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Analysis of Native Gibberellins in the Internode, Nodes, Leaves, and Inflorescence of Developing *Avena* Plants[1]

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ABSTRACT

The native gibberellins (GAs) of various organs of the *Avena* plant were analyzed by bioassay and gas chromatography-mass spectrometry (GC-MS) after silicic acid partition column chromatography. The major GA of the inflorescence was identified as GA_3 by GC-MS, and this GA also forms the major component of the nodes, p-1 internode, and roots as determined by GLC or chromatography/bioassay. The inflorescence and nodes are the major sources of native GAs, the last two leaves, internode, and roots having significantly lower amounts of GA-like substances. In the internode, less polar GAs predominated at the lag stage of development, whereas by the log and plateau stages, the more polar GAs increased significantly.

Since less polar GAs are early in oxidative interconversion sequences, this finding indicates sequential conversion to more polar and probably more active GAs, during log phase growth of the p-1 internode.

Excised stem segments of *Avena* plants are known to be very sensitive in their growth response to exogenously supplied gibberellins (GAs), especially GA_1 and GA_3 (13, 15). In contrast, they show no significant growth promotion by other plant hormones such as IAA, kinetin, and ABA (14). In light of this marked specificity of oat stem segments to GAs, especially the more oxidized GA_1 and GA_3, it is germane to ask the following questions: Where in the *Avena* plant are the primary sources of native GAs that may be regulating internodal elongation *in situ*? Are these GAs produced mainly in the internodes, or are they derived from other parts of the plant? What are the predominant forms (*i.e.* degree of oxidation) of GAs found in the *Avena* plant during rapid internode elongation?

Past studies on native GAs in other cereals are relevant to the present investigation. Radley and co-workers (12, 21, 22) have characterized GA_1- and GA_3-like substances in barley by means of TLC, fluorimetry, and bioassay. Faull *et al.* (9) reached similar conclusions for barley GAs using paper, TLC, GLC, paper electrophoresis, and bioassay. Murphy and Briggs (18) identified GA_3 and GA_7 in germinating barley by novel means using crystallization to constant specific radioactivity with unlabeled carrier GA methyl ester after initial methylation of the endogenous GA with ^{14}C-diazomethane. By isotope dilution techniques they estimated GA_3 levels to be 1.5 ng/grain, a level which correlates well with bioassay estimates. Faull *et al.* (10) showed that 11-day-old barley seedlings synthesized an A_1-like GA *de novo* from $^{14}CO_2$, and that the labeled GA was metabolized within 12 hr, implying a high rate of turnover of GAs in the seedlings. Nicholls (19) found very high levels (15 to 48 μg/g, dry weight) of GAs in barley inflorescences.

In the present study native GAs of *Avena* nodes, internodes (at 3 stages of elongation), roots, leaves, and inflorescences are analyzed by silicic acid partition column chromatography, being quantitated by bioassay, and in certain cases, GLC. Identification of one GA was made by GC-MS.[4] These analyses have allowed the questions posed above to be answered, at least in part.

MATERIALS AND METHODS

Plant material of *Avena sativa* cv. Victory was harvested in Ann Arbor, Mich., immediately frozen in liquid N_2, and lyophilized prior to shipment to Calgary, for analysis of GAs. Plants were grown in a cooled greenhouse (21.5 C day, 15.5 C night) under natural photoperiod from September to December, 1973. Plant parts sampled are given in Table I.

Batches of plant tissue, as described in Table I, were extracted in cold (<0 C) 80% methanol (10 g methanol to 1 g tissue) for GAs as described previously (3). Purification of the acidic, ethyl acetate-soluble fraction on Polyclar AT (PVP), an insoluble form of poly-N-vinylpyrrolidone (11), and charcoal-Celite columns (26) was followed by chromatography on Woelm silica gel partition columns (5, 20) which were gradient-eluted using a four-chamber Varigrad system, chambers 1 to 4 being 50%, 65%, 85%, and 100% ethyl acetate in hexane (w/w), respectively. The 26 fractions collected were diluted serially over a wide range (1/150 to 1/3600 depending upon the dry weight and biological activity present) and bioassayed for GA-like activity in 0.5 μl of 95% ethanol at each diluiton on 10 dwarf rice *cv.* Tanginbozu plants (17). Using the dilution yielding the highest GA estimate, the GA-like substances were quantitated for each fraction and expressed in GA_3 equivalents. Only dilutions differing significantly from controls (P = 0.95%) were used to estimate, relative to a standard GA_3 curve, endogenous GA levels.

