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PRESENCE OF SEROTONIN IN JUGLANS *AILANTHIFOLIA VAR. AILANTHIFOLIA* Carr. AND ITS PHYSIOLOGICAL EFFECTS ON PLANTS

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Serotonin (5-hydroxytryptamine) was established in acid extracts of the mature embryo of *Juglans ailanthifolia* var. *ailanthifolia* Carr. The biogenic amine from the neutralized extract was separated on the ion exchanger Amberlite CG-50 and detected by chromatographic, spectrophotometric and spectrofluorimetric methods. By These methods serotonin was detected in a quantity of 95 $\mu g\ g^{-1}$ fresh weight.

Serotonin influenced rhizogenesis in Aspen leaves cultivated *in vitro* to the same extent as indole-3-acetic acid. The biogenic amine also ihibited tumor formation in potato tuber discs and stimulated root formation in tumors.

Introduction

Over the last decades much attention has been devoted to the investigation of tryptamines, in particular to serotonin, in plant tissues. This biogenic amine is wide-spread in both plant and animal kingdom. Due to its possession of auxin-like activity in the *Avena* coleoptile test (Niaussat et al. 1958), *Lupinus albus* hypocotyls (Umrath and Thaler 1980) and its effect on longitudinal growth of the root (Csaba and Pál 1982) serotonin may be a plant growth hormone. Most of the accumulated serotonin is assumed to be localized in the vacuoles, particularly in the nettle stings of *Urtica* species (Collier and Chesher 1956, Regula and Devidé 1980) or in the lower epidermal cells of *Elaeagnus*

umbellata (Regula 1970). The localization of serotonin in stings or trichomes of the pods of *Mucuna pruriens* (Bowden et al. 1954) may have a protective role, like tannins, against predations.

These experiments investigate the presence of serotonin in the embryo of walnut, as well as its activity in the root induction in aspen leaves cultivated *in vitro* and its effect on crown gall tumor formation in potato tuber discs.

Materials and Methods

The embryos (20 g.) of *Juglans ailanthifolia* var. *ailanthifolia* (seeds from Litva USSR) were homogenized and extracted several times with 0.1 N HCl. The acid extracts were separated from the tissue by centrifugation and neutralized with $Zn(OH)_2$. The neutralized extract was concentrated to a smaler volume. A part of this crude extract was added to the aspen culture medium for induction of rhizogenesis and a part applied in the mixture for tumor induction in potato discs. As a crude extract soon showed physiological activity in the tissue in these experiments it was interesting to try to detect the compound responsible for it.

Identification of the active compound in the extract

The residual part of the extract was chromatographed on Whatman No 1. and on thin layers of Silica gel G and Al_2O_3 G (Table 1.). A part of the extract was passed through a column of an Amberlite CG 50 the cation exchanger in its NH_4^+ form. The column was washed with 0.02 M ammonium acetate and the basic substance eluted with 1 N HCl and absorption and fluorescence spectra were measured. Quantitative determination of the basic substance was carried out by a spectrocolorimetric method using 1-nitroso-2-naphthol reagent and measuring the absorbance at 535 nm.

Histochemical localization was achieved by p-dimethylaminobenzaldehyde on thin sections of the tissue (Regula 1972).

Leaf culture

The leaves of an aspen hybride (*Populus tremuloides* x *P. tremula*) with DP_1 label were used for detection of hormone like activity. The leaves were axcised from shoots grown *in vitro* and planted on media containing plant extract or different amounts of IAA or 5-HT. The Aspen culture medium according to Ahuja (1983) in which adenin sulphate was omitted was used as the nutrient medium. The control medium was free of IAA, 5-HT and plant extract. The cultures were incubated in a growth Chamber at 26°C exposed to artificial light of 700—1500 1x (daylight fluorescent tubes TEŽ-Zagreb, 40W, 220 V, 6500° K), with light-dark cycles of 16/8 hours.

Tumor induction

Tumors were induced on sterile potato tuber discs according to the method of Anand and Heberlein (1977). The inoculum consisted of the pure bacterial suspension (*Agrobacterium tumefaciens*, B6S3), or

Table 1. Rf values and colour reactions of the compound from the plant extract and of authentic 5-hydroxytryptamine

Substance	Paper chromatography Rf in soivent system*					Thin-layer chromatography			Reagents					
	1	2	3	4	5	6+	6++	7+	I	II	III	IV	V	VI
Plant constituent	0.48	0.52	0.36	0.62	0.09	0.66	0.80	0.13	b.	b.	b.	v.	v.	v.
5-Hydroxytryptamine	0.48	0.52	0.37	0.63	0.09	0.66	0.80	0.13	b.	b.	b.	v.	v.	v.

