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THE CORRELATION OF DIFFERENT ASPECTS OF AUXIN ACTION[1]

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An auxin has been defined by Thimann (10) as "an organic substance which promotes growth (i.e., irreversible increase in volume) along the longitudinal axis, when applied in low concentrations to shoots of plants freed as far as practical from their own inherent growth-promoting substance. Auxins may, and generally do, have other properties, but this one is critical."[2] This definition emphasizes the aspect of auxin activity which was first studied by plant physiologists and which becomes particularly obvious when a stem or petiole responds with curvature to unilateral illumination. It was this curvature that first aroused the interest of Charles Darwin (2) and led to his classical studies on the power of movement in plants. This same curvature led Boysen-Jensen (1), thirty years later to his experiments with decapitated oat coleoptiles in which he demonstrated the capacity of the curvature stimulating influence to diffuse through a gelatin barrier and finally to the experiments of Went (12) who proved that agar on which the coleoptile tips had been placed contained a substance which caused curvature in decapitated coleoptiles.

It is therefore logical from a purely historical point of view to emphasize that aspect of auxin action which manifests as elongation growth. But, as Thimann points out in the same paper, the effects of auxin on plant cells are numerous and are by no means confined to stimulating growth in length. Stimulation of cell division in the cambium, initiation of adventitious roots, inhibition of growth of axillary buds, stimulation of parthenocarpic development in fruits are all aspects of the action of auxin. It is this multiplicity of effect which makes the action of an auxin so difficult to define and its mechanism so hard to explain. Nor is the problem made any simpler by the fact that there are many unrelated chemical compounds which function as auxins.

In the present work the technique of cultivating sterile segments of sunflower hypocotyl on nutrient agar was utilized (3). Because the segments were sterile their behavior could be studied over a period of several weeks which made possible the observation of other more slowly induced auxin effects besides the rapidly manifested effect of stem elongation. These

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[2] More recent definition of auxin was given in Plant Physiol. 29: 307, 1954. This does not differ in any essential feature from the earlier definition given by Thimann.

effects were cellular proliferation and production of adventitious roots. The work was planned to demonstrate how these three aspects of auxin activity are affected: 1) by the position, apical or basal, of the fragment in the hypocotyl, 2) by the end, apical or basal, to which the auxin was applied, 3) by the concentration of auxin, 4) by the presence or absence of light, 5) by the age of the hypocotyl from which the fragments were taken.

Materials and Methods

Sunflower seeds (var. Russian giant) were sterilized with alcohol followed by Clorox. The embryos were removed aseptically and placed on 10 ml of 1 % agar containing mineral salts in the proportions used by Hildebrandt, Riker and Duggar (6) in 22 × 175 mm tubes. Embryos to be used for experiments in light were placed in the light given by four 40-watt white fluorescent lamps at a distance of 2 feet from the light source, those to be used for experiments in darkness were germinated in complete darkness. Embryos were used when 3 or 5 days old. The hypocotyl was removed aseptically, severed just below the cotyledons and immediately above the roots. The upper and lower 5 mm of the hypocotyl was then cut off. These hypocotyl segments, to be referred to as the apical and basal fragments respectively, were then placed on 1 % agar with mineral salts, as above, containing also 2 % sucrose and dispensed in 10 ml amounts in 18 × 150 mm tubes. The agar was supplemented with indole-3-acetic acid (IAA) at concentrations of 50, 10, 1, 0.1 and 0.01 μgm/ml. In the series cultured in darkness the preparation of the hypocotyl fragments was carried out by the light of a 40-watt red Safelite, and the tubes were then incubated in darkness at 24° C. The series in light was incubated at a distance of 2 feet from a group of 4 fluorescent lamps.

Growth in length was measured after 48 hours and after 7 days. Measurements were made with a microscope equipped with a moveable stage and an ocular micrometer. Determinations of fresh and dry weights and of numbers of adventitious roots were made after 7 or 14 days.

