Identification of Translocated Regulating Chemicals through Plant Response and Chromatography

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A method for identification of some absorbed and translocated plant regulators was developed. It involves comparison of a translocated substance (or substances) with the compound applied to the plant, both by chromatography and by observation of the type of morphological response induced when the translocated substance is extracted, chromatographed, and reapplied to a test plant.

In absorption and translocation studies with plant regulators it is often necessary to learn whether the regulator or a metabolite of it is moved within the plant. This has been accomplished by using a radiolabeled regulator and comparing the regulator with the translocated substance by means of paper chromatography (*3–5*). As many radiolabeled regulators are difficult to obtain, stable ones are sometimes used, and the position of the compound on a chromatogram is detected by other means such as chemical indicators or bioassay (*1, 2, 6*). These methods of locating chromatographed regulating substances which are not radiolabeled have limitations. Chemical reagents satisfactory for their detection are not always known, and bioassay methods used, for the most part, are applicable to only those regulators that affect cell elongation.

In the present method, comparison of a regulator with the translocated substance was made first on the basis of its R_f value, and second, on the basis of the type of morphological response induced by the chromatographed compound when it was eluted from the paper and applied to an intact plant. Plant responses—such as stem curvature, stem elongation, suppression of stem elongation, and formative effects of leaves—characteristic of the compound involved were used for this purpose.

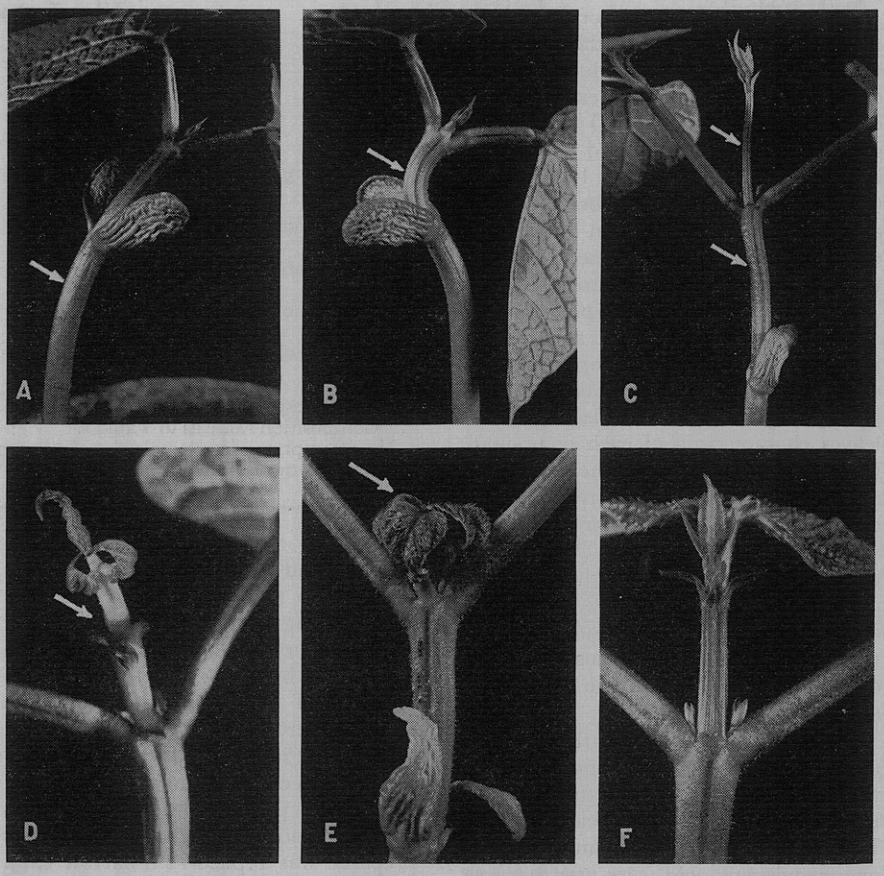

Figure 1. Characteristic responses induced by growth regulators (*A, B,* C) that influence cell elongation and those (*D, E*) that induce leaf modification when applied unilaterally to first internodes of bean plants. Arrows designate location of various responses

A. Indoleacetic acid
B. 2,4-Dichlorophenoxyacetic acid
C. Gibberellic acid
D. α-Methoxyphenylacetic acid
E. 2,3,5-Triiodobenzoic acid
F. Untreated control (to be compared with *D* and *E*)

Methods and Evaluation

Representative regulators used were indoleacetic (IAA), gibberellic, 2,4-dichlorophenoxyacetic (2,4-D), α-methoxyphenylacetic (MOPA) and 2,3,5-triiodobenzoic (2,3,5-TIBA) acids. Conventional chromatographic techniques were used in development of descending chromatograms on 1 × 18 inch strips of Whatman No. 1 paper (*7*). Known and unknown samples were always chromatographed simultaneously in the same chamber so that variation in room temperature did not affect the comparisons. The four solvents used, each in a separate chamber, were run concurrently.

Chromatograms were developed for 6 to 8 hours and, after marking the position of the fronts, the papers were dried and divided individually into pieces 1 inch long. The pieces were placed separately in marked shell vials (10 × 30 mm.). A 2.1-ml. portion of an ethyl ether–(wet) lanolin mixture (0.8 gram of lanolin per 100 ml. of ether) was added to each vial and the paper was eluted for 30 minutes. Additional ether (without lanolin) was added to maintain the volume during this period. The papers were removed, drained, and discarded, and the ether was evaporated (50° C.). The lanolin, then mainly in the bottom of a vial, contained the eluent from a segment of the chromatogram. The small amount of lanolin that adhered to the side of the vial was negligible.

