Magnitude and Kinetics of Stem Elongation Induced by Exogenous Indole-3-Acetic Acid in Intact Light-Grown Pea Seedlings

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Exogenously applied indole-3-acetic acid (IAA) strongly promoted stem elongation over the long term in intact light-grown seedlings of both dwarf (cv Progress No. 9) and tall (cv Alaska) peas (Pisum sativum L.), with the relative promotion being far greater in dwarf plants. In dwarf seedlings, solutions of IAA (between 10^{-4} and 10^{-3} M), when continuously applied to the uppermost two internodes via a cotton wick, increased whole-stem growth by at least 6-fold over the first 24 h. The magnitude of growth promotion correlated with the applied IAA concentration from 10^{-6} to 10^{-3} M, particularly over the first 6 h of application. IAA applied only to the apical bud or the uppermost internode of the seedling stimulated a biphasic growth response in the uppermost internode and the immediately lower internode, with the response in the latter being greatly delayed. This demonstrates that exogenous IAA effectively promotes growth as it is transported through intact stems. IAA withdrawal and reaplication at various times enabled the separation of the initial growth response (IGR) and prolonged growth response (PGR) induced by auxin. The IGR was inducible by at least 1 order of magnitude lower IAA concentrations than the PGR, suggesting that the process underlying the IGR is more sensitive to auxin induction. In contrast to the magnitude of the IAA effect in dwarf seedlings, applied IAA only doubled the growth in tall seedlings. These results suggest that endogenous IAA is more growth limiting in dwarf than in tall plants, and that auxin promotes stem elongation in the intact plant probably by the same mechanism of action as in isolated stem segments. However, since dwarf plants to which IAA was applied failed to reach the growth rate of tall plants, auxin cannot be the only limiting factor for stem growth in peas.

Auxin often strongly stimulates elongation in isolated stem segments, and much progress has been made in understanding the mode of auxin action in this system (Cleland, 1987; Rayle and Cleland, 1992). However, owing to a lack of direct evidence, it remains uncertain whether the results obtained from studies on isolated stem segments are applicable to the intact stem, and whether endogenous auxin directly participates in regulating stem elongation in intact plants. In fact, in an increasing number of species, GA has been shown to play an essential role in the control of vegetative stem elongation in intact plants, with GA_{3} as the prime effector (Reid, 1987). Nonetheless, several recent studies have directly related endogenous auxin contents of elongating tissues to growth (e.g. bean stem [Bialek et al., 1983]; lupin hypocotyl [Ortuño et al., 1990]). In particular, Law and Davies (1990) found that there was a close correlation between the endogenous level of IAA and stem growth in a range of genetic lines of pea (Pisum sativum L.) differing in height, including slender phenotypes, which are ultratall regardless of their GA_{3} content. Thus, this line of evidence contends that auxin is also involved in regulating the growth of intact plant stems.

The major argument against such a role for auxin is that auxin applications to intact plants fail to elicit an appreciable growth response (Hanson and Trewavas, 1982). More recently, the growth response of intact plants to auxin application has been reexamined in dark-grown pea epicotyls (Hall et al., 1985) and in hypocotyls of sunflower, marrow (Tamimi and Fim, 1985), and watermelon (Carrington and Esnard, 1988). It was consistently found in all of these studies that exogenous auxin did strongly promote stem elongation, but that the promoting effect was short-lived (3-5 h), and auxin subsequently became inhibitory. The lack of a prolonged growth promotion by applied auxin seems to support the contention of Hanson and Trewavas (1982) that the effect of applied auxin on intact stem growth is too insignificant to account for any role of auxin in regulating stem growth. An alternative view proposes that endogenous auxin may be active, but its supply in intact plants is not a limiting factor for stem growth (Tamimi and Fim, 1983). It should be noted, however, that previous studies on the effect of applied auxin on the growth of intact stems were performed almost exclusively with dark-grown plants. In view of many differences in developmental morphology and physiology between dark-grown and light-grown tissues, it seems invalid to extend the implications of the growth response to applied auxin in dark-grown plants to the situation in light-grown plants.

