ACTIVITY OF THE IAA-SYNTHESIZING SYSTEM IN RELATION TO SYNERGISM BETWEEN AUXINS AND NON-AUXINIC CHEMICALS IN THE ROOTING OF CUTTINGS

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SUMMARY

In vitro studies on the activity of the indoleacetic acid (IAA)-synthesizing system of *Phaseolus vulgaris* L., *P. aureus* Roxb. and *Ipomoea carnea* Jacq. have shown that the root-promoting effects of phenolic and allied simple aromatic compounds can not be interpreted on the basis of the stimulation of the synthesis of IAA from the precursor tryptophan. Caffeic acid greatly stimulated the *in vitro* enzymatic synthesis of IAA from tryptophan but did not synergize the rooting of cuttings in the presence of tryptophan. Catechol, *p*-benzoquinone and *p*-coumaric acid also showed the stimulation of IAA synthesis. *P*-coumaric acid, in particular, was very effective in the presence of the mung bean enzyme but the monophenol showed no synergism with tryptophan in the rooting of mung bean cuttings.

INTRODUCTION

Basu (1970) showed that the synergism between auxins and non-auxinic chemicals in the rooting of cuttings could not be explained on the basis of an IAA-protection mechanism by the synergistic chemicals. While a number of non-auxinic chemicals that stimulated the activity of IAA-oxidizing system, promoted auxin-induced root formation as well, there were others that did not promote rooting in the presence of auxins even though, they significantly inhibited the IAA-oxidizing system. Gorter (1969) and Basu (1969, 1970) suggested that the root-promoting effects of the synergistic chemicals cannot possibly be interpreted in terms of enhanced IAA synthesis from the precursor tryptophan by the endogenous IAA-synthesizing system, as the chemicals which are known to stimulate the IAA-synthesizing system, did not

show any major effect on the rooting of cuttings of *Phaseolus vulgaris* L. In the present investigation, the direct enzymatic synthesis of IAA from tryptophan in the presence of a number of non-auxinic chemicals has been studied *in vitro* and the root-promoting effects of the different non-auxinic chemicals have been related to their respective capacities to stimulate the synthesis of IAA from tryptophan.

Materials and Methods

Rooting experiments.—Seeds of French bean (*P. vulgaris* L.) and mung bean (*P. aureus* Roxb.) were sown in washed sand under natural outdoor conditions and 10-day old seedlings were harvested for the preparation of cuttings following the methods as described by Basu (1970) and Basu *et al.* (1970). Softwood *Ipomoea (Ipomoea carnea* Jacq.) cuttings, each 25 cm long, with about 1 cm basal diameter and having one pair of young expanding leaves and the upper unfolded bud, were prepared from vigorously-growing young shoots. Ten cuttings of each material were treated with the non-auxinic chemicals singly or in combination with IAA or tryptophan in 10-ml vials for French bean and mung bean and in 250-ml beakers for *Ipomoea* cuttings for 24 hr and the data on the number of roots produced were recorded after 10 days in case of French and mung bean and after 20 days in case of *Ipomoea*. Synergism and antagonism between the interacting chemicals were calculated following the methods described by Basu (1972).

Enzymatic studies.—The IAA-synthesizing system was extracted from the cuttings of each of the species of plants following in principle, the methods of Gordon (1958) and Gordon and Paleg (1961). Whole cuttings were chopped into small pieces and ground in a mortar with equal volume of buffer at pH 7.2 (0.1 M phosphate buffer containing 0.3 M sucrose). The slurry was strained through muslin cloth and centrifuged at 3500 × g for 10 min and the supernatant was used immediately.

The incubation mixture consisted of 1 ml of enzyme solution (equivalent to 0.5 g fresh weight of the tissue), 0.6 ml of the respective non-auxinic chemicals (5×10^{-3}, 5×10^{-4} or 5×10^{-5} M), with or without 1 ml of 0.02 M L-tryptophan, finally making a total volume of 3.0 ml by adding requisite quantity of phosphate buffer pH 7.2 (0.1 M). The incubation was done at 25±1°C for 2 hr after which the reaction was stopped by heating the mixture for 2 min. The mixture was centrifuged at a high speed and to the clear supernatant, 3.0 ml of Gordon and Weber's reagent was added. The intensity of the pink colour was read

at 530 mμ on a AIMIL Hilger-Biochem Absorptiometer. The IAA in the incubation mixture was also chromatographically purified and assayed colorimetrically using the Salkowski' s reagent.

