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Gibberellins in Apical Shoot Meristems of Flowering and Vegetative Sugarcane

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Abstract. Gibberellins A_1, A_3, iso-A_3, A_4, A_{19}, A_{20}, and A_{36} were identified by gas chromatography–selected ion monitoring in apices of sugarcane (*Saccharum* spp. hybrids). Flowering apices (i.e., 2–4 cm panicle) contained 8–9 times more (estimated by bioassay) endogenous gibberellins $A_{1/3}$ and iso-GA_3 (ratio of 1:6:8, respectively; in total 51 ng g^{-1} fresh weight) than vegetative apices (6.4 ng g^{-1} fresh weight). Vegetative apices contained small but significant levels of GA_{19}, which could not be detected in flowering apices; vegetative apices also contained approximately four times more of a GA_{36}-like substance than flowering apices. Since the two apex types developed under the same photoperiod, the increased levels of $GA_{1/3}$ and iso-GA_3 and the reduced levels of GA_{19} and GA_{36}-like substances are correlated with the flowering state rather than with photoperiod or photoperiod changes per se. Since there were relatively high levels of C_{19} GAs along with low levels of C_{20} GAs in flowering apices, and since the converse is true in vegetative apices, metabolism of C_{20} to C_{19} GAs may be enhanced in flowering apices.

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Abbreviations: GA, gibberellin(s); MS, mass spectrometry; fw, fresh weight; GC, gas liquid chromatography; GC/MS, combined gas chromatography–mass spectrometry; MeOH, methanol; MeTMSi, methyl ester–trimethylsilyl ether; PVP, polyvinylpyrrolidone; SIM, selected ion monitoring; Rt, retention time; SiO_2, silica gel.

Gibberellins A_1, A_3, A_4, A_{19}, A_{20}, and A_{36} have now been identified in extracts of sugarcane leaf and shoot apical meristem tissues by GC-MS (Kuhnle et al. 1983, Koshioka et al. 1984). Bioassay of the SiO_2 column fractions by dwarf rice (cv. Tan-ginbozu) showed greater GA-like activity in extracts of apical tissues below a 2-cm panicle than in extracts of non-flowering apical meristems (Kuhnle et al. 1983). We have now analyzed additional extracts using a sequential SiO_2 partition column → bioassay → reverse-phase C_{18} HPLC → bioassay → GC-SIM procedure to identify and quantify (by bioassay) GA_1, GA_3, iso-GA_3, GA_{19}, and GA_{36} from vegetative and/or flowering apices of sugarcane.

Materials and Methods

A comparative study was conducted on extracts of shoot apical meristems of vegetative and flowering plants of sugarcane (*Saccharum* spp. hybrids) clone H59-3775. Field-grown plants were harvested Jan. 15 at 10.5 months of age, 3.5 months after the flowering induction period of September. The apical meristems were visually classified using a hand lens as vegetative or flowering just after dissection from the shoot. Each sample, vegetative and flowering, was a composite from 20 plants and had a total fw of 120 g.

Detailed methods used for collection, lyophilization, extraction, separation, and analysis were described previously (Kuhnle et al. 1983). Briefly, the acidic ethyl acetate fractions were purified using PVP (Glenn et al. 1972) followed by chromatography on SiO_2 partition columns (Durley et al. 1972). The thirty 20-ml fractions collected from the SiO_2 column were bioassayed (1/200 dilution) on dwarf rice cv. Tan-ginbozu (Murakami 1968) as modified in Kaufman et al. (1976).

Fractions from the SiO_2 partition columns (Fig. 1) were grouped on the basis of bioassay activity on dwarf rice, with fractions 14–20 (flowering) and 15–19 (vegetative) being grouped separately. Bioactivity in other SiO_2 partition column fractions was low (Fig. 1), and to facilitate characterization by GC-SIM fraction, residues were combined for induced plus vegetative apices (see Koshioka et al., 1984, for details on GC-SIM of these combined fractions). The pooled fractions were then chromatographed on a reversed-phase C_{18} Bondapak (Waters Associates) HPLC column (Koshioka et al. 1983) with the following gradient: 32.5% MeOH in 1% Aq. acetic acid, isocratic 0–20 min, linear gradient from 32.5% MeOH in 1% Aq. acetic acid to 73% MeOH in 1% acetic acid in 20–45 min). Three-minute fractions (6 ml) were collected, and taken to dryness *in vacuo* before a second bioassay on dwarf rice at 1/200 and 1/400 aliquot per rice plant.

