DISTRIBUTION OF ENDOGENOUS GROWTH REGULATORS WITHIN THE GEOTROPICALLY STIMULATED ROOT-TIPS

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Distribution of the acid growth substances in the upper and lower halves of geotropically stimulated root-tips (0·5 cm apical portion) of *Vicia faba* has been studied following paper-partition chromatography. Quantitative measurements (adapting oat mesocotyl segment bio-assay) of the three growth substances thus separated indicate that the substance occurring at the IAA region of chromatograms is present in higher quantity in the upper segments of stimulated tips, a situation contrary to Cholodny and Went's theory. A slow-moving compound was also found to be more in the upper segment. The third substance occurring at a high Rf value was found to be present in relatively greater quantity in the lower half of the root-tips in comparison with that in the upper half. It has been speculated that the inhibitory role of this compound on the root-tissue may not be manifested as the inhibition caused by this compound is known to be almost insignificant in the presence of high concentration of IAA-like substance. The significance of the findings has been discussed in the light of present knowledge on geostimulation of roots.

The Cholodny (1924) and Went's (1927) theory of internal redistribution of auxins has for a long time been accepted as a basic mechanism of geotropic curvature in plants. Rufelt (1957), however, suggested the possibility of an increased production of auxin in the lower half in relation to the upper half. Whichever the case may be, very few experimental evidences have been furnished to support that geostimulation actually brings about a greater accumulation of growth substances in the lower half of the reacting organ. Hawker (1932) reported that greater positive curvature can be induced in an unstimulated root of broad bean by hormones extracted (by gelatin diffusion method) from the lower halves of geotropically stimulated root-tips than by hormones extracted from the upper halves of the same root-tips. However, Ching and Fang (1958) observed no differences in the distribution of IAA-1-C^{14} in the geotropically stimulated roots of pea, lima bean and corn, and in consideration of the diverse fate of administrated IAA within the tissue they admitted that their results will be more useful for the purpose of speculation regarding the validity of the redistribution theory.

It is understandable that quantitative determination of the endogenous growth substances in the upper and lower halves of the geostimulated organs

is the only possible way of resolving this problem. In view of this, the present investigation was undertaken to assay the acid growth substances of the upper and the lower portions of the geotropically stimulated root-tips following paper-partition chromatography. Since this technique is more refined than that adopted by Hawker (1932), it was felt that the present findings will be more reliable and therefore one may know more about the distribution of the IAA-like substance, which happened to be the pivoting regulator in the opinion of the classical workers.

Material and Methods

Seedlings of *Vicia faba* were raised on washed and sterilized sand for three days in the dark at 25°±1° C. Fifteen to twenty seedlings having straight roots (*ca* 3 to 4 cm long) were selected, washed in distilled water and then pinned through the cotyledons on small cork mats covered with filter paper. The mats were placed on glass tanks containing small quantities of water so that the roots were vertical. To stimulate the roots, individual mats were turned through 90° in a predetermined sequence and at appropriate intervals, to allow for the time taken to harvest and for slicing of root-tips at the end of stimulation period (i.e. one hour). The upper portions of the stimulated tips were marked with India ink and at harvest 0·5 cm of the apical portions of roots were sliced into two equal (upper and lower) halves with a thin half-piece of razor blade held vertical on a *V*-shaped perspex holder. For slicing the tip, broader basal portion of the tip was first lightly pushed with the blade placed centrally, both the sides coming to rest on either side of the *V*-holder. Then it was pushed further and the holder guided the apex to split into halves. Although bean roots are broad and therefore easier to handle for such experiments, splitting of the tapering tip requires precise manipulation. The upper and lower portions of root-tips were collected in ethanol in flasks (pre-weighed to obtain the weight of the tissue) kept at 0° C in an ice bath. After extraction for 20 hours at −15° C the alcohol was evaporated off under reduced pressure and the acid fraction (Kefford 1955) was chromatographed in the iso-butanol : methanol : water (80 : 5 : 15) solvent system. The developed chromatograms were divided into 20 equal portions and oat (var. Victory) mesocotyl segments were used for bio-assay.

