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THE RELATIONSHIP BETWEEN GROWTH AND INDOLE-3-ACETIC ACID CONTENT OF ROOTS OF PISUM SATIVUM L.

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The indole-3-acetic acid (IAA) content of roots and shoots of light-grown pea seedlings (*Pisum sativum* L. 'Little Marvel') growing at different rates was studied by radioimmunoassay during the first week of germination. Different growth rates were obtained by daily irrigation with either deionized water or a dilute Hoagland's mineral nutrient solution. Mineral nutrient-grown roots grew more rapidly, had more lateral roots, and initiated lateral roots at a greater distance from the apex than did water-grown roots. Growth kinetics with both treatments were biphasic. There was an initial phase of rapid cell expansion lasting about 3 days during which growth was insensitive to external mineral nutrient supply. This was followed by a slower growth phase consisting of a balance between cell division and cell expansion. Withholding nutrients resulted in the progressive inhibition of cell division during this second phase. At no time did the amount of IAA per gram fresh weight or the number of cells per gram fresh weight differ significantly in roots treated with water or mineral nutrients, whereas the total amount of IAA per organ and the total number or cells per organ were greater in roots provided with nutrients. The amount of IAA per gram fresh weight changed dramatically in roots during the first week of germination, but this was correlated with changes in the relative contributions of cell division and cell expansion to fresh weight growth rather than to growth rate. By contrast, the amount of IAA per gram fresh weight in 3-mm root tips showed a direct relationship with growth rate. The relationship between IAA content and growth in whole shoots was qualitatively and quantitatively similar to that found in whole roots.

Introduction

The role of indole-3-acetic acid (IAA) in the growth and development of roots has been the subject of considerable controversy. Early bioassays of tissue-extracted auxin showed that the amount and distribution of auxin in the root and shoot were similar (THIMANN 1934). More recently, IAA has been determined in roots by combined gas chromatography–mass spectrometry (GC-MS) (BRIDGES, HILLMAN, and WILKINS 1973; ELLIOT and GREENWOOD 1974; RIVIER and PILET 1974). A comparison of results using GC-MS and bioassay (BRIDGES et al. 1973; GREENWOOD et al. 1973) indicated that, in *Zea*, amounts of IAA in roots were similar to those in coleoptiles.

THIMANN (1937, 1977) emphasized the greater sensitivity of roots than shoots to externally applied auxin, the former responding to 100–1,000 times lower concentrations of IAA applied in solution. These observations led to the view that auxin relations are fundamentally different in the root and shoot, namely, that shoots normally contain suboptimal amounts of IAA and hence are limited in their growth by auxin while roots contain supraoptimal amounts of IAA and are actively inhibited by auxin.

In view of the continuing uncertainty surrounding IAA and root growth, we examined in detail the IAA content of *Pisum sativum* roots using a sensitive and specific radioimmunoassay (RIA) (PENGELLY and MEINS 1977). To relate IAA content to root growth, we studied the distribution of IAA in the root, changes in IAA content during growth and development, and IAA levels in plant material of the same age growing at different rates, the latter experimental condition being achieved by varying the external mineral nutrient supply. For comparison, similar studies with pea shoots were also carried out.

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Material and methods

PLANTS.—Pea seeds (*Pisum sativum* L. 'Little Marvel,' Asgrow Seed Co., New Haven, Conn.) were rinsed briefly in tap water and then surface-sterilized for 10 min with a 10% (wt/vol) filtered calcium hypochlorite solution containing either a few drops of Tween 80 (Polyoxyethylene [20] sorbitan monooleate, Fisher Scientific, Springfield, N.J.) or a small amount of commercial detergent as a surfactant. The seeds were rinsed four times, soaked for 8 h in distilled water, sown in fine-particle washed river sand (special grade #OON, Kesseli and Morse Co., Worcester, Mass.) that had been previously washed with deionized water, and watered thoroughly with deionized water. The seeds were grown in a chamber (Sherer Model CEL 511-38) illuminated with warm-white fluorescent lamps supplemented with incandescent lamps with an average of ca. 350 μE m^{-2} s^{-1}. The chamber was operated on a 16-h light-cycle with temperatures of 25 C day and 19 C dark. On the beginning of day 2 (24–27 h of germination) and daily thereafter, the plants were watered with either a 0.25-strength Hoagland's solution (HOAGLAND and ARNON 1950) or deionized water.