Groupings of biologically active fractions from HPLC were made prior to derivatization and analysis by GC-SIM. These groupings were further analyzed by GC-SIM as the MeTMSi derivative (made with ethereal diazomethane and TRI-SIL; Pierce Chemical Company). The derivatized fraction groupings noted above were dissolved in 15 μl of dichloromethane, and 1-μl aliquots were injected into a Hewlett-Packard 5790A GC and 5790A series Mass Selective Detector, using a cross-linked 5% phenylmethyl silicone

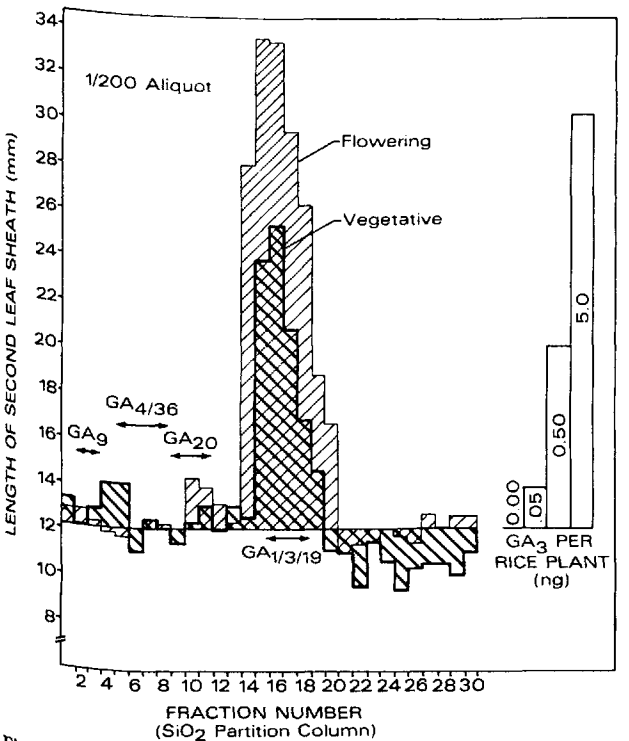

Fig. 1. Biological activity (on dwarf rice cv. Tan-ginbozu) of acidic ethyl acetate soluble extracts from 20 apical meristems (vegetative) and 20 apical meristems (flowering) sampled on January 15 from shoots of sugarcane clone H59-3775. The Rts of authentic GAs on the SiO_2 partition column are shown as ↔. The activity of authentic GA_3 standards, expressed as 0–5.0 ng GA_3/rice plant, is shown on the right. Each SiO_2 partition column fraction was assayed at 1/200 aliquot. Quantities have been converted to GA_3 equivalent g^{-1} fw in Table 1.

column, film thickness 0.33 μm, internal diameter 0.2 mm, length 25 m, E.M. volts 1400, head pressure 10.0 psi, temperature programmed from 60°C to 270°C at 24°C/min.

Results and Discussion

The Qualitative Spectrum of Endogenous Gibberellins and GA-like Substances Present in Sugarcane Apices

SiO_2 Partition Column Analysis

The Rts of authentic GAs are shown above the appropriate fractions in Fig. 1. They are derived both from the published literature (Durley et al. 1972) and from the identification of endogenous GAs in these extracts by GC-SIM (see later).

The growth of dwarf rice, which can be expressed in GA_3 equivalents rela-

tive to authentic GA_3 standards, was most stimulated by fractions 14–20, where GA_1, GA_3, iso-GA, and GA_{19} would elute from the SiO_2 column. The bioassay activity of GA_1, GA_3, iso-GA_3, and GA_{19} on dwarf rice is similar (Crozier et al. 1970, Hoad et al. 1976). Hence, neither the SiO_2 partition column nor the bioassay will differentiate between the C_{20} GA, GA_{19}, and the C_{19} GAs, GA_1 and GA_3, or iso-GA_3. Most other GAs will have generally lower activity than GA_3 on the bioassay (Crozier et al. 1970), so the amounts present may be significantly more than those expressed in GA_3 equivalents.

