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Estimates of Free and Bound Indole-3-Acetic Acid and Zeatin Levels in Relation to Regulation of Apical Dominance and Tiller Release in Oat Shoots*

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Abstract. Oat stem segments containing quiescent lateral (tiller) buds during times of strong apical dominance, and growing buds released from this inhibition, were collected for analysis of native auxin and cytokinins. Free IAA and IAA conjugates were determined by a ^{14}C-IAA and ^{14}C-IBA double isotope dilution assay. Free zeatin (Z), zeatin riboside (Z-r), and their glucoside conjugates were purified from butanol-soluble fractions by means of a cellulose phosphate exchanger and thin-layer chromatography. Hormones were analyzed by gas chromatography and mass spectrometry (GC-MS). Results of these analyses indicate that changes in free and bound IAA within the stem do not correlate well with the release of tiller buds (as brought about by decapitation, gravistimulation, or the emergence of the inflorescence). However, increases in Z-r levels are well correlated with tiller release. The glucoside conjugate of Z-r may act as a storage form of cytokinin in quiescent tiller buds. In light of these results, we find that the auxin-cytokinin ratio in oat stem segments is shifted during tiller

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Abbreviations: IAA, indole-3-acetic acid; Z, zeatin; BA, benzyl adenine; Z-r, zeatin riboside; Me-IAA, methyl ester of indole-3-acetic acid; Me-IBA, methyl ester of indole-3-butyric acid; ABA, abscissic acid; NPD, nitrogen-phosphorus detector for gas chromatograph; HPLC, high-performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; TLC, thin-layer chromatography.

release, primarily as a result of increases in Z-r levels. The increase in Z-r is accompanied by a decrease in Z-r glucoside.

Lateral buds (tillers) are released from apical dominance in oats after gravistimulation, decapitation, or the emergence of the flag leaf and inflorescence (Harrison and Kaufman 1980). Also, in oats, cytokinin (kinetin) induces significant tiller bud release from apical dominance, and auxin inhibits kinetin-induced tiller release (Harrison and Kaufman 1980).

In the present study, endogenous levels of free and bound auxin (IAA) and cytokinin (Z and Z-r) were analyzed in oat stem segments containing quiescent tiller buds derived from upright, decapitated, and gravistimulated intact oat plants and from segments containing growing (releasing) tiller buds coincident with inflorescence emergence.

Materials and Methods

Plant Material

Oats (*Avena sativa* L. cv. 'Victory') obtained from the Swedish Seed Association (Svalöf, Sweden) were germinated and grown in a soil mixture of loam:peat:Perlite (4:1:1) in a greenhouse programmed for 20°–23°C day and 17°C night temperatures. Plants were illuminated with overhead incandescent and fluorescent lamps, giving a photosynthetic photon flux density of 150–1,100 $\mu E \cdot m^{-2} \cdot s^{-1}$ at plant level for 20 h a day. Plants were fertilized once a week with 5-10-5 N-P-K fertilizer. Thirty-five to 46 days after sowing, plants were selected for all the experiments. Entire flats of oats were either left upright, gravistimulated for 24 h, or used 24 h after decapitation. Hormone analyses were conducted on stem segments containing tiller buds excised at the p-5 node (fifth node below the peduncular node; the peduncle is the internode that supports the inflorescence) (Harrison and Kaufman 1980).

IAA Analysis

Oat stem segments (10 g fresh weight) were extracted by homogenizing the segments in 70% aqueous acetone (v/v) and reextracted with ether, as described by Bandurski and Schulze (1974). (2-^{14}C)-indole-3-acetic acid, 0.1–0.3 μg, 55 mCi/mmol specific activity, (gift of Robert Bandurski, Michigan State University, East Lansing, Michigan) was added immediately after homogenization of the tissue. Bound IAA is defined as the IAA that is liberated after hydrolysis with 7 N NaOH for 3 h at 100°C (Bandurski and Schulze 1977). After hydrolysis, the extract was acidified and extracted with ether. Ether extracts (from hydrolyzed or nonhydrolyzed extracts) were reduced, partitioned against $NaHCO_3$, and reextracted into ether as cited in Bandurski and Schulze (1977). The final ether extract was dried, redissolved in 50% aqueous ethanol (v/v), placed on a DEAE Sephadex acetate A-25 column, and eluted with a 0.5% acetic acid gradient in 50% ethanol (Cohen and Schulze 1981).

