TRANSPORT OF INDOLEACETIC ACID IN BEAN CUTTINGS IN RELATION TO ROOT FORMATION

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ABSTRACT

Transport experiments with carboxyl-^{14}C-labelled indoleacetic acid have shown that chemicals which interacted negatively with IAA in rooting of *Phaseolus vulgaris* L. cuttings significantly 'promoted upward movement and accumulation of radio-carbon of IAA-^{14}C applied to the base of the cuttings. The results suggest that increased acropetal movement of basally applied IAA adversely affects adventitious root formation in cuttings.

RECENT evidences indicate that the synergistic effects of phenalic and many other aromatic compounds in rooting cannot be explained on the basis of inhibition of the indoleacetic acid oxidizing system and consequent 'IAA-sparing action' of the synergistic chemicals.[1-4] It has been shown that a number of monophenolic chemicals which did not inhibit the activity of the IAA-oxidizing system promoted auxin-induced rooting, on the other hand, many of the polyphenolic compounds which inhibited the enzyme system did not synergise auxin-induced root formation. The information, so far available, does not give any definite idea of the mechanism of synergism or antagonism in rooting and further research on the physiological and biochemical basis of the positive or negative interactions between auxins and non-auxinic chemicals should be undertaken. The present study was taken up to see how far the synergistic or antagonistic effects of different chemicals are related to transport and distribution of exogenously applied IAA in the cuttings, employing carboxyl-^{14}C-labelled IAA and 15 non-auxinic chemicals listed in Tables I and II.

Seedlings of French bean (*Phaseolus vulgaris* L.) cv. Tender green' were grown in vermiculite under controlled light (16 hours light of 1,800 F.C. at plant level and 8 hours dark period in a 24-hour cycle) and temperature (light temperature 21 ± 1° C and dark temperature 18 ± 1° C) conditions in a growth room. When the seedlings were 12 days old, 12-15 cm tall with two primary leaves and the trifoliate bud, 10 cm long cuttings with about 4-6 cm hypocotyl portions and leaf area kept uniform at 10 sq cm per cutting, were made from them. The cuttings were allowed to form adventitious roots in glass vials (Fig. 1), eight cuttings were placed in each vial which contained 6 ml rooting solution of auxin and non-auxinic chemicals at concentrations mentioned in Table I. A separate set of glass vials was employed for the transport experiment in which carboxyl-^{14}C-labelled IAA was employed in place of non-radioactive IAA. The ^{14}C-labelled IAA, 3-indolyl (acetic acid-1-^{14}C), specific activity 33·0 mCi/mM was obtained from the Radio Chemical Centre, Amersham. The specific activity of the labelled auxin was lowered to 3·3 mCi/mM by diluting the radioactive stock with non-radioactive IAA. The cuttings treated with labelled IAA were kept in a radiochemical fumehood to avoid contamination and the cuttings of the rooting experiment on a nearby working table for 24 hours but subsequently transferred to the growth room at the light and temperature conditions mentioned above and kept there till root initiation was complete, watering with distilled water whenever necessary. Data on root furmation were taken 10 days after starting the experiment.

FIG. 1. Photographs showing (above) bean seedlings growing in vermiculite and cuttings in glass vials in the growth room and, (below) rooted cuttings in glass vials ready for taking data on root formation.

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TABLE I

Interaction of IAA with the different non-auxinic chemicals on rooting of Phaseolus vulgaris *cuttings*