*1. n-BuOH-AcOH-H_2O /60 : 15 : 25/
2. i-PrOH-NH_3-H_2O /10 : 1 : 1/
3. n-BuOH-EtOH-H_2O /4 : 1 : 1/
4. MeOH-BuOH-C_6H_6-H_2O /4 : 2 : 2 : 2/
5. Dest. H_2O
6. i-PrOH-NH_3-EtAc /35 : 20 : 45/
7. $CHCl_3$-C_5H_6 /1 : 1/
+ Silica gel G
++ Al_2O_3 G

I = Ehrlich
II = p-Dimethylaminocinnamaldehyde
III = Xanthydrol
IV = 1-Nitroso-2-Naphthol
V = Ninhydrin
VI = Ninhydrin-Acetic Acid
b. = blue
v. = violet

of a mixture containing bacteria and a 0.11 mM solution of the substance which had to be tested: tryptophan, tryptamine, indole-3-acetic acid, serotonin-creatininsulphate or plant extract.

Results and Discussion

Crude extracts of *Juglans ailanthifolia var. ailanthifolia* or different concentrations of authentic samples of IAA or 5-HT respectively exhibited comparable stimulatory effects on rhizogenesis in all concentrations tested. The roots were induced on the remaining 5 mm long part of the leaf petiole, directly, without callus formation (Fig. 1., 2.). One to three, or even more roots were formed per leaf. The roots induced by the optimal concentration of the substance (0.1, 0.5, 1.0 μM) were mainly laterally branched (Table 2.).

Table 2. Effect of IAA and 5-HT on root formation of hybride aspen leaves. Each class consisted of 15 leves.

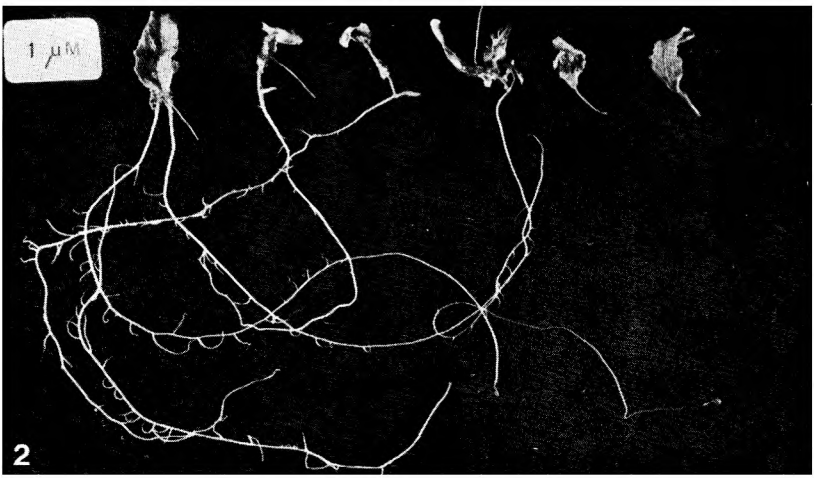
Substances	Concentration (μM)	Number of rooted leaves		Number of unrooted leaves	
		two or more long laterally branched roots	one or two short laterally unbranched roots	healthy	necrotic
Control	0	0	0	0	0
IAA	0.05	0	5	4	6
5-HT		0	5	3	7
IAA	0.1	3	3	4	5
5-HT		3	2	4	6
IAA	0.5	4	3	4	4
5-HT		5	3	4	3
IAA	1.0	3	5	2	5
5-HT		4	3	3	5
IAA	1.5	2	5	5	3
5-HT		3	4	4	4
IAA	2.0	0	3	6	6
5-HT		1	3	5	6
IAA	3.0	0	2	6	7
5-HT		0	3	7	5

Tumor formation on potato tuber discs was stimulated by indole-3--acetic acid but inhibited by plant extract, tryptophan, tryptamine, and particularly by serotonin (Table 3. Fig. 3.). Although IAA and serotonin

Fig. 1 and 2. Root formation in hybride aspen leaves in response to 1μM IAA (Fig. 1) of serotonin (Fig. 2).

Fig. 3. Tumors on potato tuber discs 18 days after infection. Abbreviations as in Table 3.


