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The Effect of Indol-3-yllactic Acid on Maize Seedling Growth

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Abstract. D,L-indol-3-yllactic acid was applied at concentrations 0.2–20 mg l^{-1} (10^{-6}–10^{-4}M) to the roots of 3-d-old intact maize seedlings grown in the dark and in the light. By day 3 after application, ILA at lower concentrations (0.2–1 mg l^{-1}) caused slight increase in the mass of etiolated plants, mainly roots. Shoot and root length was not increased. ILA at concentrations equal to, or greater than, 2 mg l^{-1} reduced the length of the main roots and from 10 mg l^{-1} on also the length of the shoots. In contrast, root mass was decreased by higher ILA concentrations to a lesser extent than shoot mass. The response to ILA application of etiolated seedlings differed from that of the seedlings grown in the light only at 20 mg l^{-1}. The inhibitory effect was more pronounced in the case of quickly growing etiolated plants.

Indol-3-yllactic acid (ILA) was identified as a natural indole compound in higher plants in tomato shoots (SCHNEIDER *et al.* 1972). In this species its biosynthesis from tryptophan (Trp) and indol-3-ylpyruvic acid (IPyA) was also demonstrated in feeding experiments with ^{14}C-Trp (WIGHTMAN 1964, GIBSON *et al.* 1972).

The role of ILA in plant growth regulation is unknown. Its biological activity was tested only in assays devised for the determination of auxin activity in which ILA showed only low effect when compared to indol-3-ylacetic acid (IAA). THIMANN (1958) found with ILA isolated from *Oidium lactis* 0.16 % efficiency in the *Avena* coleoptile test and 0.4 % in the *Pisum* straight growth and 1.8 % in the *Pisum* curvature tests. WIGHTMAN (1962) followed on wheat coleoptile segments the effects of synthetic ILA at concentrations ranging from 10^{-7} to 10^{-4} M. A significant increase in growth, by 9 and 12 %, was caused by 10^{-5} M (2 mg l^{-1}) and 10^{-4} M (20 mg l^{-1}) concentrations, respectively. The same concentrations of IAA enhanced growth by 50 and 52 %, respectively. BALLIN (1967) tested the effects of ILA on segments from oat, wheat and maize coleoptiles and from pea stems. Only wheat coleoptile and pea segment growth was significantly enhanced by 10 mg l^{-1} (5×10^{-5}M) ILA concentration, the other plant objects responded to 100 mg l^{-1} (5×10^{-4}M).

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In experiments described in this paper ILA was applied to the roots of intact maize seedlings and its effect on root and shoot growth was followed.

MATERIAL AND METHODS

D,L-indol-3-yllactic acid (Sigma Chemical Co) was added to Knop's nutrient solution and applied to the roots of three-day-old maize (*Zea mays* L. cv. CE 240) seedlings germinated in the dark at 25 °C; eight seedlings were grown in vessels containing 100 ml of the cultivation solution. There were two sets of experiments:

1. ILA was applied at concentrations from 0.2 to 10 mg l^{-1}, seedlings were kept in the dark at 25 °C after ILA application;
2. ILA was applied at 5 to 20 mg l^{-1}; seedlings were grown after ILA application either in the dark at 25 °C or transferred to the light [8h darkness, 16 h light, irradiation (400—700 nm) 60 W m^{-2}] at 21 to 23 °C.

Changes in root and shoot length and in shoot and root mass caused by ILA application were determined by day three after ILA application. The experiments were replicated three times in both series.

RESULTS

ILA at concentrations of 0.2 to 1.0 mg l^{-1} caused slight stimulation of growth of etiolated maize seedlings (Table 1). Root and shoot length was not influenced, however their mass was increased, root mass more than shoot mass. The highest stimulation of both root and shoot growth was recorded at 0.5 mg ILA l^{-1}, however the increase in plant mass was only at the boundary of the $P = 0.05$ statistical level.

ILA at concentrations equal to, and greater than, 2 mg l^{-1} inhibited growth. The length of the main roots decreased with increasing ILA concentration from 2 mg l^{-1} on and that of the shoots from 10 mg l^{-1} on. Root mass decrease was slower than shoot mass decrease. Whereas both fresh mass and dry mass of shoots significantly decreased already at 10 mg l^{-1}, in the case of roots only fresh mass was affected at the highest concentration applied, *i.e.* 20 mg l^{-1}.

