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Short Communication

Effects of Gibberellic Acid on Endogenous Indole-3-Acetic Acid and Indoleacetyl Aspartic Acid Levels in a Dwarf Pea[1]

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ABSTRACT

Two-week-old dwarf peas (*Pisum sativum* cv Little Marvel) were sprayed with gibberellic acid (GA_3), and after 3 or 4 days the upper stem and young leaf samples were analyzed for indole-3-acetic acid (IAA) and indole-3-acetyl aspartic acid by an isotope dilution high performance liquid chromatography method. GA_3 increased IAA levels as much as 8-fold and decreased indole-3-acetyl aspartic acid levels.

IAA is thought to have a major role in the regulation of elongation in young stems, and application of gibberellins may result in greatly increased stem elongation rates, particularly in dwarf varieties. Lantican and Muir (4) reported that application of GA_3 to green 8-d-old Little Marvel seedlings increased diffusible auxin collected from the apical portion of the epicotyl 3 d after treatment. Using HPLC with electrochemical detection, we have measured free IAA and IAAsp[2] levels in selected stem and leaf samples after GA_3 treatment of 14-d-old light-grown seedlings of Little Marvel pea and find that GA treatment greatly increases free IAA levels.

MATERIALS AND METHODS

Seeds of *Pisum sativum* cv little Marvel were soaked for 5 h with aeration, sown in trays of vermiculite, and placed in a greenhouse. Seedlings received Hoagland solution at 7 and 12 d, and otherwise were watered as needed. At 14 d, plants were sprayed with either 0.45 mM GA_3 (Sigma) or water (15 ml/tray of 40 seedlings, 0.1% v/v Tween 20) and returned to the greenhouse. At 17 or 18 d, epicotyls were harvested and divided into three samples which consisted of the youngest visible leaf (with the enclosed apical meristem), the uppermost 8 mm of the stem (beginning at the base of the folded stipules of the youngest leaf), and the next 8-mm stem segment. Samples were kept on ice during harvesting, weighed, and stored at −20°C until analysis. Samples for each replicate were taken from 30 to 90 plants and weighed from 0.57 to 2.64 g.

Our procedure for analysis of endogenous IAA and IAAsp has been reported previously (5). Briefly, the tissue was extracted with 70% methyl alcohol containing BHT (0.4 mg/ml). Small amounts of [1-^{14}C]IAA (Amersham Corp.) and [1-^{14}C]IAAsp (a gift from Dr. J. Cohen [3]) were added for isotope dilution analysis. After reduction of volume *in vacuo*, the extract was applied to a small trilayered column of PVP:carboxymethyl cellulose:Sephadex G-10 (10:5:10 ml) and eluted with 10 mM ammonium acetate. Labeled fractions were then applied to a DEAE-cellulose column (15 ml) and washed with the acetate buffer. IAA was eluted with 1 N acetic acid, followed by elution of IAAsp with 0.15 N HCl. Acid was removed and volumes reduced by immediately loading the IAA and IAAsp samples on a small C_{18} cartridge, washing with water, and eluting with 2 ml HPLC-grade methyl alcohol. Samples were analyzed by HPLC equipped with an electrochemical detector as previously described in (5) and calculations of endogenous levels were made by a ^{14}C fractional recovery formula. Three or more injections (45–90 μl) per sample were made for peak area and ^{14}C determinations. The eluting solvent was 20% methanol in buffer (50 mM $NaClO_4$, 5 mM sodium acetate, 1 mM EDTA) adjusted to either pH 5.0 for IAA or pH 3.5 for IAAsp (5).

RESULTS AND DISCUSSION

Typical determinations of IAA in pea stem segments are illustrated in Table I for three injections of 45 μl from the final 2-ml sample. If the IAA or IAAsp peak overlapped other peaks, separation could be achieved by a slight adjustment of the pH. On occasion, confirmation that the peaks were IAA or IAAsp was obtained as follows. (a) Samples and standards were analyzed at a lower electrochemical cell potential and the peak area was divided by the peak area at the higher cell potential. These peak area ratios were the same for standards and samples. (b) Other elution solvents were sometimes used to examine standards and samples and in all cases the retention time of standards and samples was the same. (c) Samples and standards were sensitive to 3 N HCl at 60°C for 20 min but were not sensitive to 3 N NH_4OH under the same conditions. (d) In one case, methyl esters were made with diazomethane which chromatographed like the methylated standards on HPLC (50% methanol buffer, pH 5.0).

GA_3 induced a large increase in IAA levels in the stem as well as in the apex and young leaves (Table II). The greatest increase occurred in the rapidly elongating upper stem sections, but free IAA levels were still up to 3-fold greater in the lower stem segments of treated plants compared to untreated ones. The young leaves, plus apical meristems, should include the sites of most IAA synthesis, and levels of IAA are increased up to 5-fold by GA_3 in this sample. It is interesting that the less responsive plants (experiment B) also gave a lower GA_3-induced IAA increase (Table II).