The difference in stem height between tall and dwarf pea varieties is relatively small in etiolated seedlings, but it becomes striking if they are grown in the light, with stem growth in the dwarf being much more reduced than that of the tall (Muir, 1972; Ross and Reid, 1989). Furthermore, auxin levels are often lower in apices and elongating stems of light-grown dwarf plants than in their tall counterparts (Law and Davies, 1990). Therefore, should applied auxin...
effectively promote growth in intact plants, the effect ought to be clearly manifested in a light-grown dwarf pea.

We have developed a precisely controlled system for auxin application to intact plants. This, along with a high-resolution growth recording system (Behringer et al., 1990), allowed us to characterize the stem elongation responses of intact light-grown dwarf and tall pea seedlings to exogenously applied IAA. We have already shown that IAA can indeed provide a long-term stimulation of stem elongation in intact pea plants (Behringer et al., 1992). Herein, we report on the comparative responses of dwarf and tall plants and the kinetics of IAA-induced growth in the whole plant stem.

**MATERIALS AND METHODS**

**Plant Material**

Seeds of *Pisum sativum* L. cv Progress No. 9 (Agway, Syracuse, NY) and cv Alaska (Burpee, Warminster, PA) were sown individually in moist vermiculite in 100-mL plastic pots and grown and treated at 23°C under continuous light (30 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) at plant level) from cool-white fluorescent lamps (General Electric). Thirteen to 15-d-old dwarf Progress No. 9 and tall Alaska seedlings that exhibited healthy growth and uniformity were selected for experiments. At this stage, the uppermost internode (sixth, counting the cotyledons as zero) of both varieties was about 20 to 30% fully expanded, or between 2 to 3 mm long in the dwarf and around 15 mm long in the tall plant.

**Auxin Application**

To ensure precise control of dose-application and treatment duration, we developed a method for applying aqueous auxin solutions to intact stems. Solutions of IAA (Sigma) were freshly prepared in 1 mM Na\(_2\)HPO\(_4\)-citrate (pH 6.0) with 0.2% Tween-20. Control plants received the same solution without IAA. Treatment of a seedling was accomplished by a flow of solution through thin (approximately 0.1 mm), absorbent cotton strings that were evenly wrapped around the uppermost one or two internodes, depending upon the experiment type (Fig. 1). The solution was delivered at a constant rate of 0.15 mL min\(^{-1}\) by a computerized pump (Cole-Parmer Instrumental Co., Chicago, IL) through plastic tubing to the upper end of the cotton wick. After flowing around the wrapped region of the apical shoot, the solution was diverted laterally off the plant into a waste container by the extending cotton wick beneath the site of application, so as to avoid contact between the solution and any other part of the plant or soil. The addition of Tween-20 as a surfactant in all treatment solutions greatly facilitated the flow-through of solution along the cotton wick, and presumably also aided in the uptake of auxin through the cuticle around the stem surface. In this way, a seedling could be exposed to a constant strength of treatment solution throughout a prolonged period. Interchange between IAA and control solutions was completed within 30 s using computer-driven valves, which allowed withdrawal and readdition of a treatment solution with great precision.

**Figure 1.** A 14-d-old dwarf cv Progress No. 9 pea seedling illustrating the application method and stem-growth measurement. The closest stipule at the second node below the apical bud was removed to show the apical bud and uppermost internode. A continual flow of treatment solution was supplied to the seedling from the top via a cotton wick, which then wrapped around the uppermost or two internodes (depending on the experiment type), and was then diverted away from the plant to waste by the extending cotton wick beneath the site of application (down-pointing arrows). Whole-stem elongation was recorded by a transducer (T) attached above the uppermost internode (horizontal arrow, T1). Extension in the stem below the uppermost internode was simultaneously measured in some experiments by a co-attached transducer located on the second node below the apical bud (horizontal arrow, T2). Bar = 10 mm.