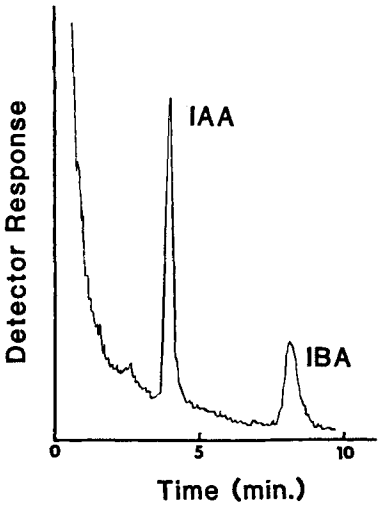

Fig. 1. Recorder tracing from Varian TSD nitrogen-specific detector of a methylated sample from *Avena* stem segments with added ^{14}C-IAA and ^{14}C-IBA internal standards.

The radioactive fractions were pooled, dried, redissolved in methanol, and placed on a Partisil-10 ODS HPLC column and eluted with 30% aqueous methanol (v/v). Radioactive fractions were reduced and methylated with diazomethane (Cohen and Schulze 1981). ^{14}C-IBA, 0.8–1.5 μg, 508 μCi/mmol, (gift of Robert Bandurski) was added immediately before methylation. The ratio of peak areas of Me-IAA and Me-IBA, as detected by GC (equipped with an N/P detector), and the ratio of radioactivity in the peaks separated by GC (equipped with an FID, with the flame extinguished) were used to determine the specific activity of the reisolated IAA according to the double standard dilution assay described by Cohen and Schulze (1981). Radioactivity measurements were corrected for counter efficiency. All samples were chromatographed on a 183 cm × 4 mm i.d. glass column packed with 3% OV-17 on 100/120 Gas Chrom Q at 180°C. The Me-IAA and Me-IBA were initially chromatographed to insure absence of interfering compounds. No contaminants that would interfere with measurements of Me-IAA and Me-IBA peak areas appeared in the plant samples (Fig. 1). Each experiment for the determination of free and bound IAA was repeated three times.

Z and Z-r Analysis

Stem segments (10 g fresh weight) were extracted in methyl alcohol, $CHCl_3$, and HCOOH according to the procedure of Bieleski (1964). ^{14}C-BA, 0.10–0.13 μg, 54 mCi/mmol specific activity, (Amersham) was added immediately after extraction. The extract plus internal standard was adjusted to pH 8.2 and partitioned with water-saturated butanol three times (Dekhuijzen 1980). The combined organic phases were reduced, placed on a cation exchange cellulose phosphate column, and eluted with 0.3 N NH_4OH at pH 8–10 (Dekhuijzen 1980). The NH_4OH was removed under vacuum and the residue, dissolved in methanol, was applied to a silica gel TLC plate, and developed in methanol:

$CHCl_3$ (9:1). The zones co-chromatographing with Z and Z-r were eluted with methanol. These zones were verified to contain cytokinin activity by their antisenescence (chlorophyll retention) activity on the first leaf of oats (Kuhnle et al. 1977). The combined aqueous extracts were degraded with β-glycosidase (Dekhuijzen 1980), partitioned with butanol, and separated on a TLC plate as described above. The Z and Z-r samples plus a GC internal standard, ^{14}C-kinetin, 0.25–0.35 μg, 10 mCi/mmol specific activity, (Cal Biochem) was methylated with diazomethane as described for IAA. The ratio of peak areas of kinetin and Z or Z-r was measured on the same column as described for IAA analysis by GC (with N/P detector) at 210°C. No labeled Z or Z-r was available to enable us to perform a double standard dilution assay. Losses during purification were estimated by ^{14}C-BA internal standard and on the GC column by ^{14}C-kinetin, which may not fully represent the losses of Z and Z-r. Radioactivity measurements were corrected for counter efficiency. GC-MS was used to compare putative methylated Z and Z-r from oat stem extracts with methylated Z and Z-r standards (Figs. 2 and 3). Each experiment was repeated three times.