	Mean No. of roots per cutting Control (no auxin)	IAA	Increase (+) or decrease (−) in number of roots per cutting due to synergism or antagonism
Control ..	12·2	14·2	..
Phenol ..	8·2	15·6	+ 5·4
2, 4-Dichlorophenol ..	9·1	13·1	+ 2·0
Guaiacol ..	10·4	15·3	+ 2·9
Hydroquinone ..	8·6	13·8	+ 3·2
Pyrogallol ..	41·3	24·6	−18·7†
p-Hydroxybenzoic acid	11·4	14·6	+ 1·2
Salicylic acid ..	9·1	16·3	+ 5·2
p-Coumaric acid ..	11·8	14·9	+ 1·1
o-Coumaric acid ..	13·1	21·5	+ 6·4
Cinnamic acid ..	14·1	24·1	+ 8·0*
Ferulic acid ..	10·2	18·1	+ 5·9
Caffeic acid ..	13·9	18·4	+ 2·5
Coumarin ..	30·0	24·3	− 7·7*
Indole ..	16·8	31·8	+13·0†
α-Naphthol ..	15·1	24·6	+ 7·5*
L.S.D. at 0·05 P ..	6·1	6·8	..
„ 0·01 P ..	8·4	9·4	..

* Significant at 0·05 P, † Significant at 0·01 P.
Concentration of IAA 5×10^{-5} M, non-auxinic chemicals at 10^{-3} M except 2, 4-DCP at 3×10^{-4} M and salicylic and cinnamic acids at 5×10^{-4} M.

TABLE II

Distribution of radioactivity (CPM) in different sectors of Phaseolus vulgaris *cuttings 24 hours after treatment with IAA-*^{14}C

	Sectors					Total per cutting
	I	II	III	IV	V	
Control ..	3542	600	225	90	63	4520
Phenol ..	2936	463	191	117	62	3769
2, 4-Dichlorophenol	3598	351	179	88	56	4272
Guaiacol ..	4747	497	139	54	47	5484
Hydroquinone ..	4472	631	256	98	63	5520
Pyrogallol ..	3724	955	548	443	115	5785
p-Hydroxybenzoic acid	3287	451	179	104	75	4096
Salicylic acid ..	3475	600	225	90	89	4479
p Coumaric acid ..	3629	376	160	78	72	4315
o-Coumaric acid ..	3317	501	117	62	75	4072
Cinnamic acid ..	3081	499	170	62	65	3877
Ferulic acid ..	5007	545	143	66	72	5833
Caffeic acid ..	4440	295	130	50	48	4963
Coumarin ..	2959	617	401	206	87	4270
Indole ..	3107	534	222	80	44	3987
α-Naphthol ..	4675	520	120	71	56	5442
L.S.D. at 0·05 P ..	153	40	27	12	14	149
0·01 P ..	206	54	37	16	19	200

Concentration of IAA-^{14}C and non-auxinic chemicals same as in Table I.

Values for synergism or antagonism were obtained by subtracting individual *effects* of IAA and any non-auxinic chemical from the *effect* due to IAA-*cum*-non-auxinic chemical, using the formula $(d-a)-[(b-a)+(c-a)]$, which resolves to $(d+a)-(b+c)$; a, b, c and d being the number of roots per cutting under control, IAA, any non-auxinic chemical and IAA-*cum*-non-auxinic chemical, respectively. Positive values (+) indicate synergism, negative values (−) denote antagonism.

Samples for the assay of radioactivity in cuttings were taken 24 hours after treatment with IAA-^{14}C, by which time the whole of the rooting solution was taken up by the cuttings and the vials had to be supplied with distilled water, and 48 hours after starting the experiment. On each sampling occasion, four cuttings were sampled. The cuttings were divided into five sectors equal in length (each sector 2 cm long); the topmost sector (sector V) included the two leaves (Fig. 2). For the estimation of