TISSUE EXTRACTION.—The pea seedlings were harvested and rinsed in distilled water in the darkened laboratory. The cotyledons were removed with a razor blade at the base of their petioles, and the remainder of the seedling was bisected into root and shoot by a single transverse cut through the cotyledonary node. Root or shoot pieces were blotted dry with paper towels, weighed, and immediately immersed in aqueous methanol (80% vol/vol, analytical reagent grade) prechilled to −15 C and held on ice.

For studies of the IAA content in the root apex, 12-mm root tips of intact seedlings were sliced into 3-mm sections with a razor blade cutter, weighed rapidly in groups of five, and immersed in the cold methanol. Typically, 0.07–2.0 g fresh weight of tissue were used for each IAA measurement. Plant tissues in 25 volumes of cold methanol were ground for 15 min on ice with a mortar and pestle together with a small amount of washed and ignited sand and ca. 10^5 dpm (6×10^6 Bq) of freshly purified [5-^{3}H]IAA (27 Ci mmol^{-1}, Schwarz-Mann, Orangeburg, N.Y.) added for recovery estimates (PENGELLY and MEINS 1977). During grinding, a second 25 volumes of methanol were added. The homogenate was vacuum filtered in a Büchner funnel. The residue was washed twice with 25 volumes of methanol, scraped from the filter paper, and homogenized for a second 15 min, first with a small amount of methanol to insure complete homogenization and then with 50 volumes of methanol. The second homogenate was filtered and washed as the first; filtrates and washes were pooled; and the methanol was removed by rotary evaporation at 37 C. The aqueous residue (10–20 ml) was brought to pH 8.5 with the addition of 10 ml of 0.5 M K_2HPO_4 and partially purified by repeated diethyl ether-buffer partitioning (PENGELLY and MEINS 1977) to yield a final 1.0-ml extract in phosphate-buffered saline (PBS; 0.1 M K_2HPO_4, 0.14 M NaCl, pH 8.0). Recovery of radiolabeled tracer averaged ca. 60%.

RIA.—Plant extracts in PBS were analyzed for IAA content by RIA, using a procedure modified from PENGELLY and MEINS (1977). The standard assay mixture consisted of 25 μl of [^{3}H]IAA (3×10^4 dpm), 150 μl of sample in PBS, and 25 μl of anti-IAA antiserum previously diluted with PBS to give a final antiserum dilution in the assay of 1/200. Tissue extracts were assayed in replicate tubes containing 10–150 μl of the plant extract and 140-0 μl, respectively, of PBS. Assays of 0, 0.2, 0.5, 1, 2, 5, 10, and 20 ng IAA in 150 μl of PBS were used as external standards. The solutions were mixed and incubated for 1 h at 4 C in the dark. The IAA-antibody complex that forms was precipitated by the addition of 200 μl of saturated $(NH_4)_2SO_4$ with mixing, and the solutions were cleared by centrifugation at 2,000 *g* for 40 min at 4 C. Aliquots of 100 μl of the supernatant were mixed with 1.1 ml NCS tissue solubilizer (Amersham, Arlington Heights, Ill.) and 5.0 ml of toluene containing, per 1 liter, 6 g 2,5-diphenyloxazole (PPO) and 2.5 mg 1, 4-bis-2-(5-phenyloxazolyl) benzene (POPOP).

Radioactive determinations were made using the liquid scintillation method in the Isotope Facilities of the Biological Laboratories, Harvard University, Cambridge, Massachusetts, and samples were corrected for quenching by the channels ratio method. The amount of IAA in the extract was determined by comparing the fraction of [^{3}H]IAA bound with the external standard curve. Assays were validated either by comparing the values obtained using different volumes of the plant extract or by adding internal standards of unlabeled IAA to assays of plant extracts (PENGELLY and MEINS 1977).