**Growth Measurement**

Stem elongation of intact seedlings was recorded using position transducers that were interfaced with a microcomputer for data acquisition and analysis, details of which have been previously described (Behringer et al., 1990). In brief, a very fine barb at the end of the transducer arm was pressed into the uppermost node (just below the apical bud) to record growth in the whole stem. In some experiments, a second transducer was attached to the penultimate node to measure stem growth below the uppermost internode (Fig. 1). Electric signals from the transducers were amplified, converted into digital form, and logged by a microcomputer. Stem growth was monitored over various time periods at 1-min intervals, and the data were computationally analyzed with smoothing.
on the running average of 10 data points. The weight imposed by wicking on the upper stem did not appear to affect seedling growth. After attachment to the apparatus, seedlings were allowed to equilibrate for about 1 h, during which a flow of buffer solution without IAA was applied. All treatments were performed at least five times. To exhibit the distinct features of the kinetic response, we did not average the experiment replicates and used a typical response curve for figure presentation.

RESULTS

Characteristics of IAA-Induced Stem Elongation Response in Dwarf cv Progress No. 9 Seedlings

Dwarf seedlings treated with buffer solution elongated at a nearly constant rate of about 1 μm/min. Continuous application of IAA (10^-2 M or higher concentrations) around the two uppermost internodes promoted the elongation in the whole stem by at least 6-fold over the first 20 h (Fig. 2A; Table I). However, the induced growth in the whole stem was found to be distributed only in the two uppermost internodes, because little or no growth could be recorded by a transducer attached below these internodes. Following the application of 2.5 × 10^-4 M IAA to this entire growing stem region, there was about an 18-min lag before the stem elongation rate increased sharply (Fig. 2B; Table I). The growth rose about 10 times that of the control seedling to an initial peak (IGR) approximately 50 min after the start of treatment. This was followed by a drop in the growth rate, usually to a minimum around 75 min, before a second increase to a steady-state PGR at 2 to 3 h after the application. The PGR was subsequently maintained for a period of more than 20 h at a rate about 6-fold higher than that of control seedlings (Fig. 2B). The IGR and PGR observed here in the

Table 1. Effect of IAA on stem elongation in light-grown dwarf cv Progress No. 9 pea seedlings

Buffered solution (1 mM Na₂HPO₄-citrate, pH 6.0, 0.2% Tween-20) with or without IAA was continuously applied to the intact stem by a flow of solution around the two uppermost internodes. Whole stem elongation response at selected time points after start of the treatment is shown. Data are means ± se (number of replicates shown in parentheses). NS, Not specific.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IAA Concentration (M)</th>
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<tbody>
<tr>
<td></td>
<td>0 (8)</td>
</tr>
<tr>
<td>Rate at 0 h (μm/min)</td>
<td>1.48 ± 0.19</td>
</tr>
<tr>
<td>Latent period (min)</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum rate (μm/min)</td>
<td>NS</td>
</tr>
<tr>
<td>Time until maximum rate (min)</td>
<td>NS</td>
</tr>
<tr>
<td>Rate during treatment (μm/min)</td>
<td></td>
</tr>
<tr>
<td>At 3 h</td>
<td>1.22 ± 0.17</td>
</tr>
<tr>
<td>At 6 h</td>
<td>1.24 ± 0.25</td>
</tr>
<tr>
<td>At 9 h</td>
<td>1.31 ± 0.28</td>
</tr>
<tr>
<td>At 12 h</td>
<td>1.02 ± 0.19</td>
</tr>
<tr>
<td>At 15 h</td>
<td>0.66 ± 0.17</td>
</tr>
<tr>
<td>At 20 h</td>
<td>0.56 ± 0.17</td>
</tr>
<tr>
<td>Total elongation (mm)</td>
<td></td>
</tr>
<tr>
<td>By 6 h</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>By 20 h</td>
<td>1.14 ± 0.09</td>
</tr>
</tbody>
</table>
whole stem clearly resemble those of the IAA-induced growth response in isolated stem segments (Cleland, 1987).

The promotion of stem growth tended to be progressively dampened after seedlings had been continuously treated for more than 24 h, and there was little increase after 48 h of treatment. However, if IAA was withdrawn at this time, and 3 to 4 d were allowed to elapse for the formation of new stem tissue, reaplication of IAA to the apical bud or newly formed internode again substantially promoted stem elongation as recorded above the new uppermost internode (results not shown).