Results

In figure 1 the response of the sunflower hypocotyl fragments to IAA has been summarized. Elongation occurred only in the apical fragments and was a rapid process, completed within the first 48 hours. Basal fragments did not elongate at all. Maximum elongation was obtained when the apical fragment was placed with the apical end on the agar. When the basal end was placed on the agar very little elongation occurred. This was no doubt the result of polarity in auxin transport. Continuous light reduced elongation but did not eliminate it. The peak of the response occurred at a concentration of from 0.1 to 0.01 μgm/ml IAA. It was strongly inhibited by concentrations of 1 μgm/ml or over. Fragments from 3-day-old hypocotyls had a slightly greater capacity to elongate than had those from 5-day-old hypocotyls.

Table I

Growth in 14 Days of Sunflower Hypocotyl Fragments Cultivated in Darkness on Agar Containing IAA (Means of 10 Values)

End in contact with agar	Measurements	IAA conc, μgm/ml 50	10	1	0.1	0.01	0
		Apical fragment					
Apical	Fresh wt, mg	86	247	195	109	77	55
	Length, mm	9.2	9.5	10.8	15.2	14.4	10.4
	Roots/fragment	0	0	0	0	0	0
Basal	Fresh wt, mg	122	379	227	202	114	43
	Length, mm	9.2	10.6	10.4	10.0	10.4	8.0
	Roots/fragment	0	0	0	1.3	0	0
		Basal fragment					
Apical	Fresh wt, mg	41	80	101	117	92	52
	Length, mm	5.4	6.0	6.4	6.4	6.4	6.0
	Roots/fragment	0	0	0.3	2.3	2.3	0.2
Basal	Fresh wt, mg	48	92	123	113	104	59
	Length, mm	5.0	5.0	6.4	6.4	6.0	5.8
	Roots/fragment	0	0	0	0.9	1.8	0.3

Fresh and dry weights of the fragments were influenced by IAA in a different way from elongation. Apical fragments, in general, showed a peak response at from 1 to 10 μgm/ml IAA whereas the peak for basal fragments was at 0.1 to 1 μgm/ml. Response in both cases was greater when the basal end of the fragments rested on the agar. Response to the lower concentrations of auxin was reduced by light.

Root initiation was greater in basal than in apical fragments and reached a maximum value when from 0.01 to 0.1 μgm IAA was applied to the apical end of the basal fragments (table I). Application of auxin to the apical end of such fragments resulted in the emergence of roots from both top and bottom of the fragment and values as high as ten roots per fragment were sometimes recorded. When auxin was applied to the basal end of the fragment only one set of roots was produced.

The appearance of fragments grown on various concentrations of IAA is shown in figure 2. These fragments were cultured in darkness for 14 days on agar containing 50, 10, 1, 0.1, 0.01 μgm/ml IAA. The quantitative aspects of their response are shown in table I. The highest IAA concentration (50 μgm/ml) was toxic, the fragments becoming converted to waterlogged masses of tissue incapable of further growth; 10 μgm/ml was toxic for the basal fragments but growth-promoting for the apical fragments; 1 μgm/ml was growth-promoting for both apical and basal fragments but inhibited elongation of the apical fragments. The concentration of 0.1 μgm/ml stimulated elongation of the apical fragment when applied to the apical end. When applied to the basal end it