In the second set of experiments in which the inhibitory action of ILA on the mass of etiolated seedlings and seedlings grown in the light was compared (Table 2), the changes in mass caused by ILA action at the concentrations of 5 to 10 mg l^{-1}, expressed in absolute values (mg fresh or dry mass, respectively) were approximately the same in plants grown either in the light or in the dark. Further increase in ILA concentration to 20 mg l^{-1} manifested itself differentially in the two types of experiments. A much more marked inhibition of growth was caused by ILA in etiolated seedlings growing at a faster rate than in seedlings grown in the light, although when expressed in relative values, the inhibitory action of ILA was more marked in experiments with plants grown in the light.

Root and shoot dry matter contents increased with increasing ILA concentration in both etiolated plants and plants grown in the light, and the ratio of shoot mass to root mass decreased.

Abbreviations used: IAA = indol-3-ylacetic acid; ILA = indol-3-yllactic acid; IPyA = indol-3-ylpyruvic acid; PLA = phenyllactic acid; Trp = tryptophan.

TABLE 1

The effect of indol-3-yllactic acid on shoot and root growth of maize seedlings grown in the dark in nutrient solution ($n = 3$; $s_{\bar{x}}$ = standard mean error; + denotes significant change at the 0.05 level of probability)

ILA [mg l⁻¹]	Shoots									Roots								
	Fresh mass [mg]			Dry mass [mg]			Length [mm]			Fresh mass [mg]			Dry mass [mg]			Length [mm]		
	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]
0	2994	108	100.0	190	10	100.0	110	2	100.0	796	61	100.0	49	6	100.0	130	6	100.0
0.2	3030	126	101.2	204	17	107.4	111	2	100.9	917	54	115.2	58	4	118.3+	143	4	110.0
0.5	3032	118	101.3	220	11	115.8+	111	3	100.9	935	41	117.5+	59	2	120.4+	138	5	106.0
1.0	2866	65	95.7	201	7	105.8	107	2	97.3	850	56	106.8	56	1	114.3	130	6	100.0
2.0	2821	103	91.8	188	9	98.9	108	2	98.2	780	27	98.0	48	3	98.0	117	6	90.0+
5.0	2858	55	95.5	182	5	95.8	106	2	96.4	744	15	93.5	48	3	98.0	107	4	82.3+
10.0	2250	150	75.2+	162	9	85.3+	91	3	82.7+	639	53	80.3+	47	2	95.2	82	4	63.1+
$LSD_{(0.05)}$	297			28			8			128			9			13		

Table 2

The effect of indol-3-yllactic acid on fresh and dry mass of shoots and roots of maize seedlings grown either in the light or in the dark, ($n = 3$; for other details see Table 1)

	ILA [mg l^{-1}]	Shoots						Roots						Shoot/Root ratio		Dry mass [%]	
		Fresh mass [mg]			Dry mass [mg]			Fresh mass [mg]			Dry mass [mg]						
		$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	$\bar{x}$	$s_{\bar{x}}$	[%]	fresh m.	dry m.	Shoots	Roots
Darkness	0	5512	143	100.0	398	9	100.0	2583	73	100.0	170	2	100.0	2.13	2.34	7.2	6.6
	5	5200	130	94.0	382	9	95.5	2523	140	97.7	166	8	97.6	2.06	2.30	7.3	6.6
	10	4843	157	87.9+	347	8	87.2+	2424	150	93.8	163	10	95.9	2.00	2.12	7.1	6.7
	20	3931	41	71.3+	304	4	76.6+	2123	136	82.2+	155	7	91.2	1.85	1.96	7.7	7.3
LSD$_{(0.05)}$			491			26			376								
Light	0	1933	17	100.0	181	2	100.0	1039	10	100.0	72	1	100.0	1.86	2.51	9.4	6.9
	5	1613	34	83.4+	156	3	86.2+	944	14	90.9	73	1	101.3	1.71	2.14	9.7	7.7
	10	1353	89	70.0+	135	2	74.6+	839	47	80.8+	68	2	94.4	1.61	1.99	9.8	8.1
	20	1034	87	53.5+	112	10	61.9+	777	30	74.8+	62	3	86.1+	1.33	1.81	10.8	8.0
LSD$_{(0.05)}$			254			22			115			7					

DISCUSSION

The effects of ILA on plant growth were studied only by THIMANN (1958), WIGHTMAN (1962) and BALLIN (1967). ILA concentrations, which caused growth stimulation of shoot segments in their experiments, resulted in our experiments with ILA applied to roots of intact maize seedlings, in a marked inhibition of shoot growth. However, a longer time of action of ILA in our experiments and above all a different way of its application must be taken into account.

