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Influence of 3-metoxycarbonylmethylbenzothiazolium bromide on growth and mitotic activity of *Vicia sativa* L. roots

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Abstract

3-Metoxycarbonylmethylbenzothiazolium bromide (MMB) influences the growth of the roots seedlings of *Vicia sativa* L. It stimulates growth within a broad concentration range of 10^{-13}-10^{-7} M, but inhibits growth at 10^{-3} M and shows similar stimulating and inhibiting effects on the mitotic activity of root tip cells.

Key words: MMB, Vicia sativa, mitotic activity

INTRODUCTION

Benzothiazol derivatives as auxinoid analogues (Giannella et al. 1971) and above all N-substituted benzothiazolic salts (Monsanto 1979, 1980 a, b), characterized by their solubility in various solvents, the best, however, in water, have a stimulating or inhibiting effect, when applied to the seeds or leaves in the form of sprays in concentrations in the scale of 10^{-3}-10^{-16} M (Sutorise et al. 1980). Their action is the object of our present investigations.

MATERIAL AND METHODS

For this study the water soluble benzothiazolium salt 3-metoxycarbonylmethylbenzothiazolium bromide (MMB) was used. Its formula was shown on Fig. 1.

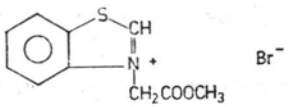

Fig. 1. Structural formula of 3-metoxycarbonylmethylbenzothiazolium bromide (MMB)

The growth tests were performed on *Vicia sativa* L. var. Fatima (HS-245-8-E) originating from the plant breeding station at Horná Streda.

In the first experimental series (Table 1), seeds of approximately equal size and equal colour were soaked in flowing water of 25°C temperature for 6 hours. After sowing into perlite, the seeds germinated in the dark for 48 hours at 25°C. Thereafter the seedlings were washed from the perlite and blotted with paperwadding. Seedlings with roots of 25-30 ± 1 mm length were selected — twenty root lengths were measured. Sets of seedlings were planted on filter paper, wetted with 30 cm^3 of MMB solution in 17-cm Petri dishes. The control series of seedlings grew on filter paper saturated with distilled water. After 24 hours, the roots were remeasured.

In the first series of experiments, the mitotic activity was determined. Root tips were cut off after 0, 2, 6 and 24 hours, and fixed in the mixture ethanol:acetic acid (3:1), hydrolyzed in 5 N MCl at room temperature for 9-10 min and stained in the Feulgen reagent for 60 min. Squashes of the root tips were made by the cellophane technique (M u r i n 1960). After removing the cellophane foil, the slides were dried and embedded in Emulsogen N-090 (alkylarylpolyglycolether — manufactured by Hoechst Ltd). The mitotic index (MI‰) was then evaluated. For each treatment 5000-7000 cells were evaluated.

In the second experimental series (Table 2), seedlings were cultured under similar conditions. After selecting seedlings with roots of 25-30 ± ± 1 mm, the apical zone of the primary root was decapitated at the standard length of 15 mm. Twenty root segments were placed on a 9-cm filter paper disk wetted with distilled water, or 10^{-11} M MMB. The increase in length of the root segments was measured after 2, 6 and 24 hours.

In the 3rd experimental series (Table 3) decapitated seedlings were treated with MMB for 2 hours. The decapitated root segments were tested under conditions analogous to those of the second experimental series. Replication of each set was threefold. The experiments were evaluated biometrically, the significance of the results was extablished by Student's t-test.

RESULTS AND DISCUSSION

INFLUENCE OF MMB ON ROOT GROWTH

In our experiments, intact and decapitated roots of *Vicia sativa* var. Fatima were used to determine the effect of MMB on growth. This system was chosen for its high homogeneity in germination and growth

of the seedlings, and for the high sensitivity of the roots to exogenous stimulation, as has been shown in the literature (G a m b u r g 1976).

In the first series of our experiments (Table 1), intact primary roots of the vetch were incubated for 24 hours. MMB stimulated growth within the range of 10^{-13}-10^{-7} M, with maximum at the concentration of 10^{-11} M. At this concentration, stimulation was increased by 29% as compared with the control. On the contrary, the most concentrated solution of 10^{-3} M MMB had an inhibiting effect. Root growth was retarded by 25 per cent.

Table 1

Growth of intact primary roots of *Vicia sativa* var. Fatima in continuous contact with 3-metoxycarbonylmethylbenzothiazolium bromide (MMB)

MMB, M	Initial length, mm	Root elongation, mm · 24 h^{-1}	Stimulation, % of control	Significance
Control	25.15±1.58	23.85±1.17	0	—
10^{-13}	27.15±1.19	28.35±1.48	+18.87	2.39 +
10^{-11}	29.55±1.33	30.65±1.01	+28.51	4.40 ++
10^{-9}	24.35±1.46	30.20±1.98	+26.62	2.11 +
10^{-7}	25.80±1.23	30.20±1.45	+26.62	3.41 ++
10^{-5}	27.30±0.86	27.10±1.38	+13.62	1.80 n.s.
10^{-3}	24.80±1.00	17.85±1.40	−25.16	2.33 +

n.s. — not significant, + — significant, ++ — high significant.