After conversion to the methyl ester, MeTMSi derivatives (2), those fractions co-chromatographing with GA_3 from the nodes (fractions 13 to 15), inflorescences (fractions 14 to 18), and log phase internodes (fractions 13 to 15) were analyzed by GLC. Fractions 14 to 18 from the inflorescences were subsequently subjected to GC-MS. GLC utilized glass columns (1.8 × 3 mm i.d.) packed with either 2% QFI or 2% SE30 on Gas-chrom Q at

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[4] Abbreviations: GC-MS: gas chromatography-mass spectrometry; MeTMSi: trimethylsilyl ether.

Table I. *Description of Plant Part, Weights, and Number of Avena Plants Utilized in GA Extractions.*

Plant Part[1]	Dry Weight	Plants
	g	No
Last leaf	38.40	232
Young inflorescence	29.45	308
Next-to-last leaf	33.80	257
Nodes	31.47	1960
p-1 internode		
lag phase 1-2 cm	6.50	4060
log phase 2-5 cm	25.73	4680
plateau phase 5-10 cm	32.90	570
Root	29.20	260

[1] See diagrammatic representation of *Avena* plant in Fig. 3.

190 C with helium carrier gas flowing at 45 ml/min. Peaks were compared to MeTMSi derivatives of various GAs. Examination by GC-MS utilized a Varian 1200 GLC connected via a Biemann-Watson double stage separator to a Varian CH5 mass spectrometer. The glass column (1.6 m × 2 mm i.d.) containing 2% QFI or 2% SE30 on Gas-chrom Q was maintained at 198 C with helium flowing at 20 ml/min.

RESULTS AND DISCUSSION

The qualitative spectrum of endogenous GA-like substances in various organs of *Avena* is shown in Figure 1. There are obvious qualitative and quantitative differences between the organs, but, with the exception of the last leaf, a GA_3-like substance (fractions 13 to 18) appears to predominate. However, it should be kept in mind that the results are expressed in GA_3 equivalents, and the bioassay measures most other GAs at much lower efficacy than GA_3 (4).

The inflorescence and nodes are exceptionally high in a GA_3-like substance, the inflorescence having large amounts of other GA-like substances as well (Fig. 1). The p-1 internode, which is quite responsive to exogenous application of GA_3 (13–16) is relatively low in GA-like substances except for the GA_3-like peak. The roots and next-to-last leaf show only modest amounts of GA-like substances. As well, the roots possess a rather limited qualitative spectrum of GA-like substances (Fig. 1).

Gibberellin A_3 was isolated and characterized by GLC and GC-MS from fractions 15 to 19 of the inflorescence. Since this zone of activity was wide (Fig. 1), it was examined in three parts, fractions 15 and 16, 17, and 18 to 19 by GLC, using two columns, 2% QFI and 2% SE30. In all three samples, peaks were observed (Fig. 2) which corresponded to those of GA_3MeTMSi, the largest being in fractions 15 and 16. The samples were next examined by GC-MS. From fractions 15 and 16 of the inflorescence the mass spectrum was found to be identical with the published spectrum (1) of GA_3MeTMSi. Similarly, GA_3MeTMSi was confirmed in fractions 17 and 18 and 19 of the inflorescence. Numerous other peaks in the samples were also scanned, but none could be identified as GA derivatives.