Results and Discussion

Content of Free IAA and IAA Conjugate in Oat Stem-Bud Segments

Exogenous auxin is known to inhibit cytokinin-induced lateral bud release in oats (Harrison and Kaufman 1980) and in peas (Wickson and Thimann 1958). Tucker (1977) found that a high auxin level in the stem of the 'Rogue' cultivar of tomato (as determined by the *Avena* coleoptile straight growth bioassay) strongly inhibits the degree of branching. However, in the present study on oat stem-bud segments, the level of free IAA increases in segments obtained from plants 24 h after decapitation, and from those with inflorescences emerging (41–44 days old) compared with stem segments containing quiescent buds (Table 1). During these stages of inflorescence emergence and decapitation, tiller buds begin to swell, denoting the onset of tiller release (Harrison and Kaufman 1982, Harrison 1982). Gravistimulation for 24 h, which also initiates tiller bud swelling (Harrison and Kaufman 1982), does not appear to cause a change in the amount of free IAA in such stem-bud segments. Levels of ester and peptidyl IAA, primarily peptidyl conjugates in vegetative oat tissue, (Bandurski and Schulze 1977) decrease in stem segments derived from decapitated or gravistimulated shoots or in those with releasing tiller buds. Thus, in oat stem segments, no high concentration of free IAA occurs during the period of strong apical dominance, nor do IAA levels decrease during tiller release. Jablanovic and Neskovic (1977) also found a low auxin level (as determined by the *Avena* first internode test) during periods of strong lateral bud inhibition in peas and an increase in auxin concentration as the lateral buds began to grow. This auxin increase agrees with our findings in oat tiller buds that are being released at the time of inflorescence emergence. The increase in free IAA in growing buds may be due to *de novo* synthesis of IAA in the growing (swelling) buds subsequent to their release from inhibition or, alternatively, the

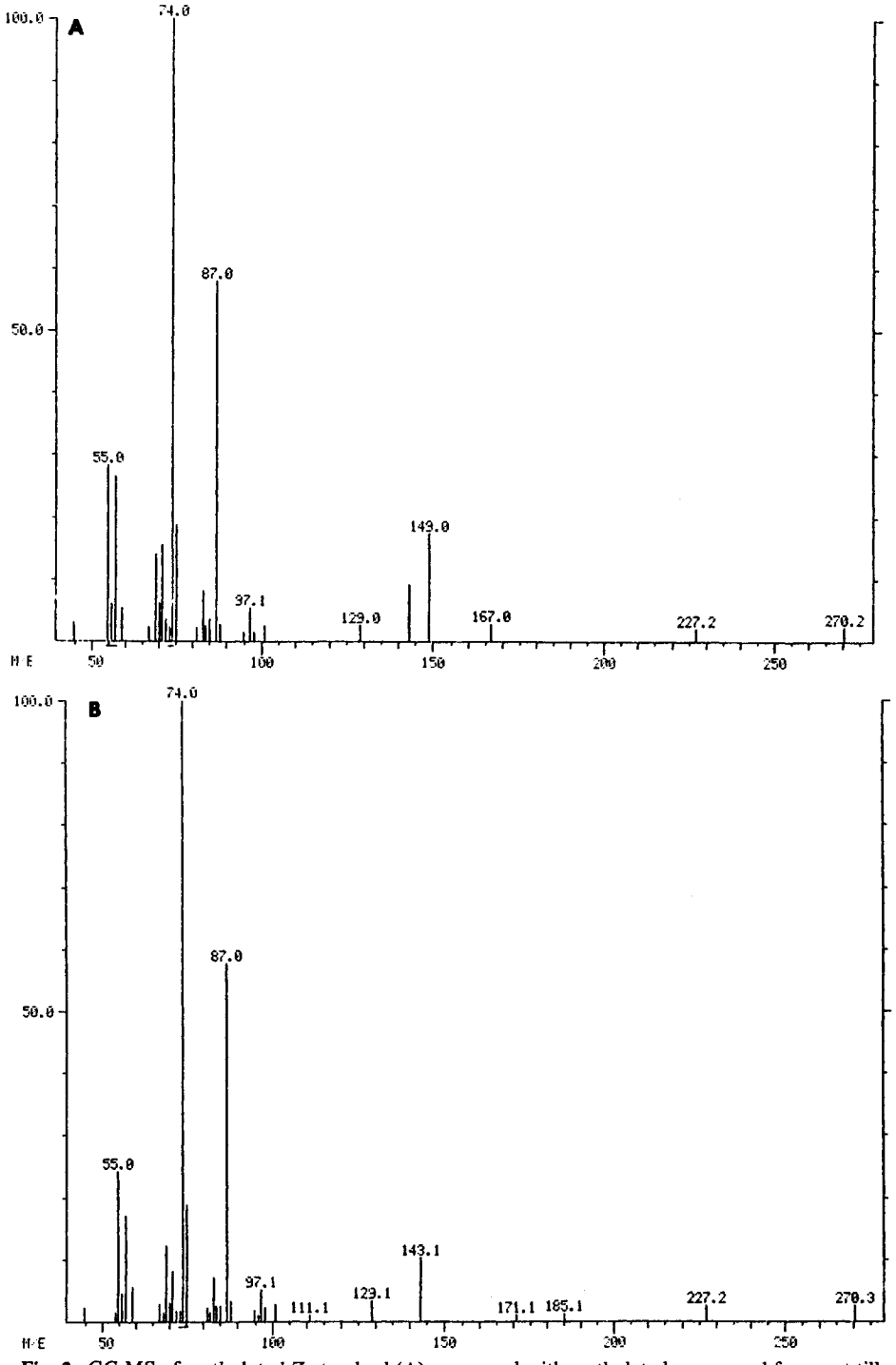

Fig. 2. GC-MS of methylated Z standard (A) compared with methylated compound from oat tiller bud extract that co-chromatographs with Z (B).