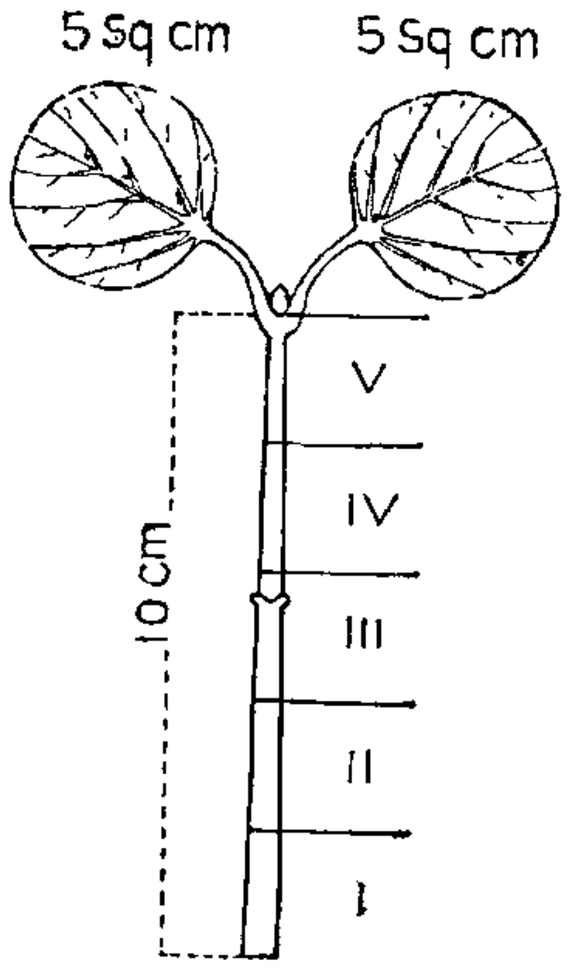

FIG 2 Diagram showing sectioning of cutting into five equal sectors for the extraction and assay of radioactivity; the fifth (topmost) sector included the leaves.

radiocarbon in tissues, the sectors were separately extracted with ethanol and radioactivity in the ethanolic extracts was assayed on a low background Nuclear-Chicago gas-flow G.M. counting system.

Pyrogallol and coumarin greatly promoted rooting of bean cuttings when applied alone but interacted negatively with IAA (Table I). The promotion due to indole and α-naphthol did not reach the level of statistical significance in absence of IAA but in combination with the auxin, indole and α-naphthol significantly synergised root formation. Synergism between

cinnamic acid and IAA was also significant ($P = 0{\cdot}05$). The synergistic effects of ferulic acid, o-coumaric acid, salicylic acid and phenol were not statistically significant. In all other cases, the effects were either inconspicuous or simply additive.

The trend of distribution and accumulation of radiocarbon in the cuttings under the different treatments was similar in the two sampling occasions. The results obtained with samples taken 24 hours after treatment with IAA-^{14}C are presented in Table II. Total radioactivity per cutting was found to be significantly influenced by the different non-auxinic chemicals. α-Naphthol and the polyphenolic chemicals, guaiacol, pyrogallol, ferulic acid, caffeic acid, and hydroquinone maintained greater radioactivity in cuttings compared to indole, coumarin and the monophenolic compounds such as *p*-hydroxybenzoic acid, salicylic acid, *p*-coumaric acid, o-coumaric acid and others. Such differences were due to the differential effects of the chemicals on the activity of the indoleacetic acid oxidizing system.[5] The results confirm the earlier findings[1,4] that synergism in rooting is not dependent on the activity of the IAA-oxidizing system and there is no direct relationship between total radioactivty per cutting and rootability.

As regards the distribution of radioactivity in the different sectors of cuttings, it was noted that pyrogallol and coumarin, the two chemicals which showed negative interaction with IAA in rooting exhibited greater upward movement and accumulation of ^{14}C in the upper sectors of the cuttings. None of the synergistic chemicals showed such promotion of upward movement of radiocarbon. The synergistic chemicals, however, showed minor differences amongst themselves but such differences were not consistently correlated with the degree of synergistic effects of the respective chemicals. The non-promotion of accumulation of radiocarbon in the tops of cuttings in presence of synergistic chemicals and the association of antagonism with increased upward movement and accumulation of radiocarbon of IAA-^{14}C suggest that for optimum rooting the exogenously applied auxin should be confined to the basal root-forming region of cuttings and distributon of basally applied IAA over a longer region of the cutting would adversely affect adventitious root formation.