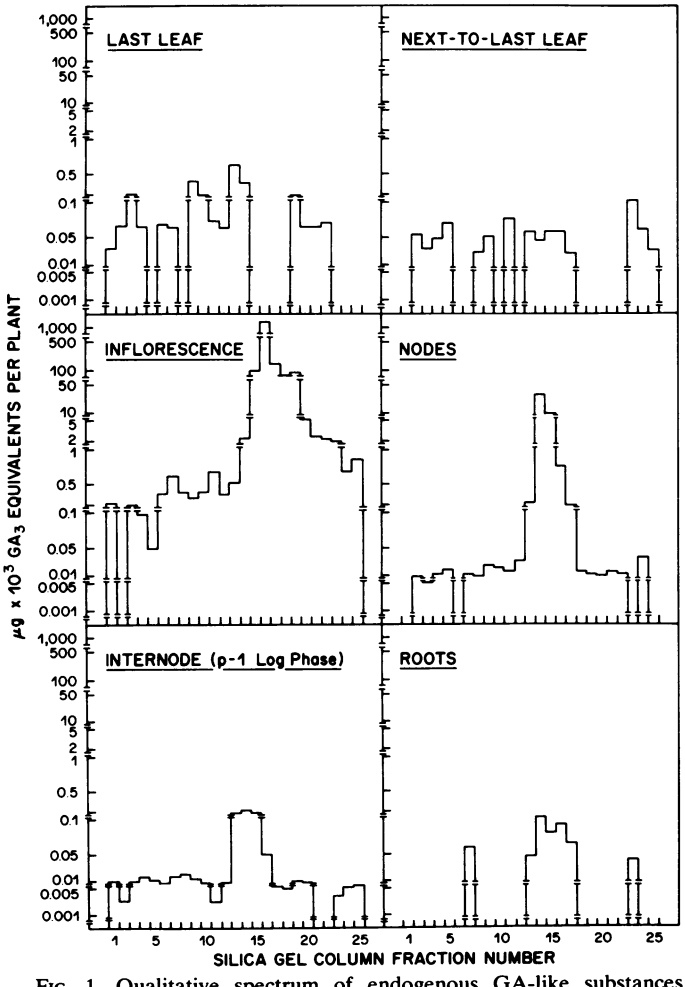

FIG. 1. Qualitative spectrum of endogenous GA-like substances from *Avena* plant parts as determined by bioassay on dwarf rice *cv.* Tanginbozu. Gibberellin A_3 was characterized from fractions 15 to 18 by GC-MS. Reference GAs would be eluted as follows from this silica partition column: GA_9: fractions 2 and 3; GA_4: fractions 4 and 5; GA_3: fractions 14 to 16; GA_8: fractions 20 to 22.

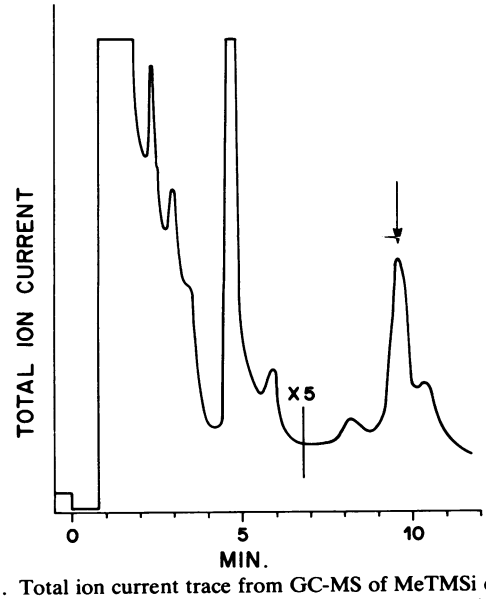

FIG. 2. Total ion current trace from GC-MS of MeTMSi derivatized fractions 15 and 16 from inflorescence. GLC conditions: glass column (1.6 m × 2.0 mm i.d.) packed with 2% QF1 on Gas-chrom Q at 198 C and helium flowing at 20 ml/min. A scan of the indicated peak gave a mass spectrum identical with GA_3MeTMSi.