Results and Discussion

While in French bean cuttings, indole, ferulic acid, *p*-coumaric acid and caffeic acid all synergized rooting in the presence of IAA but not with tryptophan (Table I), in the mung bean cuttings, rooting was not much influenced by the non-auxinic chemicals. The non-auxinic chemicals did not give any synergistic effect in combination with tryptophan (Table II). In case of *Ipœnoea* cuttings, however, indole, catechol, *p*-coumaric acid and *p*-hydroxybenzoic acid showed synergism with IAA, the effect of indole being maximum. There was no synergism between tryptophan and any of the non-auxinic chemicals (Table III).

Table I. Mean number of roots per Cutting (± S.E.) produced on French bean cuttings when treated with the non-auxinic chemicals singly and in combination with IAA or tryptophan. Values in parentheses denote synergism (+) or antagonism (—)

Non-auxinic chemical	Control	IAA	Tryptophan
Control	16.5±0.57	23.6±1.41	19.4±0.57
Indole	24.2±2.40	60.0±8.50 (+28.7**)	25.4±3.45 (—1.7)
Catechol	42.4±4.59	55.5±1.22 (+6.0)	37.3±1.35 (—8.0)
p-Benzoquinone	20.8±1.47	33.2±0.28 (+5.3)	19.7±1.58 (—4.0)
p-Hydroxybenzoic acid	18.8±4.81	32.7±2.37 (+6.8)	19.4±1.95 (—2.3)
p-Coumaric acid	18.9±2.36	37.5±3.09 (+11.5*)	21.9±0.31 (+0.1)
Ferulic acid	14.5±2.05	37.1±2.00 (+15.5**)	19.4±1.68 (—2.0)
Caffeic acid	25.1±2.56	39.5±2.02 (+7.3*)	32.6±1.14 (+4.6)

Concentration of IAA, 5×10^{-5}M; tryptophan, 5×10^{-5}M; non-auxinic chemicals, 10^{-3}M.

* Significant at 0.05 P, ** Significant at 0.01 P

Table II. Mean number of roots per cutting (±S.E.) produced on mung bean cuttings when treated with the non-auxinic chemicals singly and in combination with IAA or tryptophan. Values in parentheses denote synergism (+) or antagonism (−)

Non-auxinic chemical	Control	IAA	Tryptophan
Control	10.6±0.77	11.0±0.85	11.8±1.26
Indole	19.1±1.77	22.2±2.45 (+2.7)	19.7±2.15 (−0.6)
Catechol	16.6±1.50	17.1±1.89 (+0.1)	17.0±2.43 (−0.8)
p-Benzoquinone	15.5±1.89	14.0±1.25 (−1.9)	15.6±1.74 (−1.1)
p-Hydroxybenzoic acid	14.7±1.91	15.4±2.53 (+0.3)	14.4±1.62 (−1.5)
p-Coumaric acid	12.1±0.86	12.6±1.46 (+0.1)	12.7±1.28 (−0.6)
Ferulic acid	12.3±1.20	14.1±1.71 (+1.4)	12.1±1.46 (−1.4)
Caffeic acid	16.9±1.09	16.0±1.17 (−1.3)	14.5±0.36 (−3.6*)

Concentration of IAA, 10^{-5}M; tryptophan, 10^{-5}M; non-auxinic chemicals, 5×10^{-4}M.

*Significant at 0.05 P

Table III. Mean number of roots per cutting (± S.E.) produced on Ipomoea *cuttings when treated with the non-auxinic chemicals singly and in combination with IAA or tryptophan. Values in parentheses deenote synergism (+) or antagonism (−)*

Non-auxinic chemical	Control	IAA	Tryptophan
Control	12.8±1.38	45.5±1.21	14.3±0.71
Indole	11.1±0.77	94.3±9.31 (+50.5**)	16.6±0.88 (+4.0)
Catechol	17.5±0.77	69.0±1.15 (+18.8**)	13.5±1.18 (−5.5)
p-Benzoquinone	15.1±1.11	46.0±2.85 (−1.8)	19.0±1.48 (+2.4)
p-Hydroxybenzoic acid	12.3±0.95	56.0±4.29 (+11.0*)	17.4±1.11 (−3.6)
p-Coumaric acid	19.1±1.81	64.6±3.03 (+12.8**)	14.5±0.65 (−6.1)
Ferulic acid	13.0.±0.57	51.8±1.79 (+6.1)	11.4±0.89 (−3.1)
Caffeic acid	13.6±1.15	50.0±0.82 (+3.7)	13.3±0.75 (−1.8)

Concentration of IAA, 10^{-4}M; tryptophan, 10^{-4}M; non-auxinic chemicals, 10^{-3}M.

* Significant at 0.05 P; ** significant at 0.01 P

In the presence of the IAA-synthesizing system from the three plant materials, caffeic acid (10^{-3} M) significantly stimulated the synthesis of IAA from tryptophan (Tables IV-VI). *P*-benzoquinone, *p*-coumaric acid and catechol also showed some promotion of IAA synthesis in case of mung bean and *Ipomoea*, the effect in each case, however, decreased with decreasing concentration except with caffeic acid which stimulated the synthesis of IAA even at 10^{-4} M in French bean (Table IV).

