2522	418 (3)	386 (16)	371 (3)	343 (5)	296 (23)	284 (100)	268 (77)	225 (102)	
	Literature	2519	418 (3)	386 (32)	371 (7)	343 (10)	296 (32)	284 (100)	268 (85)	225 (95)	
GA_{53}*†	Sample	2501	448 (100)	433 (16)	419 (13)	416 (27)	389 (55)				23–26
	Literature	2497	448 (100)	433 (14)	419 (14)	416 (30)	389 (51)				

* Ion abundances were calculated from GC-SIM spectra.

† The relative abundance of m/z 448 was adjusted to 100, because of extraneous contamination of m/z 207.



Scheme 1. Possible biosynthetic pathways of endogenous GAs in tomato foliage. GAs not identified in this study are parenthesized. Iso-GA$_3$ was not included.

similar to the procedure used for 3-epiGA$_1$ [21] was performed. The method uses [17-$^2H_2$]GA$_3$ as an internal standard, and assumes that isomerization occurs at the same proportion for both natural GA$_3$ and the internal standard. In three independent experiments for the solarised and the non-solarised soil treatment, both iso-GA$_3$ and the recovered GA$_3$ exhibited a similar $^2H_0$:$^2H_2$ ratio (Table 2; shown for the former treatment only). It is therefore concluded that most or all of the detected iso-GA$_3$ resulted from GA$_3$ isomerization. However, the $^2H_0$:$^2H_2$ ratio obtained in experiments 2 and 3 were somewhat higher for iso-GA$_3$ than for GA$_3$. Thus, the presence of a small amount of endogenous iso-GA$_3$ cannot be ruled out. Nevertheless, most of the iso-GA$_3$ detected in our system seems to be a methodological artefact.

Some members of the early-non-3,13-hydroxylation pathway ($\rightarrow GA_{15} \rightarrow GA_{24} \rightarrow GA_9$) were earlier reported for tomato fruits [5, 6], but were not detected in leaves and shoot tips (Table 1, present study). However, possible metabolites of this pathway were identified, as follows: GA$_4$ (3β-hydroxylated GA$_9$), GA$_{34}$ (2β,3β-hydroxylated GA$_9$) and GA$_{51}$ (2β-hydroxylated GA$_9$). These GAs have not been reported earlier in the tomato.

Table 2. $^2H_0$:$^2H_2$ ratio from the isotope-dilution analysis of the M$^+$ ions of the MeTMSi derivatives of GA$_3$ and iso-GA$_3$ (solarisation treatment)

Exp	GA	$^2H_0$	$^2H_2$
1	GA$_3$	9.1	90.9
	iso-GA$_3$	9.4	90.6
2	GA$_3$	56.8	43.2
	iso-GA$_3$	48.9	51.1
3	GA$_3$	64.9	35.1
	iso-GA$_3$	58.3	41.7

A comparison of the GAs from tomato foliage revealed no qualitative differences between solarised *vs.* non-solarised soil treatments. Hence, the increased growth response in solarized soils [14] may be due to quantitative differences. This is currently under investigation.

The presence of the potentially active GA$_1$, GA$_3$ and GA$_4$ in tomato foliage indicates a role for these compounds in the plant's growth regulation. This can be demonstrated in cases such as the *procera* mutant, where overgrowth of shoots as compared to the near-isogenic wild type cannot be explained by the level of GA$_1$ [9, 10]. It was therefore suggested that the growth of this mutant is promoted by increased sensitivity to GA$_1$ [7, 10]. Alternatively, increased levels of, or hypersensitivity to, GA$_3$ and/or GA$_4$ may cause the *procera* overgrowth.

EXPERIMENTAL

Plant material and growing conditions. Uniform tomato seedlings (*Lycopersicon esculentum* Mill. cv Rehovot 13; Hazera Seed Company, Haifa, Israel) were grown on vermiculite and pricked out 1–2 days after emergence, at the cotyledonary stage. The primary root was trimmed to 1.5–2 cm, and groups of five seedlings were transplanted equidistantly into 12 cm diam. pots containing sandy soil. Soil solarisation was performed as previously described [14], and the samples were collected from adjacent solarised and non-solarised plots. The pots were placed in a growth chamber at 20/28 ± 1° (night/day) and a 14 hr photoperiod, with a constant photon flux density of 150–200 μmol m^{-2} s^{-1}. Plants were watered regularly with tap water only. No disease symptoms were visible in any of the experiments.