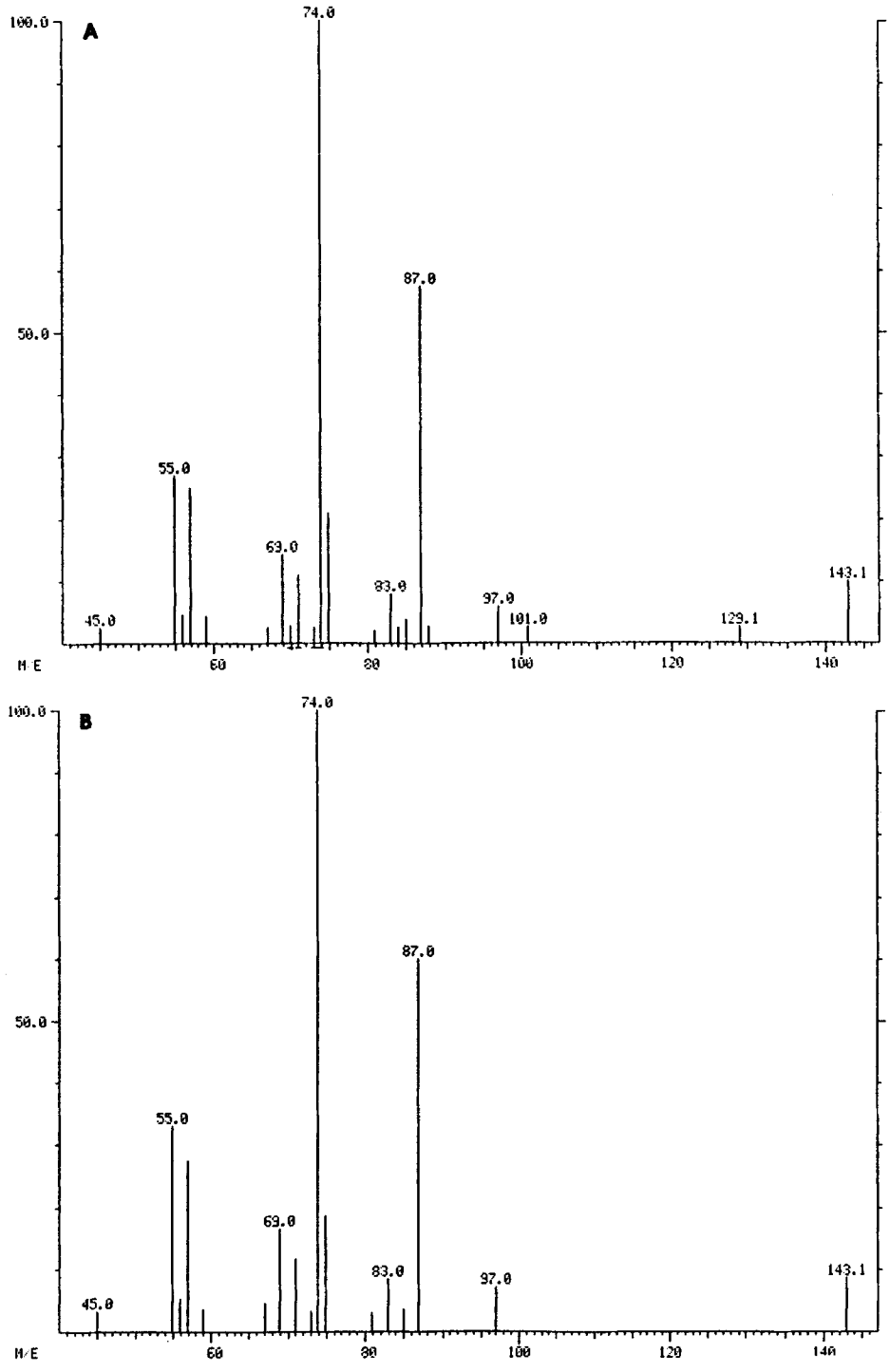

Fig. 3. GC-MS of methylated Z-r standard (A) compared with methylated compound from oat tiller bud extract that co-chromatographs with Z-r (B).