C_{18}HPLC Analysis

The Rt of authentic GAs is shown above the appropriate fractions in Figs. 2 and 3. These Rts are derived from the published literature (Koshioka et al. 1983, Jones et al. 1980), the use of authentic [^{3}H]GAs, and the identification of endogenous GAs in these extracts by GC-SIM (see later).

Nonpolar and Moderately Polar GA-like Substances

Biological activity originating from SiO_2 partition column fractions 1–14 (Fig. 1) was eluted from C_{18} HPLC at Rts similar to those noted for GA_9, $GA_{4/7}$, GA_{20}, and GA_{36} (data not shown, but see Koshioka et al., 1984, where GC-SIM characterization of GA_4 and GA_{36} is reported, and see later where GC-SIM confirmation of GA_{20} is noted).

Polar GA-like Substances

Extracts of vegetative apices had significant GA-like activity in fractions at Rts where authentic $GA_{1/3}$, GA_{19}, and GA_{36} elute (Fig. 2). Extracts of flowering apices had significantly greater GA-like activity in fractions at which authentic $GA_{1/3}$ would elute and nonsignificant activity where GA_{19} would elute (Fig. 3). Additional activity was present at a slightly more polar Rt (0–3 min; Fig. 3) and at less polar Rt (10–24 min; Fig. 3), where a variety of tri- and dihydroxylated C_{19} GAs might be expected to elute (Koshioka et al. 1983; Pharis, unpublished).

GC-SIM Analysis

Results of GC-SIM of certain biologically active fraction groupings from the C_{18} HPLC stage of analysis are noted in Koshioka et al. (1984) and in Table 2. These results, together with correlation of Rts of biologically active peaks and authentic GAs on sequential SiO_2 partition column chromatography (Fig. 1) and C_{18} reversed-phase HPLC (Figs. 2, 3), lead us to conclude that the following GAs are present: GA_1, GA_3, iso-GA_3 (produced from GA_3 during work-up? (Pryce 1973)), GA_4, GA_{19}, GA_{20}, and GA_{36}. Based on discrete peaks of biological activity (Rt 10–24 min; Fig. 3) from which no GAs could be identi-