CELL COUNTS.—The total cell number per root was determined by counting acid-macerated tissue in the microscope. Roots were soaked in a solution of 5% (wt/vol) chromium trioxide and 5% (wt/vol) hydrochloric acid (FOSKET and TORREY 1969) for 48 h. The tissue-acid mixture was then passed through a no. 18 (0.84 mm i.d.) hypodermic needle using a 10-ml glass syringe and diluted appropriately with water to give about 50–100 cells per microscopic field. Each cell number determination represents two or three replicate experiments and the counting of at least 4,000 cells.

Results

GROWTH AND THE EFFECT OF MINERAL NUTRITION.—In preparation for measurements of IAA, we first studied the growth of pea seedlings during the first week of germination. To compare IAA content with growth, we wanted to examine plant tissues of the same age growing at different rates. We found that limiting mineral nutrients to seedlings grown in sand had a marked effect on growth, and seedlings provided with only deionized water grew considerably more slowly than those provided with mineral nutrients (table 1). Both the root and shoot were affected in this way, but the effect on root growth was most striking. The shoot had greater fresh weight than roots with water treatment, whereas root fresh weight was about twice that of the shoot with nutrient treatment. At no time during the first week of germination did seedlings provided only water show any obvious symptoms of mineral deficiency other than a reduced rate of development, and water-grown seedlings would resume vigorous growth when provided mineral nutrients (data not shown).

The effect of mineral nutrients on root form was also striking. After 7 days of germination, roots provided mineral nutrients were about three times longer than those provided water (table 1). Lateral roots were more numerous and larger, and the distance from the apex to the nearest lateral root was increased with mineral nutrient treatment (table 1).

TABLE 1

THE EFFECT OF MINERAL NUTRIENT TREATMENT ON THE GROWTH AND DEVELOPMENT OF PEA SEEDLINGS GROWN FOR 7 DAYS WITH DEIONIZED WATER OR A 0.25-STRENGTH HOAGLAND'S SOLUTION

Treatment	Shoot fresh weight (mg)	Root fresh weight (mg)	Root/shoot weight ratio	Tap root length (mm)	Number of lateral roots ≥ 1 mm long	Distance from root tip to nearest lateral root (mm)
H_2O.....	157±6 (46)	125±4 (63)	.80	33±1 (64)	8.7±1.0 (20)	20±1 (20)
Hoagland.....	238±6 (38)	468±16 (42)	1.97	88±2 (67)	27.3± .8 (20)	48±2 (20)

NOTE.—Values expressed ± standard error; sample numbers in parentheses.

Logarithmic growth curves show that root growth rate was biphasic during the first week of germination (fig. 1). The rate of tissue doubling, which is directly proportional to the slope of the semi-logarithmic plot, was greatest during the first 3 days and was roughly the same for water and mineral nutrient treatments. After 3 days, root growth rate declined with both treatments. Water-grown roots grew much more slowly than nutrient-treated roots during this second growth phase.

Growth curves for shoots show a similar biphasic course (fig. 2). As with root growth, only shoot growth in the second phase was affected by mineral nutrient supply. However, the effect of mineral nutrients appeared about a day later in shoots than in roots, and the difference between growth rates during the second phase was less than found for roots.