Results and Discussion

In Fig. 1 the results of two separate experiments (*a*, *b* and *c*, *d*) have been presented. In these histograms, where the bio-assay results have been illustrated, regions above the fiducial limits have been darkened to indicate the position of growth-active compounds. In the first experiment (Fig. 1, *a*, *b*), 101 tips were used and the weights of upper and lower halves were 0·5

gm and 0·48 gm respectively. Similarly in the second experiment (Fig. 1, *c*, *d*), 104 tips were used and the weight of both upper and lower halves was 0·59 gm. This indicates that the error involved in halving was almost negligible. The histograms indicate that three acid promoters are present in the

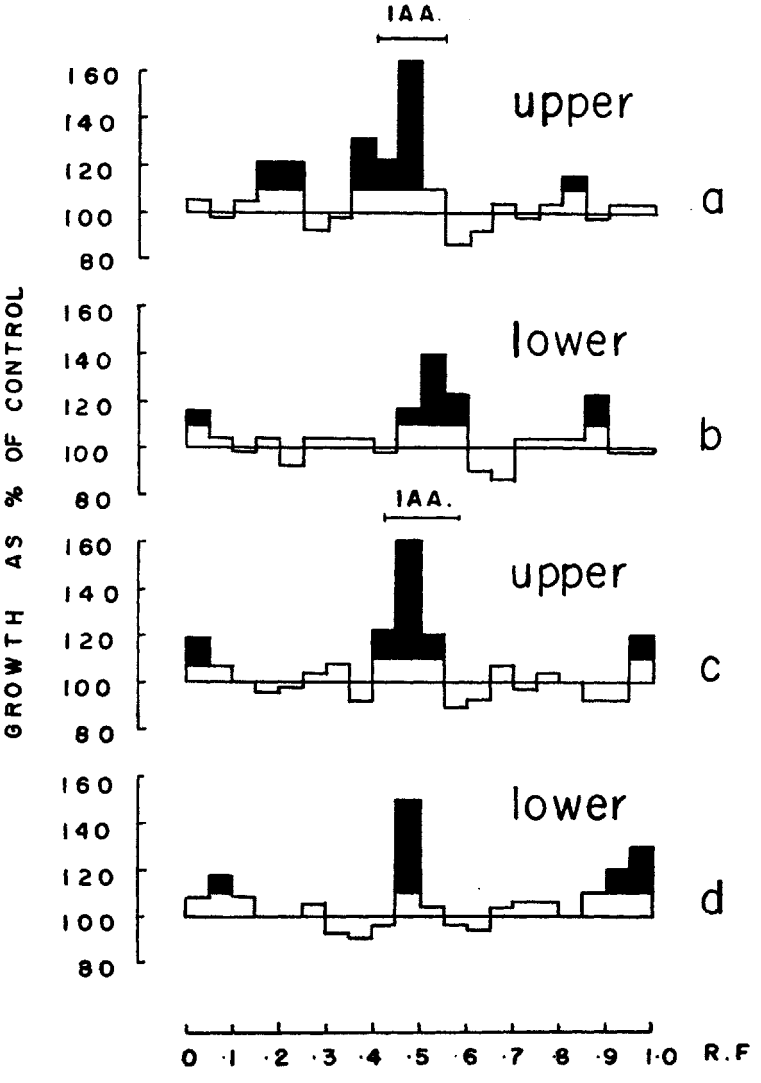

FIG. 1. Histograms illustrating the results of oat mesocotyl bio-assay of chromatograms of the acid auxins in the upper and lower halves of root-tips.

1st Expt.: *a*—upper halves; *b*—lower halves.