**IAA Dose-Response Kinetics**

IAA at concentrations below $10^{-6}$ M had no effect on stem growth. Within the range from $10^{-6}$ to $10^{-3}$ M, a higher IAA concentration correlated with a stronger growth promotion over the first 6 h of treatment, a shorter lag time, and a higher maximum elongation rate (Fig. 2B; Table I). Seedlings showed only a single, small burst of growth at about 2 h following exposure to $10^{-6}$ M IAA. This was followed by a decline to the control rate by 4 h, so the long-term growth was not clearly enhanced. Increasing IAA concentration from $10^{-6}$ to $10^{-3}$ M only increased the magnitude of the IGR and appeared largely ineffective in initiating or maintaining the PGR, since the response was only marginal after the initial peak (Table I). Higher concentrations of IAA (above $5 \times 10^{-4}$ M) effectively elicited both the IGR and PGR. IAA at $10^{-3}$ M most strongly stimulated growth over the first 6 h of treatment, but tended to be less optimal than $2.5 \times 10^{-4}$ M thereafter, and over a long term (20 h), there was no significant difference in the effect between the two concentrations (Fig. 2A; Table I). However, we observed that in some replicates $10^{-2}$ M IAA was more effective than $2.5 \times 10^{-4}$ M IAA throughout this long-term treatment. Thus, it appears that $10^{-3}$ M IAA was approaching a concentration saturating to the growth response, and the duration of its large effect was dependent upon the response capacity of the stem, which varied somewhat with individual plants.

**Growth Kinetics following IAA Application and Withdrawal**

Withdrawal of IAA ($2.5 \times 10^{-4}$ M) after the PGR was stabilized (e.g. 5 h from the start of treatment) resulted in a rapid decline in the stem growth rate following a characteristic lag period of about 25 min (Fig. 3A). The half-time for the growth-rate decline to the level of the endogenous growth was 50 to 55 min (including the lag period). Reaplication of IAA 5 h after the time of IAA withdrawal produced a kinetic growth response similar to that induced by the first IAA treatment (Fig. 3A). When IAA was reapplied only 30 to 60 min after IAA withdrawal, the stem growth rate, although rapidly declining at the time of reaplication, was again increased with a typical latent period (Fig. 3C). It was found that the magnitude of the PGR to reapplied IAA was relatively constant, regardless of the timing of IAA reaplication after the previous withdrawal, whereas the IGR to IAA reapplied within 1 h after the withdrawal was hardly visible. The magnitude of the IGR was increasingly greater as the reaplication was further delayed beyond 1 h, approaching a maximum at about 6 h (compare Fig. 3C with 3A). The typical growth rate minimum, following the initial IGR peak but before the PGR stabilized, was occasionally not seen following either the first IAA application or a reaplication (e.g. the response to the reaplication in Fig. 3A). As proposed by Vanderhoef and Stahl (1975), the lack of such a rate minimum is probably due to an increased overlap between the IGR and PGR.

An IAA treatment as short as 10 to 30 min could fully elicit the IGR and initiate the PGR, but it failed to maintain the latter (e.g. Fig. 3B). Increasing the duration of IAA treatment only extended the maintenance of the PGR and did not appear to affect the IGR (Fig. 3B). Withdrawal of IAA after a
IAA-Induced Stem Elongation in Intact Light-Grown Peas

Figure 4. Distribution of elongation induced by applied IAA in the stem of dwarf cv Progress No. 9 seedlings. IAA at $10^{-4}$ M was continuously applied around the uppermost internode, starting at time zero. Curves represent the growth rate of (a) the whole stem (solid line), (b) the uppermost internode (light dots), (c) the stem below the uppermost internode (dark dots). Whole-stem elongation of control seedlings is depicted in Figure 2.

short treatment (30–60 min) was followed by a much longer lag, before the decline of stem growth rate (Fig. 3B), than that after a prolonged treatment (Fig. 3A), suggesting that newly treated stems are much more sensitive to growth induction by IAA than those previously treated by auxin for a prolonged period.