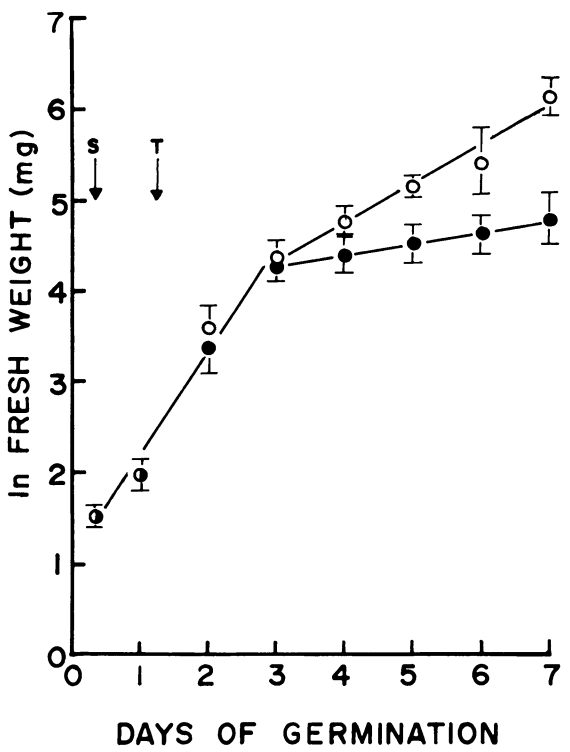

FIG. 1.—Increase in fresh weight of water-grown (●) and Hoagland-grown (○) pea roots. Arrows: *S*, end of soaking period; *T*, beginning of different nutrient treatments.

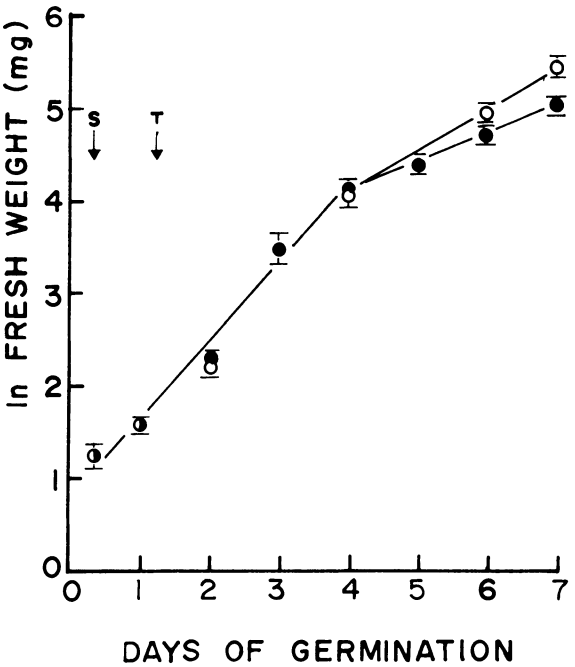

FIG. 2.—Increase in fresh weight of water-grown (●) and Hoagland-grown (○) pea shoots. Arrows as in fig. 1.

Cell counts of whole roots were performed at different stages of germination in order to determine the relationship between cell enlargement and cell division during different growth phases. The root cell number increased through the first 5 days of germination with both water and mineral nutrient treatment (fig. 3). An increase in cell number was noted as early as day 1, indicating that cell division begins early in germination, although at a low rate. By day 3, water-treated roots contained 10% fewer cells on the average than roots provided mineral nutrients, and this difference increased to 30% by day 5. On the other hand, little or no difference between treatments was noted when results were calculated on a cell per milligram fresh weight basis (fig. 3). During the first 3 days there was a rapid decline in the number of cells per milligram fresh weight, indicating that growth was based largely on cell enlargement. However, there was little or no change between days 3 and 5, indicating that growth during the second phase consisted of a balance between cell enlargement and cell division.

An important question is whether differences in cell-division frequencies were the result of an activation of cell division when nutrients were supplied or an inhibition of cell division when nutrients were withheld. To distinguish between these possibilities, cell numbers were compared before and after differences in treatment were initiated. When cell number is plotted semilogarithmically, the values for nutrient-treated roots at 3 and 5 days (after different treatments were initiated) fall on the same line as values obtained at 8 h and 1 day (before different treatments were initiated), whereas those for water-treated roots at 3 and 5 days fall below this line, the deviation from the linear relationship increasing progressively with time (fig. 4). These results indicate that mineral nutrients do not change the rate of cell division but appear instead to help maintain an exponential rate of cell division which begins very early in germination, while nutrient deprivation appears to result in progressive inhibition of cell division.

IAA LEVELS IN ROOTS AND SHOOTS.—The IAA levels in roots and shoots treated with water or mineral nutrient solutions were measured at different stages of germination by RIA. The IAA measurements are expressed in two ways. First, IAA content is expressed as the average amount per unit of tissue weight, i.e., nanogram IAA per gram fresh weight. This form of expression represents an attempt to approximate IAA concentration and, hence, hormone activity. However, because weight changes during germination, this form of expression does not reflect the net change in the amount of IAA in the organ. Therefore, IAA measurements are also expressed as the total amount of IAA per root or shoot.