Table IV. Effect of the non-auxinic chemicals on the in vitro *activity of the French bean IAA-synthesizing system. μg IAA synthesized from tryptophan per g tissue equivalent enzyme per hr*

Non-auxinic chemical	Concentration (M)		
	10^{-3}	10^{-4}	10^{-5}
Control	2.4	1.7	1.7
Indole	2.0	—	—
Catechol	3.9	2.5	1.7
p-Benzoquinone	5.6	2.2	1.5
p-Hydroxybenzoic acid	3.6	—	—
p-Coumaric acid	5.2	2.1	1.7
Ferulic acid	1.3	—	—
Caffeic acid	14.1	6.5	1.7
L.S.D. at 0.05 P	1.7	0.4	N.S.
,, 0.01 P	2.3	0.5	N.S.

N.S. = Not significant

Table V. Effect of the non-auxinic chemicals on the in vitro *activity of the mung bean IAA-synthesizing sytem. μg IAA synthesized from tryptophan per g tissue equivalent enzyme per hr*

Non-auxinic chemical	Concentration (M)		
	10^{-3}	10^{-4}	10^{-5}
Control	11.4	15.2	15.4
Indole	9.5	—	—
Catechol	19.8	15.9	17.2
p-Benzoquinone	16.9	15.7	17.2
p-Hydroxybenzoic acid	12.8	—	—
p-Coumaric acid	34.5	19.2	17.2
Ferulic acid	10.1	—	—
Caffeic acid	44.3	18.2	17.2
L.S.D.at 0.05 P	2.5	N.S.	N.S.
,, 0.01 P	3.4	N.S.	N.S.

N.S. = Not significant

Table VI. Effect of the non-auxinic chemicals on the in vitro *activity of the* Ipomoea *IAA-synthesizing system. µg IAA synthesized from tryptophan per g tissue equivalent enzyme per hr*

Non-auxinic chemical	Concentration (M)		
	10^{-3}	10^{-4}	10^{-5}
Control	7.2	9.1	9.2
Indole	7.0	—	—
Catechol	16.5	10.5	9.4
p-Benzoquinone	11.9	11.7	12.0
p-Hydroxybenzoic acid	8.5	—	—
p-Coumaric acid	10.4	12.0	10.6
Ferulic acid	5.2	—	—
Caffeic acid	48.5	21.9	11.5
L.S.D. at 0.05 P	1.1	0.4	N.S.
,, 0.01 P	1.5	0.7	N.S.

N.S. = Not significant

These results reveal the absence of any direct relationship between the root-promoting effects of the chemicals and their effects on the synthesis of IAA from tryptophan. Thus, in none of the three plant materials, there was any significant synergism between a non-auxinic chemical and tryptophan, although, *in vitro* studies on the isolated enzyme system have shown that a number of such chemicals are very effective stimulators of IAA synthesis. Had there been corresponding *in vivo* synthesis of IAA, the chemicals would have shown synergism with tryptophan, particularly in materials like *Ipomoea* in which IAA was very effective. The very high synergistic effect of indole in combination with IAA in French bean and *Ipomoea* was altogether missing in the indole + tryptophan treatment. Indole, which is neither a modifier of the IAA-oxidizing system (Basu, 1970) nor of the IAA-synthesizing system, obviously, has a different mechanism of synergism with IAA. The effect of *p*-coumaric acid in *Ipomoea* deserves special consideration in view of the high stimulating activity of the chemical on the IAA-oxidizing system (Gortner and Kent, 1958). This monophenol significantly synergized the rooting of the IAA-treated *Ipomoea* cuttings and interestingly enough, showed some promotion of IAA synthesis in this material (a highly significant promotion was noted with mung bean enzyme). Such a promotion was surprisingly associated with an antagonistic effect of the phenol in the tryptophan-treated *Ipomoea* cuttings.

The results clearly reveal the dual effect of the polyphenols, firstly by inhibiting the IAA-oxidizing system (Basu, 1970) and secondly,

through the stimulation of auxin biosynthesis, but without any link with the formatison of adventitious roots on the cuttings.

Further work is now in progress to throw more light on the role of the endogenous IAA-synthesizing system in the regeneration of roots by studying the *in vivo* IAA synthesis from tryptophan employing ^{14}C or otherwise labelled tryptophan in the presence or absence of synergistic non-auxinic chemicals. This is especially important in view of the observations of Gordon and Paleg (1961) that under natural conditions, the polyphenolases which participate in the IAA synthesis and the substrates (*i.e.* the polyphenols) may be spatially separated.

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