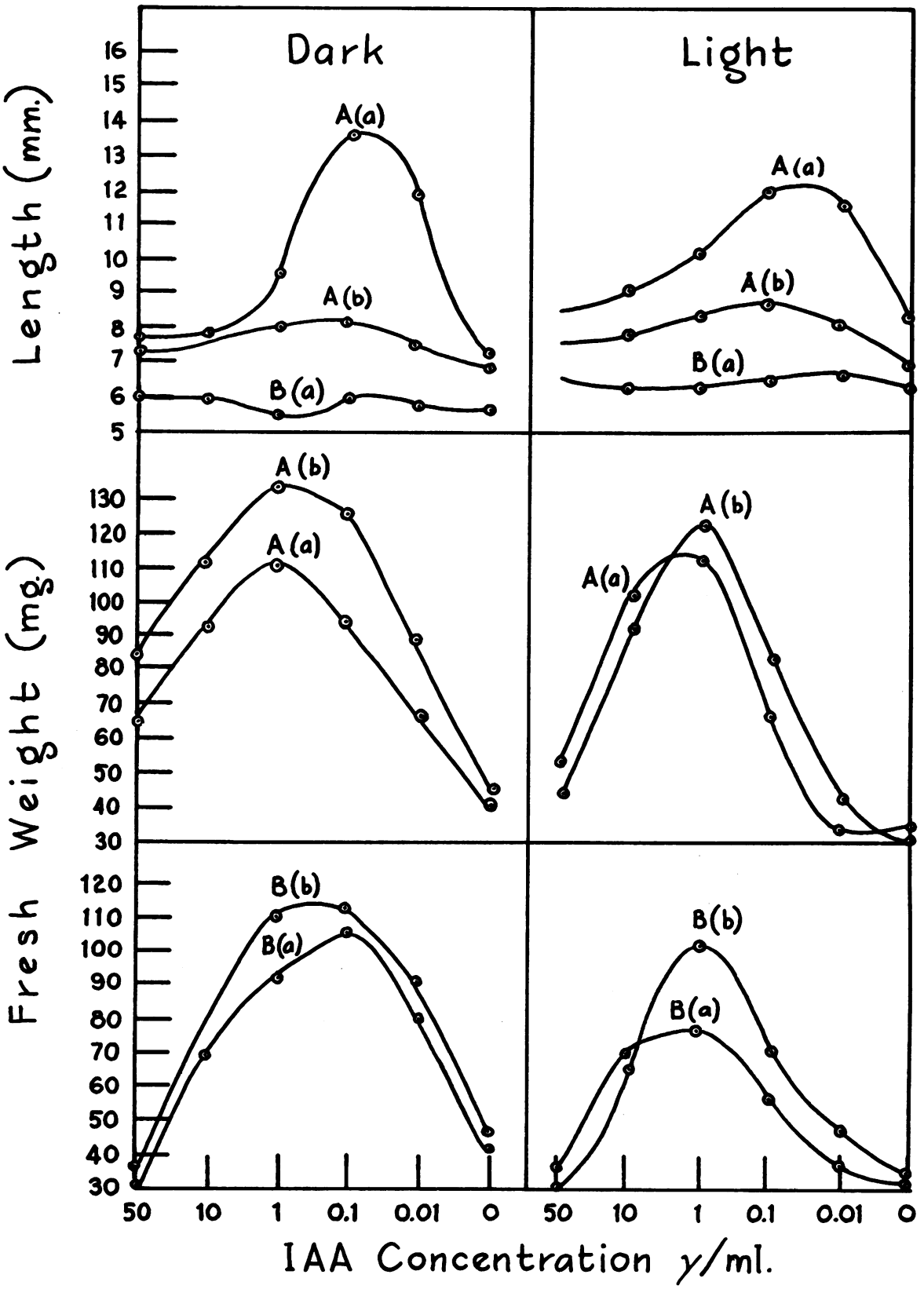

FIG. 1. Increase in length and in fresh wt in 7 days of sunflower hypocotyl fragments cultured on IAA. A—apical fragment, B—basal fragment, (a)—apical end on agar, (b)—basal end on agar.