The IAA content of roots changes dramatically during the first week of germination (table 2).

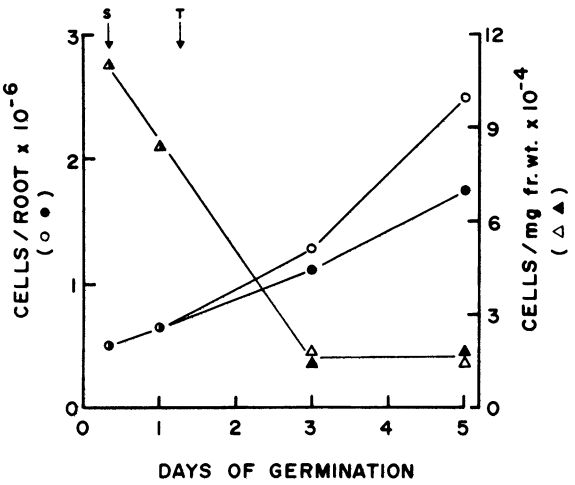

FIG. 3.—Change in the number of cells per root (○●) and the number of cells per milligram fresh weight (△▲) in water-grown (●▲) and Hoagland-grown (○△) pea roots. Arrows as in fig. 1.

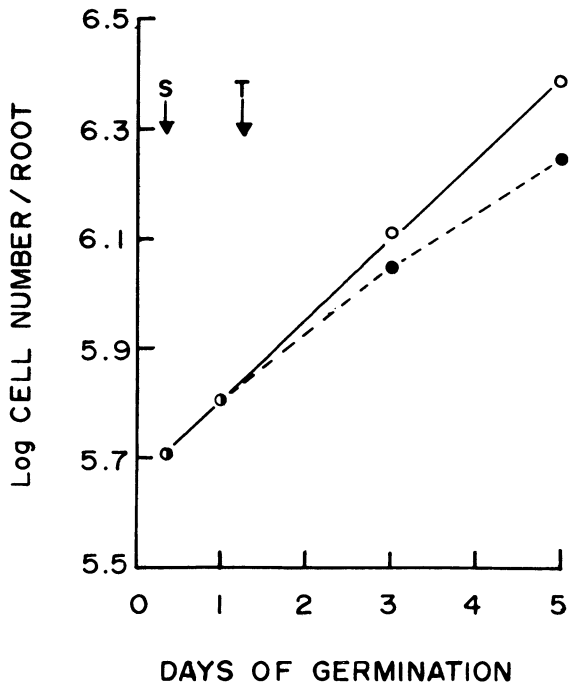

FIG. 4.—Change in cell number of water-grown (●) and Hoagland-grown (○) pea roots. Arrows as in fig. 1.

TABLE 2

IAA CONTENT OF PEA ROOTS TREATED WITH 0.25-STRENGTH HOAGLAND'S SOLUTION OR DEIONIZED WATER ONLY

GERMINATION (days)	FRESH WEIGHT		IAA CONTENT			
			ng/g fresh wt		ng/root	
	H_2O	Hoagland	H_2O	Hoagland	H_2O	Hoagland
1/3[a]	4.6±.1 (64)[b]		220±36 (4)[c]		1.03±.17 (4)	
2	31±1 (96)	38±1 (73)	46±3 (3)	51±3 (3)	1.48± .13 (3)	1.94± .11 (3)
3	73±2 (51)	79±2 (36)	60±7 (3)	66±9 (4)	4.34± .69 (3)	5.22± .57 (4)
7	125±4 (63)	468±16 (42)	38±5 (5)	36±5 (5)	5.00±1.08 (5)	14.6 ±1.97 (5)

[a] 1/3-day values represent samples taken immediately following the 8-h soaking; therefore, there is no difference in treatment.
[b] Growth values expressed ± standard error (no.), where no. = the number of roots weighed.
[c] IAA values expressed ± standard error (no.), where no. = the number of separate experiments.