Growth Distribution following IAA Application to the Apical Bud or Uppermost Internode

When IAA was applied only to the apical bud or to the uppermost internode, it produced a more complex response kinetics than did application of IAA to the uppermost two internodes; the IGR comprised two prominent peaks (Fig. 4). However, the initial lag and overall magnitude of promotion of the whole stem growth was similar between application to the uppermost one or two internodes (Fig. 2 versus Fig. 4). The only difference between application to the apical bud and uppermost internode was that the lag time following apical bud application was extended to 25 to 30 min (data not shown).

To determine the relative distribution of the induced growth in the two uppermost internodes following IAA application only to the uppermost internode, growth in the whole stem and the stem below the uppermost internode was simultaneously recorded in the same seedling (see Fig. 1). Net growth in the uppermost internode was determined by subtracting growth in the stem below it from the whole stem growth. The growth in the internode just below the uppermost internode was, in fact, fully represented by that recorded in the entire stem below the uppermost internode, given that the remaining stem below the uppermost two internodes did not show any extension in response to IAA treatment, presumably because it had reached maturity. As shown in Figure 4, the IAA-induced growth was confined mainly to the internode immediately below the uppermost internode, with a relatively small amount of growth occurring in the uppermost internode. The lag time for a growth increase in the uppermost internode that directly received applied IAA coincided with that of the whole stem response, whereas it was much longer in the lower internode (60–80 min). However, the growth responses of the two elongating internodes exhibited a similar kinetic pattern, with the characteristic IGR and PGR independently produced in each internode (Fig. 4). The multiphasic growth response of the whole stem to IAA applied to the uppermost internode thus represents a temporal and spatial summation of the unsynchronized growth responses in the two elongating internodes, which were exposed at different times to the IAA being basipetally transported after application.

Effect of IAA on Stem Growth in Tall cv Alaska Seedlings

Tall, control seedlings elongated at an average rate of 8 μm/min with large rhythmic fluctuations (Fig. 5B). The period of time for these growth oscillations was around 90 to 120 min. This contrasts with a lack of any regular growth fluctuations in dwarf cv Progress No. 9 seedlings (Fig. 2B).

IAA applied around the uppermost internode of the tall seedling enhanced the growth rate of the whole stem by as much as 3-fold during the first 6 h of continuous treatment, with the total growth reaching nearly twice that of the control by 15 h (Fig. 5; Table II). The early pattern of the whole stem growth following IAA application to the uppermost internode was characterized by multiple peaks in the growth rate (Fig.

Figure 5. Characteristic elongation response in the whole stem of light-grown tall Alaska seedlings to $10^{-4}$ M IAA continuously applied around the uppermost internode. Treatment started at time zero. A, Cumulative whole-stem elongation; B, elongation kinetics.
5B). As in the dwarf seedlings, these early peaks represent the overlapping effect of IAA-induced growth responses in individual elongating internodes, which occurred with successive delays as the applied IAA was transported down the stem. However, the induced growth was found to be distributed throughout the uppermost three internodes in tall seedlings (data not shown) instead of in the uppermost two internodes, as in dwarf seedlings, which explains why the overlapping responses in the former gave rise to more peaks during the early treatment period than in dwarf plants (Fig. 5B). The IAA-induced growth stabilized after approximately 6 h of treatment, attaining a rate of about 15 μm/min, twice the steady-state rate induced by IAA in dwarf seedlings. The steady promotion by IAA treatment clearly masked the regular growth oscillations that were typical in control seedlings, but this plateau usually began to decline slowly 12 h after the start of treatment (see Fig. 5B).

**DISCUSSION**

This study unequivocally demonstrates that exogenous auxin strongly stimulates stem elongation in intact light-grown pea seedlings over a long term. We found that the effect was particularly pronounced in dwarf seedlings, in which stem growth was promoted by at least 6-fold by a continuous IAA treatment throughout 24 h. Our finding of a prolonged growth promotion by auxin in intact plants can in large measure be ascribed to the use of light-grown plants, as well as to the method of application, namely, continuous delivery of aqueous auxin solution via a cotton wick to the apical stem parts.