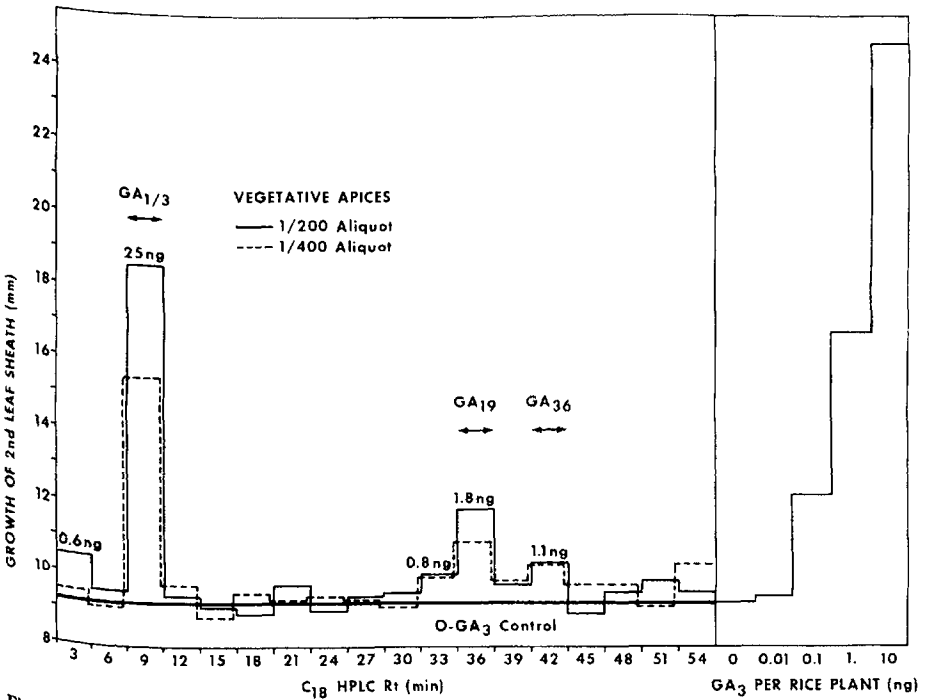

Fig. 2. Biological activity (on dwarf rice cv. Tan-ginbozu) of polar GA-like substances (i.e., Fr. 15–19 (vegetative apices) of Fig. 1) after elution from a reversed-phase C_{18} HPLC. The Rts of authentic GAs on the C_{18} HPLC are shown as ↔. The activity of authentic GA_3 standards, expressed as ng GA_3/rice plant, is shown on the right. Each HPLC fraction was assayed at 1/200 (—) and 1/400 (– – –) aliquots. Quantities (per sugarcane apex) are noted in ng (of GA_3 equiv.) above each fraction that differed significantly ($p \leq 0.05$) from the ethanol controls (i.e., 0 – GA_3 applied per rice plant). The average fresh weight for a single apex (vegetative) is 6.08 g.

fied by GC-SIM, we conclude there are at least two additional, unknown, GA-like substances present.

Quantitative Estimates of GA-like Substances in Sugarcane Apices

Estimates of GA-like activity in three grouped SiO_2 partition column fractions are given in Table 1. The qualitative GA profile is shown in Figs. 1, 2, and 3. Based on HPLC analysis and subsequent analysis of SiO_2 partition column fractions 14–19 (Figs. 2, 3) by GC-SIM, we conclude that the significant biological activity shown in fractions 14–19 (Fig. 1) is made up mainly of a combination of GA_1 and GA_3, with minor amounts of GA_{19} and GA_{36}.

Bioassay of the SiO_2 fractions in which $GA_{1/3/19}$ would elute (Fig. 1) showed flowering apices contained 51.6 ng g^{-1} fw GA_3 equiv., whereas vegetative apices contained 6.8 ng g^{-1} fw. Based on biological activity alone (Fig. 1, Table 1), it appears that there was no appreciable difference in the less polar GA-like substances (SiO_2 fractions 1–13) of flowering apices, relative to vegetative