Expressed on a fresh weight basis, IAA levels declined from 220 to about 50 ng/g during the first 2 days. Thereafter, however, IAA levels were in a relatively narrow range between 30 and 70 ng/g. Measurements made between 3 and 7 days were in this same range, indicating that IAA levels remain relatively stable following the initial rapid decline (data not shown). Significant differences between mineral nutrient and water treatments were not found, however, despite marked differences in growth rate.

Although the amount of IAA per unit of tissue weight did not reflect differences in growth rate when the whole root was measured, the question arises whether IAA concentrations differ locally in the growing region of the root. To answer this question, we divided the apical 12 mm of the primary root into 3-mm segments and measured the IAA content of these segments individually. When results were expressed on a fresh weight basis, the highest IAA level was found in the terminal 3-mm segment which contains the meristem; and, with the exception of roughly equal levels in the second and third segments, IAA levels in subapical segments declined progressively with increasing distance from the apex (fig. 5). In each respective segment we found IAA levels to be higher with mineral nutrient, as compared with water treatment. When the total IAA content per segment was calculated, the apical segment of the nutrient-treated root contained the highest amount of IAA of any segment tested, whereas the same segment in the water-treated root contained the least (fig. 5). Thus, the amount of IAA per gram fresh weight correlated with growth rate when the apical region of the root was examined.

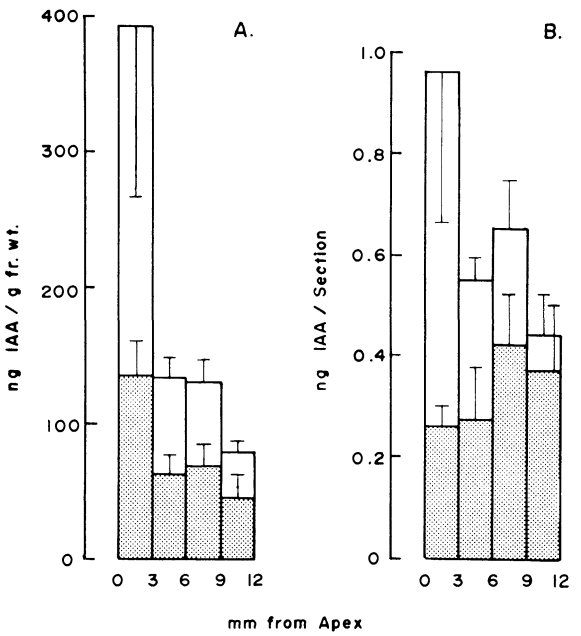

FIG. 5.—IAA concentration (*A*) and total IAA content (*B*) of 3-mm segments from 7-day-old pea root tips treated with deionized water (stippled bars) or Hoagland's solution (open bars). Error bars for the apical segment (0–3 mm) indicate the standard error for four experiments. Error bars for the remainder of the segments (3–12 mm) indicate the deviation from the mean for two experiments.

For comparison, we also measured the IAA levels in 7-day-old shoots (table 3). We found that IAA levels in the shoot, expressed on a fresh weight or per shoot basis, fell in the same range found for the root. Also, differences in IAA content between water and mineral nutrient treatments were small when expressed on a fresh weight basis, but large when expressed on a per shoot basis.

Discussion

There exists an extensive literature on efforts to determine the relationship between root or shoot growth and endogenous hormone levels in a number of different plants (THIMANN 1977; JACOBS 1980). One difficulty has been the insensitivity and unreliability of biological and chemical methods of assay. Thus, there has been considerable doubt about the natural occurrence of IAA in roots during the past 25 yr (SCOTT 1972). With the availability of analytical methods dependent on GC-MS determinations, the reliability of chemical analysis was met, but assays depended on extraction of such large amounts of tissue that experiments were difficult to perform or determinations of time courses of changing hormone levels were cumbersome and discouraging. The RIA method, with the advantage of specificity and sensitivity, can be performed rapidly on 1 g fresh weight or less of tissue. The comparability of results with GC-MS and the RIA method has now been demonstrated (PENGELLY, BANDURSKI, and SCHULZE 1981), and the RIA method can be used reliably to measure IAA in large numbers of relatively small tissue samples.