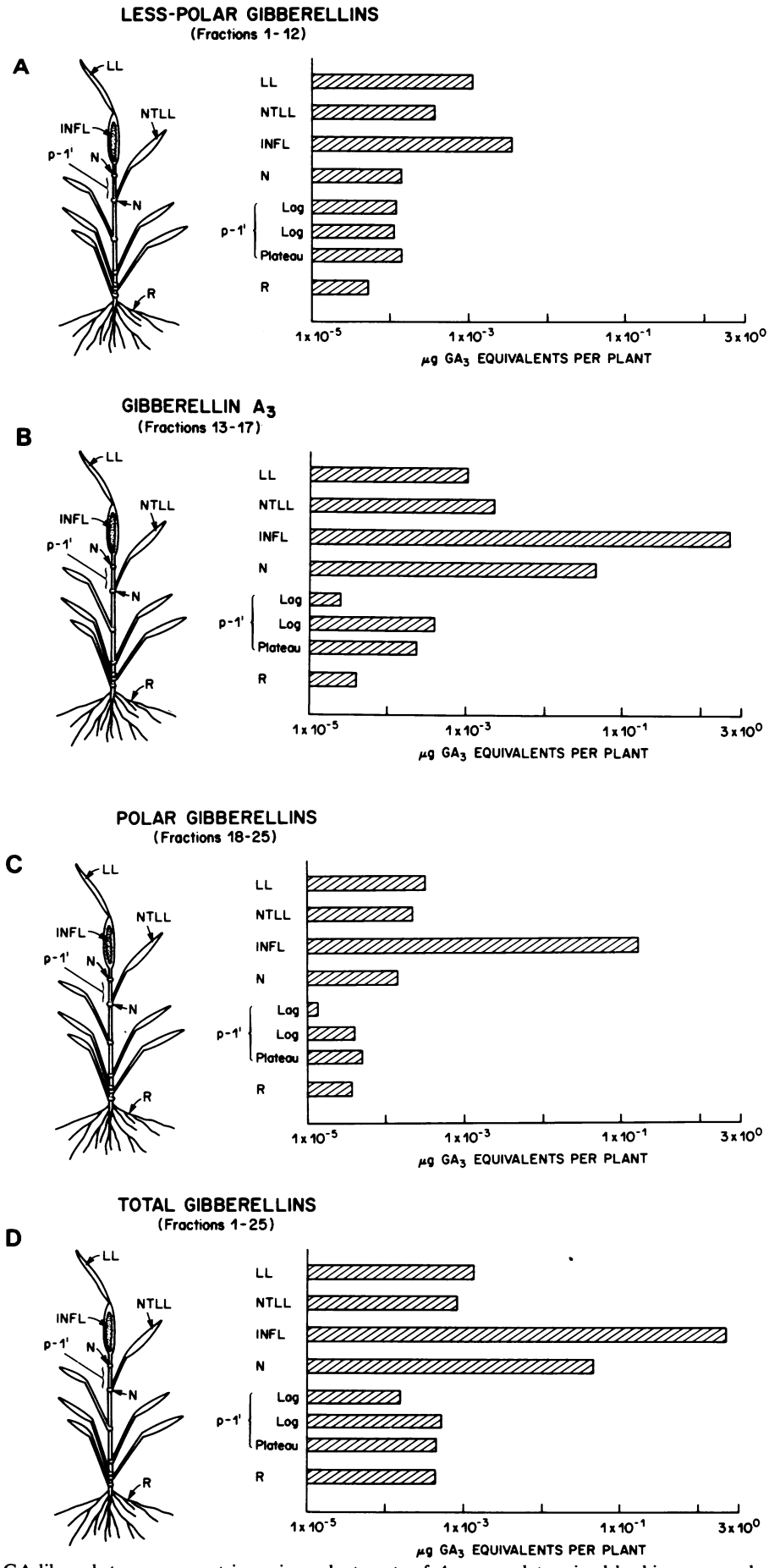

FIG. 3. Quantities of GA-like substances present in various plant parts of *Avena* as determined by bioassay on dwarf rice *cv.* Tan-ginbozu. Certain fractions were grouped for estimates as noted for each of A, B, C, and D. Note that the p-1 internode has been sampled during three different phases of growth; lag (1 to 2 cm), log (2 to 5 cm), and plateau (5 to 10 cm).