1 µM
1


1 µM
2

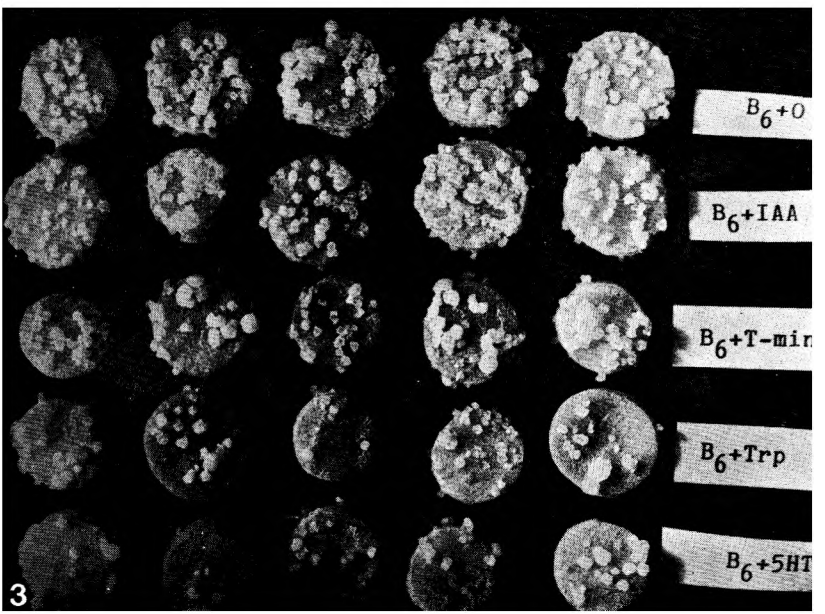

B6+0
B6+IAA
B6+T-min
B6+Trp
B6+5HT
3

Table 3. Effect of IAA, T-min (tryptamine), Trp (tryptophan), 5-HT (serotonin) and p. e. (plant extract) on tumor induction and development in potato tuber discs (monitored four weeks after infection). Each class cosisted of 50 tuber discs.

Inoculum	Number of tumors per disc	Weight of tumors per disc [mg]	Total number of rooted tumors
B6S3	49.50 ± 1.95[a]	101.50 ± 2.04[a]	6
B6S3 + IAA	87.50 ± 1.38	140.00 ± 3.34	7
B6S3 + T−min	42.44 ± 1.83	81.60 ± 2.55	10
B6S3 + Trp	34.88 ± 2.00	63.60 ± 2.22	0
B6S3 + 5−HT	28.60 ± 2.35	58.00 ± 4.59	18
B6S3 + p. e.	36.02 ± 1.48	49.73 ± 3.72	12

a — standard error of the mean.

belong to the same group of compounds (Garattini and Valzelli 1975) the former stimulated and the latter inhibited tumor induction. The oncostatic activity of serotonin has also been proved in animals (Pukhalskaya 1962). As shown in Table 3. serotonin stimulated root differentiation in tumors in a similar fashion as in aspen leaves.

One basic indolic substance was noticed in the extract of the embryo of *Juglans ailanthifolia var. ailanthifolia*. The Rf values of this substance on paper and thin-layer chromatograms as well as its colour reactions (Table 1.) were identical with those of the authentic sample of serotonin. The identity of this substance was also confirmed by measurements of U. V. Spectra in methanol and fluorescence spectra in strong acid (activ. at 295 and fluoresc. at 550 nm).

Spectrocolorimetrical measurement after addition 1-nitroso-2-naphthol reagent showed that serotonin was present in the embryo in a quantity of 95 $\mu g \cdot g^{-1}$ fresh weight.

The localization of serotonin in protein bodies of the embryo was detected by histochemical reaction with p-dimethylaminobenzaldehyde on thin sections of the embryo tissue, giving a blue color reaction, and with ninhydrin-acetic acid giving a greenish fluorescence in U. V. light. Serotonin may accumulate as a product of detoxification of ammonia derived from deamination of amino acids in the embryo, during ripening (Grosse 1982).

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SAŽETAK

PRISUTNOST SEROTONINA U ORAHU *JUGLANS AILANTHIFOLIA VAR. AILANTHIFOLLIA* CARR. I NJEGOVO DJELOVANJE U BILJCI

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Istraživana je prisutnost serotonina (5-hidroksitriptamina) u embriju oraha *Juglans ailanthifolia* var. *ailanthifolia* Carr. te njegovo fiziološko djelovanje na biljke. Sjemenke su ekstrahirane 0.1 N HCl a ekstrakti neutralizirani s $Zn(OH)_2$. Dodavanjem ovog ekstrakta mediju za indukciju korjenčića ili smjesi za indukciju crown gall tumora primjećena je fiziološka aktivnost u prisutnim biljnim tkivima. Propuštanjem ekstrakta preko ionskog izmjenjivača amberlita separirana je bazična indolska supstancija koja po svojim karakteristikama u papirnoj i tankoslojnoj kromatografiji te obojenim reakcijama, kao i spektrofotometrijskim i spektrofluorimetrijskim karakteristikama odgovara autentičnom uzorku 5-hydroxytryptamina. Ovaj biogeni amin prisutan je u tkivu embrija u količini od 95 $\mu g \cdot g^{-1}$ svježe tvari.

Indukcija korjenčića na listovima hibrida topole (*Populus tremuloides* x *P. tremula*) dobivena je osim s ekstraktom embrija također i s različitim koncentracijama autentičnog uzorka serotonina i posebno indolil-3-octene kiseline kao standardom auksinske aktivnosti. Utvrđen je jednak stupanj indukcije rizogeneze ovih supstancija kod jednake koncentracije u mediju.

Serotonin je inhibirao indukciju tumora na tkivu gomolja krumpira, dok je IAA djelovala stimulativno. Serotonin je na induciranim tumorima pospješio defirencijaciju i rastenje korijena.

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