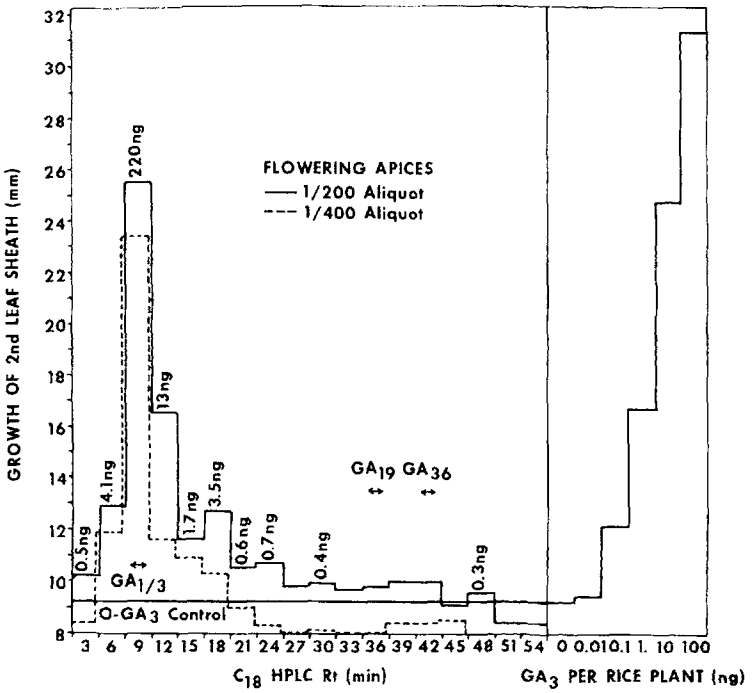

Fig. 3. Biological activity (on dwarf rice cv. Tan-ginbozu) of polar GA-like substances (i.e., Fr. 14–19 (flowering apices) of Fig. 1) after elution from a reversed-phase C_{18} HPLC. The Rt of authentic GAs on the C_{18} HPLC is shown as ↔. The activity of authentic GA_3 standards, expressed as ng GA_3/rice plant, is shown on the right. Each HPLC fraction was assayed at 1/200 (—) and 1/400 (——) aliquots. Quantities (per sugarcane apex) are noted in ng (of GA_3 equiv.) above each fraction that differed significantly ($p \leq 0.05$) from the ethanol controls (i.e., 0 – GA_3 applied per rice plant). The average fresh weight for a single apex (flowering) is 6.03 g.

apices. However, there was an eightfold increase in the more polar ($GA_{1/3/19}$-like) GA-like substances of flowering apices, relative to vegetative apices (Table 1, Fig. 1). After HPLC (which separates the C_{20} from the C_{19} gibberellins, in this case GA_{19} and GA_{36} from GA_1 and GA_3), flowering apices contained ca. 9× more $GA_{1/3}$-like substance than vegetative apices (Figs. 2, 3).

Conversely, each vegetative apex (Fig. 2) contained 1.8 ng (GA_3 equiv.) of GA_{19}-like substance, whereas flowering apices (Fig. 3) had no significant bioactivity at the GA_{19} Rt. In addition, vegetative apices contained 4× more GA_{36}-like substance than flowering apices.

Analysis by GC-SIM (Table 2) confirmed the presence of GA_{19} (Rt 34–37 min; Fig. 2), in vegetative apices, and GA_1, GA_3, and iso-GA_3 in flowering apices (Rt 7–9 min; Fig. 3). GA_{19} could not be detected by GC-SIM of extracts from flowering apices, and bioassay following HPLC indicated very low (non-significant) growth from the 34- to 37-min fraction. Since Kuhnle et al. (1983) reported GA_{19} in both flowering and vegetative apices collected earlier during panicle development (personal communication), the GA_{19} might be decreasing with advanced stages of panicle development. The $GA_{1/3}$ sample from vegetative apices (Rt 7–9 min; Fig. 2) was lost prior to GC-SIM. However, GC-SIM

Table 1. Gibberellin-like activity estimated by the dwarf rice (cv. Tan-ginbozu) microdrop bioassay in extracts of vegetative and reproductive sugarcane apical meristem tissues chromatographed on a SiO_2 partition column[a]

SiO_2 fractions	Elution profile of authentic gibberellins	Clone H59-3775[b]	
		Vegetative apices	Flowering apices
1–13	GA_4, GA_9, GA_{20}, GA_{36}	0.38	0.31
14–19	GA_1, GA_3, GA_{19}, GA_{29}, GA_{36} (tr)	6.43	50.97
20–30	$GA_{1/3}$ (tr), GA_{29}, GA_8	0.00	0.32
Total		6.81	51.60

[a] Tissue was sampled on Jan. 15, 3.5 months after the inductive photoperiods of September. Data presented as the sums of activity (GA_3 equiv. in ng g^{-1} fw) in the moderately polar (1–13), polar (14–19), and highly polar (20–30) fractions.
[b] Twenty apices per sample (120 g fw).

Table 2. GC-SIM and Rt of MeTMSi derivatives of gibberellin-like substances from vegetative and flowering sugarcane apices, clone H59-3775