Extraction and purification of gibberellins. True leaves and shoot tips (4.4–5.6 g from 70–90 and 120–145 plants grown in solarised and non-solarised soil, respectively) were harvested 13–14 days after transplanting, immediately pooled and frozen in liquid N_2, lyophilized and stored at −20° until use. GA extraction and purification was according to [6], with minor changes. Briefly, dry leaves and shoot tips were homogenized in cold 80% MeOH (40 ml g^{-1} dry wt) and extracted overnight at 4°. In preliminary experiments, samples were spiked with [^{3}H]GA$_3$ (196 GBq mmol^{-1}). Following filtration, the retained residue was re-extracted for 1 hr in cold 80% MeOH (30 ml g^{-1} dry wt) and filtered. MeOH was removed from the combined extracts *in vacuo* and the aq. residue was partitioned (× 3) with hexane (0.5 vol.). The aq. phase was acidified to pH 2.5 with 1 M HCl and partitioned (× 4) with EtOAc (1 vol.). The combined EtOAc phase was partitioned (× 3) with 0.5 M Na-Pi buffer pH 8.3 (0.5 vol.), then 5 g insoluble PVPP were added to the combined buffered phase and stirred continuously for 1 hr at 4°. The PVPP was filtered out, the buffered phase acidified to pH 2.5 with 5 M HCl and partitioned (×3) with EtOAc (0.5 vol.). Dry Na_2SO_4 was added to the combined EtOAc phase and following filtration, the extract was dried *in vacuo*. Samples were then dissolved in MeOH and dried again to assure the removal of any acid residues. After redissolving in MeOH, the sample was loaded onto a Bond Elute DEA column (500 mg) which was then washed with MeOH. The sample was eluted in MeOH containing 0.5% HOAc, filtered through a 0.22 μm nylon mesh and reduced to dryness under a stream of N_2.

HPLC fractionation. Samples were redissolved in MeOH, loaded on a Lichrospher 100 RP-18 column (10 mm i.d. × 250 mm) and eluted in a 45 to 100% gradient of aq. MeOH containing 0.1% HOAc [6]. Total elution time was 70 min with a flow rate of 2 ml min^{-1}. 35 frs (4 ml each) were collected, dried in a vacuum concentrator, and stored at −20° until bioassayed. For the identification of GA_4, frs 23–26 were subjected to a Nucleosil $N(CH_3)_2$ column (4 mm i.d. × 250 mm). GA_4 was eluted in 100% MeOH containing 0.05% HOAc at a flow rate of 1 ml min^{-1}. 25 frs (2 ml each) were collected, dried and stored at −20° until bioassayed.

Bioassay. The HPLC frs were subjected, with minor changes, to the 'modified micro-drop bioassay' [22] using dwarf rice (*Oryza sativa* L. cv Tan-ginbozu). After disinfestation with 3% NaClO for 15 min, seeds were rinsed with H_2O and imbibed in the dark for 24 hr in 80 μM uniconazole and 0.2% Tween 20. Thereafter, seeds were rinsed, imbibed and germinated in H_2O for 48 hr in the dark. Groups of 7 seedlings were placed in small beakers containing 0.8% agar and incubated for 48 hr under continuous light (80 $\mu mol\ m^{-2}\ s^{-1}$; Osram Daylight L65 W/10 S), then the most irregular seedling in each beaker was removed. The HPLC frs were dissolved in 100 μl of 95% EtOH, and aliquots of 0.5 or 1 μl per seedling, were placed between the first leaf and the coleoptile. Control plants were treated with 95% EtOH. Following a 48 hr incubation under light, the length of the second leaf sheath of each seedling was measured using an electronic caliper. Imbibition, germination and incubation were performed at 30°.

Gibberellin identification. The HPLC frs showing GA-like activity were combined into groups (frs 4–8, 15–18, 19–22, 23–26), methylated with excess ethereal CH_2N_2 [23] and evapd to dryness under a stream of N_2. After dissolving in CH_2Cl_2 and drying at 60°, samples were trimethylsilylated using pyridine-BSTFA containing 1% TMCS (1:1) at 68° for 30 min. The derivatized samples were dissolved in CH_2Cl_2 and injected (1 μl) in splitless mode into a GC-MS [24] equipped with a DB-1 capillary column (0.25 mm i.d. × 30 m). The column head pressure of the He carrier gas was 70 kPa, and the column temp. programme was according to [23], except for the initial temp. of 40°. The electron energy was 70 eV, and mass spectra were acquired every 1.3 s from 70 to 650 amu. A parafilm extract dissolved in CH_2Cl_2 was co-injected (0.1 μl) with the samples for the KRI determination [23]. Samples of GA standards as well as $[17\text{-}^2H_2]GA_3$ were kindly provided by Prof. L. N. Mander, Dr M. Sherburn and Mr B. Twitchin (Australian National University, Canberra, Australia).

Isotope dilution. The origin of iso-GA_3 was evaluated using an isotope-dilution analysis. $[17\text{-}^2H_2]GA_3$ was added as an int. reference to the leaf homogenates prior to extraction and purification. The $^2H_0$:$^2H_2$ ratio of the M^+ ion was measured by GC-SIM, and the natural abundance of $^2H_2$ was subtracted using a standard curve.

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Dedication—We would like to dedicate this report to the memory of the late Dr Maria Wodner.

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