The stimulating effect of 10^{-11} M MMB was seen also in the second experimental series (Table 2), where apical segments of primary roots of the model object were used. As early as 6 hours after addition of MMB a highly significant growth stimulation of 22 per cent occurred, after 24 hours the stimulation decreased to 10 per cent.

Table 2

Growth of apical segments of primary roots of *Vicia sativa* var. Fatima in continuous contact with solutions of 3-metoxycarbonylmethylbenzothiazolium bromide (MMB)

Time, h	Growth of root, mm		Stimulation, % of control	Significance
	control	MMB, 10^{-11} M		
0-2	1.330±0.092	1.320±0.292	0	0.03 n.s.
0-6	1.700±0.087	2.080±0.100	22.35	2.86 ++
0-24	2.700±0.096	2.980±0.101	10.37	2.01 +

Initial length of segments 15 mm.

The inhibiting efect of 10^{-3} M MMB (Table 3) was also visible in the third series of experiments. Growth of the root segments in the control series differs, at the earliest hours of the investigated time period, from

growth in the former experimental series. MMB at 10^{-3} M inhibited growth throughout the 24 hr incubation period. Maximal inhibition (33%) was observed 6 hours after the beginning of incubation. After 24 hours inhibition decreased to 25 per cent.

Table 3

Growth of apical segments of primary roots of *Vicia sativa* var. Fatima pretreated for 2 h with 3-metoxycarbonylmethylbenzothiazolium bromide (MMB)

Time, h	Growth of root, mm		Inhibition, % of control	Significance
	control	MMB, 10^{-3} M		
2-4	0.740±0.090	0.230±0.073	31.08	4.40 ++
2-6	1.440±0.083	0.470±0.060	32.64	9.47 ++
2-24	2.700±0.108	0.670±0.061	24.81	16.37 ++

Initial length of segments 15 mm.

INFLUENCE OF MMB ON THE MITOTIC ACTIVITY OF THE ROOT MERISTEM CELLS

Since growth substances influence growth not only by changing the rate of elongation growth but also by influencing cell division, we studied the influence of different concentrations of MMB on the mitotic activity in the meristematic cells of the primary root.

The results for two concentrations of MMB at various incubation times are given in Table 4 in relative values of the total number of dividing cells in the controls. At 10^{-11} M MMB stimulated mitotic activity in the first 6 hours but showed no effect after 24 hours.

Table 4

Mitotic index (MI in ‰) in root tip cells of *Vicia sativa* L. after action of 3-metoxycarbonylmethylbenzothiazolium bromide (MMB)

	Time, h	P	M	A	T	MI±S.E.
Control	0	27.87	13.11	12.93	10.39	64.30±6.61
	2	27.18	15.55	4.03	11.39	58.15±6.34
	6	37.55	16.46	8.95	11.15	74.11±8.02
	24	35.49	17.68	6.04	14.81	74.02±5.77
MMB, 10^{-11} M	0	27.87	13.11	12.93	10.39	64.30± 6.61
	2	33.77	21.89	6.77	11.84	74.84±12.9
	6	42.92	20.52	12.02	17.95	93.41± 9.74
	24	34.81	20.21	6.39	12.71	74.13±10.32
MMB, 10^{-3} M	0	27.87	13.11	12.93	10.39	64.30±6.61
	2	19.78	9.82	1.41	7.37	38.38±2.14
	6	6.49	2.16	0.45	1.75	10.85±3.26

P — prophase, M — metaphase, A — anaphase, T — telophase.

The substance inhibits significantly mitotic activity in the concentration of 10^{-3} M as early as after 2 hours. The influence of this concentration was also shown by the frequency of the particular phases of mitosis. With prolonged time of treatment, the number of cells in the particular phases of mitosis decreases.

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Wpływ bromku 3-metoksykarbonylmetylbenzotiazoliowego na wzrost i aktywność mitotyczną korzeni Vicia sativa L.

Streszczenie

Bromek 3-metoksykarbonylmetylbenzotiazoliowy (MMB) wpływa na wzrost korzeni siewek *Vicia ativa* L. Stymuluje on wzrost w szerokim zakresie stężeń od 10^{-13} do 10^{-7} M, natomiast hamuje wzrost w stężeniu 10^{-3} M. Podobne stymulujące i hamujące efekty MMB otrzymano, analizując aktywność miototy czną komórek wierzchołka wzrostu korzeni.