Origin	GC Rt (min)	Constituent ions (relative intensity)	Identity
Biologically active substances eluting from SiO_2 partition column and C_{18} HPLC coincidental with GA_{19} (Figs. 1, 2)	18.6	462 (2), 447 (trace), 434 (23), 431 (2), 402 (9), 374 (22)	GA_{19}
Authentic GA_{19}[a]	18.6	462 (2), 447 (trace), 434 (25), 431 (2), 402 (8), 374 (16)	
Biologically active substances eluting from SiO_2 partition column (fr. 14–20; flowering apices) and C_{18} HPLC coincidental with authentic $GA_{1/3}$ (Figs. 1–3)	19.7	506 (317), 491 (37), 447 (23), 416 (12), 377 (86), 313 (72)	GA_1
	20.4	504 (2129), 489 (188), 473 (55), 445 (122), 414 (45), 370 (297)	GA_3
	19.1	504 (2849), 489 (280), 473 (70), 445 (315), 414 (61), 370 (353)	iso-GA_3
Authentic GA_1[a]	19.7	506 (317), 491 (36), 447 (39), 416 (20), 377 (79), 313 (50)	
Authentic GA_3[a]	20.4	504 (2129), 489 (205), 473 (47), 445 (140), 414 (65), 370 (243)	
Authentic iso-GA_3[a]	19.1	504 (2849), 489 (284), 473 (75), 445 (319), 414 (72), 370 (353)	

[a] Relative intensity of M^+ ion adjusted to value of sugarcane sample, and intensities of other constituent ions calculated therefrom for ease of comparison.

of the Rt 7–9 min region from flowering apices (Fig. 3) yielded a ratio of GA_1:GA_3:iso-GA_3 of 1:6:8. It is possible that iso-GA_3 is a product of extraction and work-up procedures, originating from GA_3. Iso-GA_3 is almost as active as GA_3 on the dwarf rice assay at lower concentrations but less than half as active

as GA_3 at higher concentrations (Hoad et al. 1976). The relatively low level of GA_1, in comparison with GA_3/iso-GA_3, may be related to rapid conjugation of GA_1, relative to GA_3 (assumption based on unpublished results, Pharis and Koshioka).

Gibberellin A_{36} was detected by GC-SIM in combined extracts of vegetative and flowering apices (Koshioka et al. 1984). Although GA_{20} was identified from combined extracts of vegetative and flowering apices by GC-SIM (Koshioka et al. 1984), amounts were low, and a comparison between flowering and vegetative apices could not be made.

Both GA_4 and GA_{20} could be precursors to GA_1, and GA_{19} is the logical precursor of GA_{20}, just as GA_{36} is the logical precursor of GA_4 (Gianfagna et al. 1983; Graebe et al. 1974). The lowered levels of GA_{36}-like substance, and the undetectable levels of a GA_{19}-like substance in flowering apices (Fig. 3), relative to vegetative apices (Fig. 2), together with the eight- to ninefold increased level of $GA_{1/3}$-like substance present in flowering apices (Table 1; Figs. 2, 3), lends support to the hypothesis that flowering plants of sugarcane have an increased rate of metabolism (i.e., $GA_{19} \rightarrow GA_{20} \rightarrow GA_1$ or $GA_{36} \rightarrow GA_4 \rightarrow GA_1$, and $GA_? \rightarrow GA_3$). A somewhat analogous situation has been noted for spinach, where a long-day photoperiod allowed the conversion of GA_{19} (produced from [^{2}H]GA_{53}) to GA_{20}, the short-day plants converting GA_{53} (via GA_{44}) only as far as GA_{19} (Gianfagna et al. 1983). However, in the present case (with sugarcane), the photoperiod was the same for both flowering and vegetative plants. Hence, the reduced level of GA_{19} and the GA_{36}-like substance, and increased levels of $GA_{1/3}$-like substance and iso-GA_3 (Figs. 2, 3), are related to the flowering condition and not to photoperiod per se.

Both GA_1 and GA_3 were previously identified in sugarcane leaf and shoot apical meristems (Kuhnle et al. 1983), and large amounts of GA_3 were found in young inflorescences of oat (Kaufman et al. 1976). The presence of both GA_1 and GA_3 has also been noted in seeds of wheat and rye (Eckert et al. 1978), although in maize tassels and rice plants and rice seeds, wild oat seeds, and barley seeds and seedlings, only GA_1 has been found (i.e., there has been no definitive identification of GA_3) (Hedden et al. 1982, Kurogochi et al. 1979, Metzger 1983, Gaskin et al. 1983). The presence of either (or both) GA_1 and GA_3 in Graminae species is thus well established, as is the presence of relatively large amounts of GA_3 in flowering apices (Table 1, Figs. 2, 3) and in inflorescences (Kaufman et al. 1976).

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