Fractions 13 to 15 from the nodes were examined by GLC on 2% QFI and 2% SE30 columns. On each column a small peak was observed which corresponded to GA_3MeTMSi. However, definitive identifications of GA_3 in this sample could not be established since the peak was insufficient for GC-MS. Similarly, fractions 13 to 15 from the log phase internode were examined, but no GLC peaks corresponding to GAs could be found, presumably due to the low level of GAs present (Fig. 1).

Gibberellin A_3 was therefore characterized as the major GA of the inflorescence. A similar, biologically active substance in the node and internode (Fig. 1) was not present in sufficient levels to obtain a definitive mass spectrum, but this is also likely to be GA_3. Other GAs were present in too small amounts for definitive characterization.

The quantities of less polar GAs (fractions 1 to 12), GA_3 (fractions 13 to 17), more polar GAs (fractions 18 to 25), and total GAs for each plant part are shown in Figure 3. On the basis of recent work with radioactive GAs of a less polar nature (*i.e.* GA_4, GA_5, GA_9, GA_{14}, and GA_{20}), it appears that the less polar GAs are generally also less oxidized and may act as precursors to more polar GAs such as GA_1, GA_3, GA_8, GA_{18}, GA_{23}, GA_{29}, GA_{34}, and GA_{38} (6-8, 23-25). Thus, relatively high levels [here note that GA_3 equivalent values for less polar GAs are only $^1/_3$ to $^1/_{10}$ of actual amounts (4)] of less polar GAs in the last leaf and next-to-last leaf, relative to levels of GA_3 and more polar GAs (Fig. 3), may indicate that these organs are exporting GA_3 to the inflorescence and nodes, both being quite high in GA_3 relative to the less polar GAs.

Within the p-1 internode, there are marked qualitative and quantitative differences with stages of growth. The lag phase internode contains large amounts of less polar GAs (Fig. 3A) relative to GA_3 (Fig. 3B) and more polar GAs (Fig. 3C). Although internodes in log and plateau phases contain relatively large amounts of less polar GAs (Fig. 3A), their levels of GA_3 (Fig. 3B) and more polar GAs (Fig. 3C) are appreciably higher than internodes in the lag phase of growth. This may indicate that the major portion of GA_3 is produced after lag phase, during log phase of growth. The GA_3 is probably derived from less polar GAs (*a*) synthesized within the internodes, and (*b*) imported from the leaves, nodes, and inflorescence (see below). Although total amounts of fractions 1 to 12 GAs do not appear to change during either lag, log, or plateau phases of growth (Fig. 3A), the level of GA_3 diminished slightly (Fig. 3B), and more polar GAs increased slightly (Fig. 3C) as the internode changed from log to plateau phases. During plateau phase, the internode may be beginning to effectively reduce the amounts of highly biologically active GA_3 by increasing its oxidative metabolism to more polar substances.

Results of the present study show that the inflorescence and nodes are the primary sources of GAs found in the *Avena* shoot. Each inflorescence contains an average of 1850 ng GA_3, and each pair of nodes contains an average of 46 ng GA_3-like substance (Fig. 3B). Thus, these two parts of the *Avena* shoot could serve as important sources of native GAs required for elongation of the next-to-last internode. More direct support for this view is obtained from "deletion" experiments (Kaufman, Koning, and Tkaczyk, unpublished data) in which the inflorescence, leaves, and two nodes associated with the next-to-last internode are excised from developing *Avena* shoots. Under these conditions, the growth of the next-to-last internode is almost completely abolished. When the leaves and nodes are present, with only the inflorescence excised, about half as much growth occurs in next-to-last internode as in the intact shoot. These unpublished results, together with results of the present study, suggest that the nodes and leaves can also serve as important sources of GAs, not only for the next-to-last internode, but also, for the much shorter internodes found lower down on the *Avena* shoot.These lower internodes elongate well before the inflorescence is initiated.

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