The results reported here show that IAA levels in pea roots are roughly the same as in the shoot, falling in a range between 10^{-7} and 10^{-6} M on a fresh weight basis. These values are similar to those for *Zea mays* roots, both with bioassay (GREENWOOD et al. 1973) and with GC-MS (BRIDGES et al. 1973;

TABLE 3

IAA CONTENT OF WHOLE SHOOTS OF 7-DAY-OLD PEA SEEDLINGS TREATED WITH 0.25-STRENGTH HOAGLAND'S SOLUTION OR DEIONIZED WATER

TREATMENT	FRESH WEIGHT (mg)	IAA CONTENT ng/g fresh wt.	IAA CONTENT ng/shoot
H_2O.....	138±4 (20)[a]	44±3 (3)[b]	6.1± .6 (3)
Hoagland....	215±7 (12)	53±5 (3)	12.0±1.2 (3)

[a] Growth values expressed ± standard error (no.), where no. = the number of shoots assayed in three experiments.

[b] IAA values expressed ± standard error (no.), where no. = the number of separate experiments.

RIVIER and PILET 1974). THIMANN (1934) had shown by bioassay that auxin levels in roots and coleoptiles of *Avena* seedlings were very similar, and GREENWOOD et al. (1973) obtained comparable results for IAA using bioassay of partially purified extracts of *Zea mays* roots and coleoptiles. Thus, there appears to be little doubt that roots contain IAA in amounts comparable to the shoot. The consistency of results obtained with different methods greatly strengthens this conclusion.

From the data presented here in which pea seedlings growing under two quite different conditions are compared, the following observations and conclusions can be made. Seedling growth rate in mineral nutrient solutions was much greater than in deionized water. The total amount of IAA per seedling was also greater in mineral nutrient solution than in water, indicating a direct relationship between IAA content and growth rate.

When the fresh weight growth of roots (R) and shoots (S) treated with mineral nutrients (n) or deionized water (w) are compared, relative growth ranks as follows:

$$R_n > S_n > S_w > R_w .$$

When the IAA contents of the different organs are compared instead of growth, this same series is obtained. Thus, the direct relationship between IAA content and growth appears to be independent of organ type.

This direct relationship between IAA content and growth depends on how IAA measurements are expressed. When the concentration of IAA in whole organs is estimated on a fresh weight basis and is used for comparison instead of the total amount of IAA per organ, then root and shoot IAA contents were about the same with water or mineral nutrient treatment, and no relationship with growth rate was seen. On the other hand, when the root tip was considered, IAA concentration differed markedly with treatment in rough proportion to differences in growth rate. Thus, striking local differences in IAA concentration were not detected when the amount of IAA per unit of fresh weight was averaged over the whole organ.

The reason for this appears to be that a balance between cell division and cell enlargement was maintained independent of nutrient supply. Although cell division frequencies were greater in roots growing in mineral nutrient solution than in deionized water, the number of cells per unit of fresh weight was about the same in the two treatments. We found a strong correlation between IAA content and cell division activity, as evidenced by high IAA levels in the root tip which contains the meristem and by the parallel between cell division frequency and changes in the total IAA content per root. Changes in IAA "concentration" of the whole root, however, were not related to cell division frequency but were correlated instead with changes in the relative contribution of cell division and cell enlargement to fresh weight growth. During the first 3 days of germination, growth resulted primarily from cell enlargement, and both the number of cells per gram fresh weight and the amount of IAA per gram fresh weight declined dramatically. After 3 days, however, growth depended on the rate of cell division and both the number of cells per gram fresh weight and the amount of IAA per gram fresh weight stabilized. Thus, the IAA concentration of whole roots seems to reflect the stage of growth and development rather than growth rate.

Our measurements of endogenous IAA content in pea seedlings do not support the view that auxin relations are fundamentally different in roots and shoots. We found that IAA content was directly related to growth rate, and this relationship was independent of organ type.

Acknowledgment

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