**Comparison of IAA Effects on Light- and Dark-Grown Plants**

In intact etiolated seedlings, it has been shown previously that applied auxin effectively promotes stem growth for only a few hours and is subsequently growth inhibiting, accompanied by immense swelling in the actively elongating stem (e.g. Sargent et al., 1974; Hall et al., 1985). However, we found that elongation in light-grown seedlings was not inhibited by continuously applied aqueous IAA, even at a concentration as high as 10⁻³ M over the first 48 h of treatment. This lack of stem-growth inhibition by high concentrations of auxin in intact, light-grown plants is consistent with the early findings from studies on isolated, light-grown stem segments (Galston and Kaur, 1961; Burg and Burg, 1968). Thus, it appears that light-grown stem tissue is far less sensitive to growth inhibition by auxin than is dark-grown tissue.

**Characteristics of IAA Action in Intact Stems**

Auxin-depleted, isolated pea stem segments are capable of large growth responses to added exogenous IAA (Penny et al., 1972; Parrish and Davies, 1975). Interestingly, we found that intact stems of dwarf cv Progress No. 9 pea, possessing a low content of endogenous IAA compared with tall cv Alaska (Law and Davies, 1990; R.H. Hamilton, personal communication), are remarkably similar to isolated stem segments in their response to exogenous IAA, with respect to both dose-response (Brian and Hemming, 1955; Galston and Kaur, 1961) and short-term kinetics (Barkley and Leopold, 1972; Penny et al., 1972). This may suggest that elongation induced by exogenous IAA in intact stems involves the same mechanism of auxin action as in isolated stem segments.

Based on studies on isolated stem segments, it has been proposed that the two phases of growth response induced by auxin (IGR and PGR) may arise from different mechanisms: the IGR is effected by a turgor-driven burst of cell extension following auxin-stimulated wall loosening, which might be mediated by wall acidification, whereas the PGR represents a late spectrum of auxin action involving steady regeneration of the capacity of the walls to undergo wall loosening (Vanderhoef and Dute, 1981; Rayle and Cleland, 1992). These notions may be corroborated by our results showing the patterns of the IGR and PGR in intact stems following addition and withdrawal of IAA at various time points (Fig. 3). The variable magnitude of the IGR, as regulated by the time between IAA withdrawal and reapplication, may provide a measure of the amount of extensible tissue within the stem readily available for immediate elongation induction by IAA. It seems that the IGR is a spontaneous process once it is initiated, whereas the PGR is heavily dependent on the continued presence of IAA for maintenance. Despite current uncertainty on the biochemical nature underlying the two responses, IAA dose-response kinetics in intact stems recorded in our study (e.g. Fig. 2B; Table 1) suggest that the IGR is far more sensitive than the PGR to auxin induction and can be separated from the latter by a much lower inductive concentration threshold.

IAA-induced growth in the intact stem was steadily maintained for a prolonged period (>20 h) by a continuous treatment (Fig. 2). The withdrawal of IAA resulted in a rapid decline in the stem-growth rate after a lag of about 25 min (Fig. 3A). This confirms that the action of exogenous IAA in intact stems is intrinsically transient. The transient action of IAA might be occasioned by the ephemeral nature of IAA-inducible mRNAs and proteins, since the combined half-life of IAA-induced mRNA (30 min) and growth-limiting proteins (20 min) estimated in maize coleoptile segments (Edelman and Schopfer, 1989) is consistent with that of IAA-induced growth response (50–55 min) as noted in our study.

The responsiveness of the intact stem to applied IAA decreased consistently with time after more than 24 h of continuous treatment. However, the responsiveness could be

**Table II. Comparison of the stem elongation response of light-grown tall (cv Alaska) and dwarf (cv Progress No. 9) pea seedlings to exogenous IAA**

<table>
<thead>
<tr>
<th>Treatment Period</th>
<th>Dwarf</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+IAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.48 ± 0.07</td>
<td>2.54 ± 0.19</td>
</tr>
<tr>
<td>+IAA</td>
<td>3.45 ± 0.24</td>
<td>7.22 ± 0.29</td>
</tr>
<tr>
<td>Control</td>
<td>0.97 ± 0.11</td>
<td>6.28 ± 0.21</td>
</tr>
<tr>
<td>+IAA</td>
<td>8.17 ± 1.08</td>
<td>15.04 ± 0.91</td>
</tr>
</tbody>
</table>

Buffered solution with or without IAA (10⁻⁴ M) was continuously applied around the uppermost internode.
largely restored in the seedling after a long interruption of the IAA treatment, during which a new internode was formed, since the reaplication of IAA to the newly formed internode became effective in promoting stem growth. It seems that the eventual lack of growth response to continuously applied IAA may be due to a shortage of extensible cells within the stem after the preexisting tissue has been fully extended. This suggests that the cellular basis of auxin-induced elongation in intact stems conforms to that in isolated stem segments, i.e. mainly through an increase in cell elongation rather than cell division (Evans, 1984).