The possibility of formation of different IAA-non-auxinic chemical complexes[6,7] and subsequent differential movement of such complexes to the upper parts of the cuttings is doubtful in view of the almost identical pattern of incorporation of radiocarbon of IAA-^{14}C in presence of different non-auxinic chemicals (Fig. 3). It was noted that even after

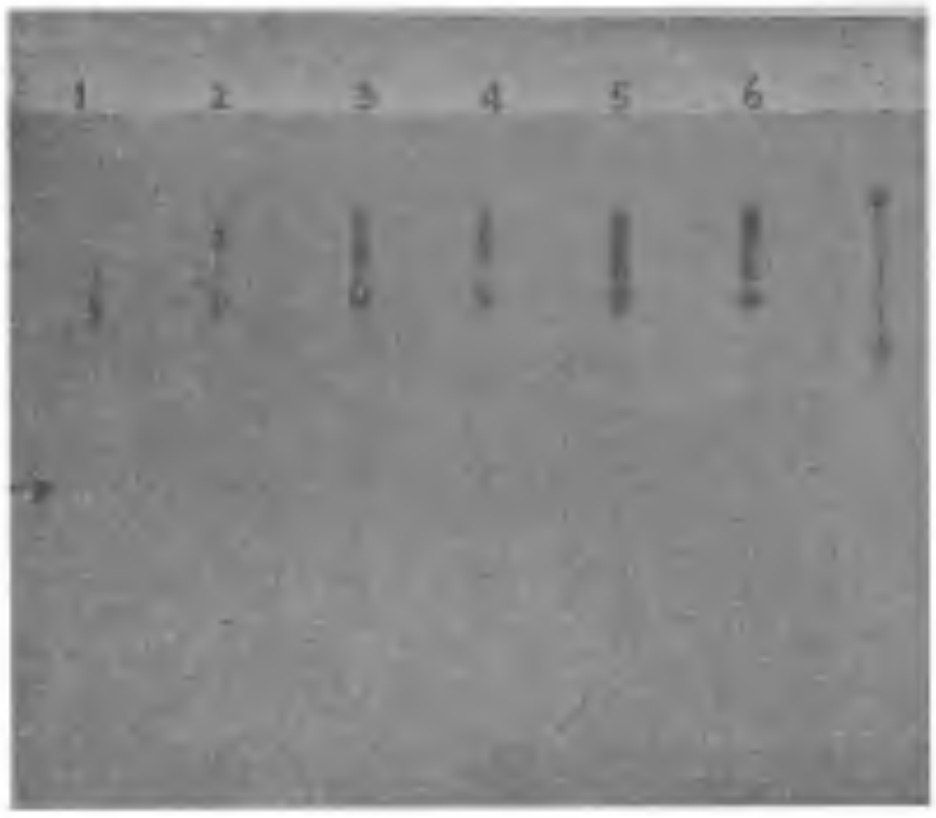

FIG. 3. Radioautograms of chromatograms of ethanolic extracts of IAA-^{14}C-treated cuttings showing incorporation of radiocarbon into different compounds from left to right (1) IAA-^{14}C + Indole, 24 hours after treatment, (2) same afrer 48 hours, (3) IAA-^{14}C + α-Napthol after 24 hours, (4) same after 48 hours, (5) IAA-^{14}C + Ferulic acid after 24 hours, (6) same after 48 hours; arrow on ieft shows the zone of resolution of synthetic IAA-^{14}C, arrow on right indicates the direction of solvent run.

24 hours, most of the radiocarbon of IAA-^{14}C was incorporated into different compounds and after such incorporation of the ^{14}C into other compounds, there was little change in total radioactivity. The differences in the relative intensities of spots on the radioautograms were due to differential effect of the non-auxinic chemicals on IAA decarboxylation prior to incorporation into other compounds. Whether in presence of antagonistic chemicals, certain of the products into which ^{14}C of the labelled IAA is incorporated show greater upward movement and accumulation in upper portions of the cuttings requires further investigation.

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