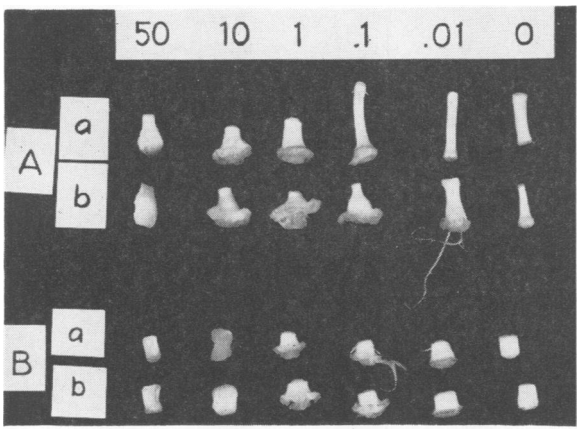

FIG. 2. Appearance of sunflower hypocotyl fragments after 14 days culture on IAA in darkness. Concentration of IAA in μgm/ml. A—apical fragment, B—basal fragment, (a)—apical end on agar, (b)—basal end on agar.

stimulated intense cellular proliferation which caused the basal end of the apical fragment to swell to several times its original diameter though very little elongation occurred. In the basal fragments this concentration of IAA stimulated the greatest root initiation. Even 0.01 μgm/ml was capable of stimulating elongation in the apical fragments when applied to the apical end and root initiation when applied to the basal end.

DISCUSSION

All the aspects of auxin activity which have been studied here have been well known for years and were described in full by Went and Thimann (13) in their classical monograph on phytohormones. The problem is essentially one of explaining why a single chemical substance, indole acetic acid, evokes more than one type of response from the tissue on which it acts. Under one set of conditions growth in length occurs, under another set of conditions cambial proliferation predominates and under a third production of adventitious roots is the outstanding characteristic of the reaction. Undoubtedly auxin concentration is of great importance in determining the kind of response which occurs. Gautheret (4) emphasized this point in 1945 and drew up a general scheme of auxin activity defining a concentration from 10 to 1000 μgm/ml as producing isodiametric growth of cells and bud inhibition, from 0.1 to 10 μgm/ml as rhizogenic, from 0.001 to 0.1 μgm/ml as inducing cellular multiplication and from 0.0001 to 0.01 μgm as promoting cellular elongation. The behavior of the sunflower hypocotyl fragment fits fairly well into this scheme. Isodiametric cell enlargement was observed close to the toxic concentration of IAA at about 10 μgm/ml, root initiation reached its peak between 0.1 and 0.01 μgm/ml, elongation was greatest at from 0.1 to 0.01 μgm/ml. The growth-promoting effect of IAA, using this term to refer only to weight increase, reached its peak between 1 and 10 μgm/ml.

Concentration of auxin is only one of the factors which influence the mode of response of plant tissue. That different regions of a growing stem elongate at different rates has been known to botanists ever since the studies of Sachs (8). The difference is not due to the fact that tissue near the apex receives more auxin than tissue near the base. As the present experiments show even the highest concentrations of IAA will not cause the elongation of fragments taken from the basal end of the stem. An explanation of the gradient of response to auxin might be sought in the findings of Galston and Dahlberg (5) who have shown that IAA oxidase, which converts IAA to a physiologically inactive product (9), increases in concentration in the tissues of the etiolated pea epicotyl as one proceeds from the apex towards the base. The basal fragments of the sunflower hypocotyl, however, have not lost their capacity to respond to IAA. What they have lost is their capacity to elongate. They respond to auxin only by increase in weight and by root initiation.

So far as root initiation by auxin is concerned, it is hard to avoid the conclusion put forward by many students of auxin activity, especially by Went (11), that auxin-induced rooting results from the interaction of auxin and some other factor which tends to accumulate in the basal portions of the stem and to be almost totally lacking in the apical regions. Previous work on the growth capacity of the sunflower hypocotyl (3) suggests that this substance needed for auxin induced root initiation reaches its maximum concentration between 30 and 60 mm from the apex of a 100 mm hypocotyl and is somewhat reduced in the lowest 5 mm of the stem. Although Went's concept of rhizocaline may require some modification, it does appear that some such factor must interact with auxin before adventitious roots can be initiated and that this co-factor tends to accumulate in the lower portion of the stem and to be absent or present in reduced amounts in the upper portion.