Table 1. Quantification of endogenous free and bound IAA in stem segments containing quiescent, i.e. controls (C), or releasing (R) oat tiller buds. Treatments: decapitation (D), gravistimulation (G). Mean ± SE.

Treatment	Free IAA[a] ng/g FW[c]	Bound IAA[b] ng/g FW[c]
C	1.3 ± 1.6	3.0 ± 1.8
G	1.0 ± 0.2	0.9 ± 0.1
R	4.3 ± 1.4	1.7 ± 1.0
D	7.8 ± 1.2	0

[a] No alkaline hydrolysis.
[b] IAA after hydrolysis with 7N NaOH minus free IAA.
[c] Fresh weight of tissue.

conjugated forms of IAA may release free IAA, as suggested by the studies of Hangarter and Good (1981).

Content of Zeatin, Zeatin Riboside, and Their Glucoside Conjugate in Oat Stem-Bud Segments

Jablanovic and Neskovic (1977) found a high cytokinin level (as determined by tobacco callus bioassay) in quiescent buds of peas. However, their analyses do not include the levels of conjugated forms of cytokinins. Tucker (1977) found that the cytokinin level (as determined by the cucumber cotyledon bioassay) increases during bud growth. In our studies with oat shoots, the level of free Z does not appear to change in stem segments obtained from shoots gravistimulated for 24 h, or from those with releasing tiller buds coincident with inflorescence emergence, with respect to free Z levels in control segments containing quiescent buds (Table 2). However, a marked change in Z-r occurs in stem-bud segments obtained from gravistimulated shoots and from shoots with emerging inflorescences. This increase in Z-r is accompanied by a reduction in glucoside conjugates of Z and Z-r (Table 2). Based on these findings, endogenous free Z levels do not appear to play a major role in stimulating tiller

Table 2. Quantification of endogenous free Z, Z-r, and their glycoside conjugates in oat stem segments. Segments were taken from upright controls (C), from decapitated (D) or gravistimulated (G) shoots containing quiescent tiller buds (34–38 days old), and from shoots with releasing (R) tiller buds (41–44 days old). Mean ± SE.

Treatment	ng/g FW[c] Z[a]	Z-r[a]	ng/g FW[c] Glycosyl Z[b]	Glycosyl Z-r[b]
C	16.9 ± 2.3	7.0 ± 1.0	50.0 ± 25.0	42.0 ± 20.0
G	14.2 ± 2.2	225.0 ± 110.0	16.0 ± 8.0	6.0 ± 1.8
R	12.7 ± 2.0	85.0 ± 35.0	22.0 ± 4.0	24.0 ± 8.0

[a] No β-glycosidase degradation.
[b] Z and Z-r in aqueous phase after β-glycosidase degradation.
[c] Fresh weight of tissue.

release due to gravistimulation or inflorescence emergence. However, an increase in Z-r may contribute to tiller release, and glucoside conjugation may act to store cytokinin in quiescent tiller buds. The greater increase in Z-r than loss of Z-r glucoside may also indicate *de novo* synthesis of Z-r. Morris and Akiyoshi (1983) found that in crown gall tumors, inactivation of the loci within the T-DNA, which controls root and shoot production, results in large changes in zeatin and ribosylzeatin levels. These results are of special interest in connection with our previously published results with exogenous Z and Z-r, which indicate that Z-r is significantly more effective in eliciting the release of quiescent oat tiller buds than is Z (Harrison and Kaufman 1980).

In conclusion, changes in auxin content in the stem may not play a decisive role in regulating apical dominance in oat shoots. Rather, our results indicate that the level of endogenous Z-r may control lateral bud growth. Also, the level of Z-r may itself be controlled by turnover from glucoside conjugates of the hormone. However, comparison of auxin and cytokinin levels are difficult because of differences in their biological activity and effectiveness in regulating tiller release. Further analysis is needed to determine the variation in auxin and cytokinin levels at the tiller bud site in relation to times of quiescence and release from apical dominance. The kinetics of changes in the amounts of free and conjugated forms of auxin and cytokinin during the period of release from apical dominance will be necessary in order to elucidate the roles of auxin and cytokinin in this process. Further, the possible involvement of other hormones, such as the other forms of cytokinins or of ABA, may be important in the elucidation of the process of maintenance of or release from apical dominance in shoots of the oat plant.

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