IAAsp is the only IAA conjugate we have detected in green

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[2] Abbreviations: IAAsp, indoleacetyl aspartic acid; BHT, butylated hydroxytoluene.

Table I. *Typical Determinations of Endogenous IAA in the GA_3-Treated Stem Sample*

A stem segment of 8 to 16 mm from experiment B (Table II) was analyzed by HPLC electrochemical-isotope recovery method. (Sample pmol = $\left[\frac{\text{pCi added}}{\text{pCi recovered}} \cdot \text{pmol recovered}\right] - \text{pmol added}$).

Amount Injected	Peak Area	Equivalent Standard IAA[a]	Recovered	[^{14}C]IAA[b]	Sample Calculated[c]
μl	*units*^{2}	*pmol*	*pCi*	%	*pmol*
45	67.2	11.05	141.9	57.9	664.1
45	68.4	11.25	145.9	59.5	655.7
45	66.8	10.98	144.6	59.0	642.9

[a] Based on a linear detector response of 6.08 (chart units)^2 per pmol IAA determined by injection of IAA standards on the same day samples were run.

[b] 10,900 pCi [^{14}C]IAA added initially (245.25 pCi/45 μl). This is equivalent to 184.75 pmol [^{14}C]IAA (59 pCi/pmol).

[c] Mean = 654.2 pmol ÷ 1.01 g = 647.7 pmol/g ~ 650 pmol/g.

Table II. *Effects of GA_3 on Endogenous IAA and IAAsp Levels in 14-Day-Old Little Marvel Pea in Two Experiments*

Each stem sample consisted of 8-mm-long segments cut from either the base of the youngest leaves (apical sample) or the next 8-mm stem segment (8–16 mm from the leaf base). The leaf sample consisted of the youngest visible leaves, with enclosed apical meristems. Experiment A employed 55 control and 90 GA_3-treated plants, harvested 3 d after treatment. GA_3-treated plants were 62% taller than controls. Experiment B employed 30 control and 68 GA_3-treated plants, harvested 4 d after treatment. Treated plants were 30% taller than controls.

Sample	Experiment	Fresh Wt		IAA		IAAsp	
		Control	GA_3	Control	GA_3	Control	GA_3
		g		*pmol/g*			
Apical stem segment (0–8 mm)	A	1.10	1.46	340	2860	970	790
	B	0.57	0.70	380	1180	840	720
Next stem segment (8–16 mm)	A	1.67	2.00	220	810	620	500
	B	0.74	1.01	360	650	470	360
Leaves plus meristem	A	1.78	2.64	150	820	1740	1540
	B	1.34	2.24	160	350	1280	710

Little Marvel seedlings following application of [^{14}C]IAA or high specific activity [^{3}H]IAA to intact plants and stem segments (5). The IAAsp apparently has auxin activity due to enzymic hydrolysis to release IAA (1) and may represent a storage form of auxin. In about 12 analyses of various stem and leaf samples from these seedlings (ages 13–18 d), IAAsp levels have always exceeded those of IAA, by 2- to 12-fold. Therefore, it is striking that, while GA_3 induces very high IAA levels, the amount of IAAsp decreases. Application of [^{14}C]IAA to the apices of GA_3-treated plants, followed by paper chromatography of extracts, did not reveal formation of any other conjugates (unpublished results).

We are currently examining the mechanism(s) by which GA_3 induces these changes in IAA and IAAsp levels. One possibility is that GA_3 promotes hydrolysis of the conjugate, though the free IAA amounts seem too high to be the result of conjugate hydrolysis alone. Lantican and Muir (4), based upon work with enzyme preparations derived from Little Marvel tissue and upon colorimetric analysis of IAAsp, concluded that enhanced IAA synthesis, not enzymic hydrolysis of IAAsp, was the mechanism by which diffusible auxin increased in GA_3-treated plants. The results of preliminary experiments we have performed with labeled precursors support the idea that GA_3 does indeed enhance IAA synthesis, though reduced IAAsp synthesis and/or increased conjugate hydrolysis may be factors. Another possible mechanism for the increased IAA levels in GA_3-treated plants is reduction of IAA oxidase activity.

The decrease in IAAsp would seem to be due to an inactivation or suppression of the normal conjugation system, or perhaps to a redirection of IAA from a pool in which conjugation normally occurs to one in which it does not.

It is plausible that GA_3 might act through auxin in the promotion of stem elongation. If that were the case, elevated auxin levels should occur before the growth response and auxin alone, without gibberellin, should induce growth. We are studying the time course of GA_3-induced elongation and its relationship to endogenous auxin. The second criterion generally has not been demonstrated, in that application of auxin to an intact plant does not induce elongation comparable to that from GA_3. However, this could be due to rapid inactivation through conjugation of the pulse of exogenous auxin. If GA_3 prevents IAA conjugation while enhancing synthesis, then it is possible that GA_3 acts at least in part through IAA. Brian and Hemming (2) showed that pea stem segments incubated in IAA will elongate substantially, while GA_3 alone has no effect. We find that Little Marvel stem segments respond in the same way.

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