2nd Expt.: *c*—upper halves; *d*—lower halves.

root-tips of broad bean occurring around 0–0·1 (substance A), 0·4–0·6 (substance B) and 0·85–1·0 (substance C) Rf. The mobility of the synthetic

IAA was almost the same as the substance occurring at 0·4–0·6 Rf. The characteristics of these compounds have already been reported elsewhere (Lahiri and Audus 1960).

The histograms of upper and lower halves clearly suggest that the quantity of the substance occurring at the IAA-region of chromatograms is relatively more in the upper halves in both the experiments. The quantities of the acid growth substances have been determined here on the basis of a IAA-calibration curve and the results have been presented in Table I. It may

TABLE I

Quantities of acid growth promoters (oat mesocotyl bio-assay) in the upper and lower halves of geostimulated root-tips (0·5 cm from apex)

Tissue/Treatments	Weight of tissue extracted in gm	Age of seedlings in days	Total acid auxin μ gm in IAA Eq.	Quantities of individual growth substances (gm μ in IAA Eq.)		
				Substance A (0–0·1 Rf)	Substance B (0·4–0·6 Rf)	Substance C (0·85–1·0 Rf)
1. Stimulation for an hour:						
(*a*) Upper halves—101 tips	0·50	3	0·0803	0·002	0·078	0·0003
(*b*) Lower halves—101 tips	0·48	3	0·0123	0·0003	0·011	0·001
2. Stimulation for an hour:						
(*a*) Upper halves—104 tips	0·59	3	0·0535	0·0006	0·052	0·0009
(*b*) Lower halves—104 tips	0·59	3	0·0274	0·0004	0·022	0·005

be observed that the total acid-auxin was relatively more in the upper halves in comparison to lower halves in both the experiments. Again, the quantities of the substances A and B were relatively more in the upper halves in both the cases. The differences in the actual amounts of substance A in the upper halves in two experiments may be due to biological variations. Only the quantity of substance C was relatively more in the lower halves of tips. If one assumes that the substance occurring at the IAA-region of the chromatogram is really IAA (it gives positive colour reaction with Ehrlich's reagent), the absence of its greater accumulation in the lower half of the geotropically stimulated tips is a contradiction of Cholodny and Went's theory.

In relation to roots, the basic assumption that the endogenous level of auxin is supraoptimal has not been proved without doubt (Audus and Das 1955). Again, difficulties have been encountered to explain the geotropic

responses of *Pisum* roots (Audus and Brownbridge 1957) as well as of plagio- and dia-geotropic organs (Bennet-Clark and Ball 1951) in terms of the auxin redistribution theory.

Apart from the earlier experiments of Hawker (1932), greater accumulation of growth substances (particularly of IAA) could not be demonstrated either by Ching and Fang (1958) or in the present investigation. However, results of the present study indicate that the substance C occurs in relatively higher concentration in the under-side of geostimulated root-tips and it is also known to inhibit the root growth (Lahiri and Audus 1960). Therefore it provokes speculation whether this substance is responsible for the geotropic curvature. But earlier studies (Lahiri 1959) have shown that substance C does not inhibit the extension growth of cells till the concentration of the substance B decreases to about 0·004 μ gm/gm, a situation which often arises due to the ageing of tissue. But in this case substance B was present in the lower halves of roots-tips in much higher concentrations (0·0229 and 0·0372 μ gm/gm) in both the experiments. This suggests that effective inhibition by substance C may not be possible under such circumstances.

The results of the present investigation therefore suggest that the mechanism of geotropic bending of roots as envisaged by Cholodny and Went's theory may not be operating in broad bean roots. In recent years there has been a renewed interest in explaining gravity effects by the starch-statolith theory (Larsen 1962; Audus 1962, 1964). Whether the answer to geostimulation of roots lies there is still a matter of speculation. It is hoped that the results of the present finding will provoke further thinking regarding the mechanism involved in the geotropic curvature of roots.

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