As regards theories of the mechanism of auxin action such as the two-point attachment theory (7), it would seem necessary to take into account the fact that different aspects of auxin activity show peak responses at widely separated concentration levels. Thus the peak for elongation occurs, for sunflower hypocotyl tissue, at between 0.1 and 0.01 μgm/ml, but the peak for weight increase occurs between 10 and 1 μgm/ml for apical fragments and 1 and 0.1 μgm/ml for basal fragments. The action of IAA does not merely become inhibitory as the concentration is raised. It inhibits one form of growth (elongation) but enhances another type of growth (weight increase due to cellular proliferation). Any satisfactory theory of auxin action must take this fact into account.

SUMMARY

1. Three aspects of auxin activity, namely, stem elongation, increase in fresh and dry weight and initiation of adventitious roots, have been studied.

2. Stem elongation took place only when the auxin was applied to the apical end of the apical 5 mm of the hypocotyl. Maximum elongation occurred at a concentration of from 0.1 to 0.01 μgm/ml IAA. Higher concentrations inhibited elongation. Fragments from the base of the hypocotyl failed to elongate on any concentration of auxin. Elongation was reduced but not eliminated by light.

3. Maximum increase in fresh weight of the fragments took place at between 1 and 10 μgm/ml IAA in the apical fragments, and this increase was greater when the IAA was applied at the basal rather than the apical end of the fragment. Fragments from the base of the hypocotyl responded with maximum weight increase to a concentration of 0.1 to 1.0 μgm/ml IAA.

4. Initiation of roots was greatest at a concentration of 0.1 μgm/ml IAA and attained a maximum value when the IAA was applied to the apical end of the basal hypocotyl fragments.

5. It is concluded that the strictly growth-promoting activity of IAA, as measured by weight increase reaches a peak at between 1 and 10 μgm/ml. Its auxin activity reaches a peak between 0.01 and 0.1 μgm/ml. The existence of two such widely separated peaks of activity must be taken into account in any comprehensive theory of auxin action.

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CALCIUM–MAGNESIUM NUTRITION WITH SPECIAL REFERENCE TO SERPENTINE SOILS[1]

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Much evidence has been presented to show that the growth of plants is markedly affected by the proportions of the exchangeable cations which are present in the soil. This evidence has been reviewed recently in detail by Mehlich and Coleman (9) and Stout and Overstreet (13). The effects on plant growth of the degree of Ca saturation of the soil colloids has been emphasized in such studies. They show that Ca absorption is decreased and plant growth limited when Ca saturation is at low levels, regardless of the complementary ions which are present on the colloids. There are definite differences, however, between the influences of adsorbed H, Mg, K, or Na on Ca availability in the soil. The present study is concerned only with the cases in which Ca and Mg are the principal exchangeable cations, and in which a raising or lowering of either of these is reflected in a complementary increase or decrease in the other. This emphasis on Ca–Mg relationships was prompted by the interest of the authors in soils derived from serpentine or related rocks, which are very high in Mg.

A number of studies have been concerned specifically with Ca–Mg interrelationships in the soil. Hunter (6) observed no effect on the yield of alfalfa when he varied the Ca/Mg ratio between 1/4 and 32/1, although Ca and Mg uptakes were altered by the changes in the soil cations. Using Ca–Mg soils, Vlamis (15) demonstrated that yields of lettuce and barley were dependent upon Ca saturation up to a level of about 20 %, above which no further response was evidenced. In experiments on several soils using Ladino clover, Giddens and Toth (2) found that reduction of the Ca saturation from 65 to 25 % restricted yields somewhat and much reduced the absorption of Ca.

It has been recognized for many years that crop plants grow poorly on serpentine soils. The early work of Loew and May (8) and the later investiga-

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