**Auxin as a Regulator of Stem Growth in Intact Plants**

Johnson and Morris (1989) have demonstrated that IAA applied to the apical bud is transported basipetally in light-grown pea seedlings at a slow rate (about 10 mm/h). Internally transported IAA from an apical exogenous source is clearly effective in promoting the growth of elongating internodes (Fig. 4). Following the application of IAA to the stem apical region, each successively lower, growing internode showed an increased lag in the growth response compared with that in the uppermost internode (Figs. 4 and 5), indicating that there was a basipetal distribution of growth increase in the elongating stem region. Thus, it appears that the growth response correlates with the profile of the polar IAA transport as reported by Johnson and Morris (1989). Apically applied auxin is mainly transported in parenchyma cells associated with the vascular tissue in intact stems (Morris and Johnson, 1985). However, the primary target cells of auxin for growth are probably located in the outer-stem tissues, i.e. the epidermis and cortex (Rayle and Cleland, 1992). According to Sánchez-Bravo et al. (1991), exogenous IAA transported in inner-stem tissues can efficiently diffuse to the outer, responsive cells to elicit a growth response. If we assume that exogenous and endogenous IAA behave similarly in intact stems in terms of transport and action, our results suggest that endogenous IAA is actively involved in promoting stem elongation as it polarly travels through the tissues of the elongating region.

The endogenous growth rate of tall seedlings was 7 to 8 times higher than that of dwarf seedlings. However, exogenous IAA promoted growth by at least 6-fold for more than 24 h in dwarf seedlings, whereas it only enhanced growth in tall seedlings by less than 1-fold and for a shorter duration (e.g. Figs. 2 and 4 versus Fig. 5). This suggests that endogenous IAA is much more limiting to growth in dwarf seedlings than in tall seedlings, and that IAA is an important regulator of stem growth in the intact plant. This assertion is supported by the close correlation between the endogenous IAA content and stem height in various pea genotypes differing in height (Law and Davies, 1990). Thus, the far greater relative promotion of growth by applied IAA in dwarf than in tall seedlings may be a consequence of the fact that the stems of tall plants have been much more extended by their higher levels of endogenous auxin than those of dwarf plants.

However, it should be noted that the absolute magnitude of this promotion was larger in tall seedlings, and that stem growth in dwarf seedlings could hardly be promoted by exogenous IAA to the level of endogenous growth in tall seedlings (Table II). This indicates that stem elongation in intact plants is not regulated solely by auxin. Compared with stems in dwarf peas, stems of tall peas exhibit a much greater number of cells (Reid et al., 1983), which has been attributed largely to an effect of their higher GAI content (Reid, 1987). As noted in our study, auxin-induced growth in the intact stem appears to be essentially confined to an increase in cell elongation in the subtending internodes; therefore, it is conceivable that tall plant stems that are endowed with a much higher number of cells showed a greater absolute elongation potential in response to exogenous IAA than their dwarf counterparts. Taken together, it appears that stem growth in peas is regulated at least by both GA and auxin. Work is currently in progress to examine the possible interaction between the two hormones in this process.

**ACKNOWLEDGMENTS**

D.M.L. dedicates this paper to Professor Robert H. Hamilton on the anniversary of his retirement, with appreciation for his important contributions to the field of plant physiology, and with gratitude for his roles as exemplary doctoral advisor, teacher, and valued friend. The application technique successfully employed in this work was developed from ideas generated in this laboratory and in conversations between D.M.L. and R.H. Hamilton. We thank Dr. Friedrich J. Behringer for initial construction of the growth-recording apparatus and for help with growth measurement, and we thank Sandra G. Brown for typing the manuscript.

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