The young beans (Pinto variety) used as test plants were carefully selected

for uniformity when the first internodes were 11 to 13 mm. in length and the trifoliate leaves were tightly folded within the terminal bud. Each lanolin-eluent mixture was removed from the vial and spread uniformly along one side of the first internode. Wooden applicator sticks (obtainable from medical supply houses) were used for this purpose, a different stick for each vial.

Growth regulatory effects were studied for lanolin, ether-lanolin eluents of Whatman paper exposed to each of the four solvents, and ether-lanolin eluents of Whatman paper not exposed to the solvents. No effect other than very slight negative curvature due to the lanolin was detected. Only lanolin controls, therefore, were included with each chromatogram.

The position of a regulator on a chromatogram was indicated by the particular type of growth response induced by the compound involved. For example, indoleacetic acid and 2,4-D chromatographed separately induced characteristic stem curvatures (Figure 1, *A* and *B*). On the other hand, gibberellic acid accelerated stem elongation (Figure 1, *C*), and this response was used to locate this chemical on the chromatogram. The positions of α-methoxyphenylacetic acid and 2,3,5-triidobenzoic acid on the papers were indicated by suppression of elongation of the second internode and by development of modified trifoliate leaves characteristic of each compound (Figure 1, *D* and *E*). The minimum detectable amount of each regulator required for bioassay of a chromatogram was indoleacetic acid, 10 γ; 2,4-D, 15 γ; gibberellic acid, 5 γ; α-methoxyphenylacetic acid, 50 γ; and 2,3,5-triiodobenzoic acid, 50 γ. The solvents used to chromatograph the various regulating compounds resulted in the R_f values shown in Table I.

To test the reliability of the method, a growth regulator was compared chromatographically with the translocated regulator extracted from treated plants. For this purpose, 2,4-D and α-methoxyphenylacetic acid were used because they are, according to previous investigations (*3*, *8*), absorbed and translocated. The present results also show that some 2,4-D and α-methoxyphenylacetic acid are absorbed and translocated without detectable changes in their composition (Table II).

In these comparisons, approximately 120 γ of one of the acids was applied as a lanolin mixture to an area (0.5 sq. cm.) near the petiole attachment and on the upper surface of both primary leaves of 40 young bean plants. Two days later (2,4-D) and 7 days later (MOPA), the primary leaves were removed and discarded; then the parts to be extracted (first internodes) were removed and frozen. After being thawed, internodes were ground in a mortar with quartz sand and 5 ml. of 95% ethyl alcohol. The resulting liquid was filtered from the ground tissue and the tissue re-extracted twice more by grinding with fresh 5-ml. portions of the alcohol. The three fractions were combined and evaporated to dryness (50° C.). Two-tenths milliliter of 95% ethyl alcohol was added and the soluble portion of the residue dissolved by stirring. Each extract thus prepared was applied as a narrow band to a chromatographic strip. For comparison, 25 γ of 2,4-D and 50 γ of α-methoxyphenylacetic acid were applied as bands to separate strips of paper. Extracts of juice from first internodes of untreated plants, prepared as previously described, were then applied to the papers on top of the regulator.

This method is of value in translocation studies of regulators not readily available in the form labeled with a radioactive isotope. It can be used not only with compounds that affect cell elongation, but also with those that result in the development of modified leaves. In addition, the cell elongation induced by 2,4-D, indoleacetic acid, and gibberellic acid, which results in different plant responses (Figure 1, *A*, *B*, and *C*), can be used in identification of these compounds both on the basis of their R_f values and their characteristic growth modifications. Furthermore, use of the method with radiolabeled regulators offers a means of determining whether or not the translocated radioactively tagged form of a compound is a regulator.

Table I. R_f Values[a] of Several Plant Regulators

Regulator and Responses	Solvents: n-Butyl alcohol saturated with buffer[b]	n-Butyl alcohol–benzene-buffer[c]	n-Butyl alcohol–formic acid–water[d]	Isopropyl alcohol–formic acid–water[d]
2,4-D, stem curvature and characteristic formative effects	0.55	0.45	0.90	0.90
Gibberellic acid, stem elongation	0.35	0.20	0.85	0.95
IAA, stem curvature	0.30	0.35	0.55	0.80
MOPA, characteristic formative effects	0.40	0.35	0.95	0.95
2,3,5-TIBA, characteristic formative effects	0.60	0.60	0.95	0.50

[a] R_f values rounded to closest 0.05. Experimental variation in R_f values approximately ±0.05.
[b] NH_4OH-NH_4CO_3 buffer composition: 4.25 grams of NH_4CO_3, 1.5 ml. of NH_4OH (28% NH_3), and 95 ml. of distilled water.
[c] 80, 5, 15 parts by volume, respectively.
[d] 4, 1, 5 parts by volume, respectively.

Table II. Comparison of R_f Values[a] Obtained with Plant Regulators before and after Translocation by Plants

Solvent[b]	Test	2,4-D: R_f of regulator	2,4-D: R_f of growth-modifying fraction of plant extract	MOPA: R_f of regulator	MOPA: R_f of growth-modifying fraction of plant extract
n-Butyl alcohol saturated with buffer	1	0.55	0.50	0.30	0.30
	2	0.65	0.60	0.45	0.45
n-Butyl alcohol–benzene-buffer	1	0.45	0.45	0.30	0.25
	2	0.55	0.50	0.35	0.40
n-Butyl alcohol–formic acid–water	1	0.80	0.80	0.70	0.80
	2	0.85	0.85	0.80	0.85
Isopropyl alcohol–formic acid–water	1	0.80	0.70	0.90	0.85
	2	0.70	0.75		

[a] See corresponding footnote of Table I.
[b] Composition same as in Table I.
No attempt was made to control temperature between Tests 1 and 2. This probably accounts for most of the variation observed.

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