As far as we know, data on the effect of ILA on root growth have not been reported. Phenyllactic acid (PLA) originating from phenylalanine and phenylpyruvic acid (CAMIRAND *et al.* 1982) in an analogical way as indol-3-yllactic acid from Trp and IPyA, stimulates root growth. PLA applied at relatively high concentrations caused marked stimulation of root growth of lettuce seedlings (TAMURA and CHANG 1965, MIKAMI *et al.* 1970). It also showed an enhancing effect on the growth of their hypocotyls, but it was inactive in the *Avena* coleoptile test.

Indol-3-yllactic acid is structurally similar to IAA. With IAA it has common precursors Trp and IPyA, but it is not a direct intermediate of its biosynthesis as proved by GIBSON *et al.* (1972) in feeding experiments with ^{14}C-Trp and ^{14}C-ILA. They found that ^{14}C-ILA was transformed in plants to Trp and only a part of ^{14}C was also incorporated into IAA and tryptophol, presumably indirectly *via* Trp. Indolelactate decarboxylase which would decarboxylate ILA to tryptophol was also not found in the plants.

In the experiments of THIMANN (1958), WIGHTMAN (1962) and BALLIN (1967) ILA showed much lower activity than IAA. In our experiments, the effects of ILA were not directly compared with those of IAA, and literature data on the action of IAA applied to the roots of intact plants can be utilized for such comparison only partially, because they include only short-term effects on root elongation growth.

The inhibitory effects of ILA on root growth seem to be negligible when compared with those of IAA. IAA starts to inhibit maize root growth already after 2 h at the concentration of 10^{-8} M, with the inhibition being about 90 % at the 10^{-6} M concentration (MULKEY *et al.* 1982). In our experiments ILA started to inhibit root growth at as high concentration as 10^{-5} M; 2×10^{-5} M ILA reduced root growth only by approximately 40 %. However, it must be emphasized that we have followed the effects of ILA on the total root length, whereas MULKEY *et al.* (1982) followed the effect of IAA on the increase in root length. Besides that, the time of sampling after the application is also important. Maize primary roots are able, in dependence on the concentration of IAA applied, to restore growth either partially or completely (GOUGLER and EVANS 1981) within a relatively short time, *i.e.* 90 min. Thus inhibitory effects of IAA on root elongation growth presumably would not be as marked in long-term experiments.

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BOOK REVIEW

BAJAJ, Y. P. S. (ed.): BIOTECHNOLOGY IN AGRICULTURE AND FORESTRY, VOL. 2. CROPS I. — Springer-Verlag, Berlin—Heidelberg—New York—Tokyo 1986. 608 pp. Hard cover DM 348.—

This second volume of the series dealing with crops comprises 33 chapters organized into 3 sections: Cereals, Vegetables, Legumes and tubers, and Future agricultural crops. A great part is devoted to wheat and rice (9 chapters, 167 pp.) with emphasis on (1) anther culture with respect to the generation of genetic variability, applications in crop improvement, haploid production and performance of doubled haploids, (2) problems of establishments of callus and regeneration of plants from callus cultures, and (3) on cryopreservation of cell cultures in rice. The next chapters deal with the following crops: corn, barley, sorghum, pearl millet, soybean, *Phaseolus* spp., tomato, pepper, eggplant, cucurbits, *Allium* spp., celery, butter-bur, potato, sweet potato, sugar beet, globe artichoke, triticale, hordecale, winged bean, amarants and buckwheat. The selected biotechnological methods described differ according to species and include: anther culture for induction of haploids and of genetic variability, establishment of cultures and plant regeneration, induction and use of somaclonal variability, and embryo cultures for hybrid production.

The large volume of accumulating information and considerable progress achieved in the field of plant cell and tissue culture are promising for the future practical applications of the *in vitro* techniques in research programms of the genetics and breeding. With the increasing interest in this and related areas of research activity this book will surely become an invaluable source of information for scientists working in various fields of botany, as well as for university teachers and students.

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