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Short-term effects of Mn^{2+} on elongation growth and H^+-extrusion in *Zea mays* L. coleoptile segments

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Abstract

The effect of 10^{-2}-10^{-6} M Mn^{+2} on the growth and acidification of the incubation medium by *Zea mays* L. coleoptile segments was studied. It is shown that 10^{-5}M manganese stimulated growth (in comparison to a control without Mn^{+2}) while concentrations from 10^{-2} to 10^{-4}M clearly inhibited it. It was also found that manganese at the studied concentration range significantly inhibited acidification of the incubation medium. Auxin, at a concentration of 10^{-5}M, reversed the inhibitory effect of Mn^{+2} (with the exception of 10^{-2}M) on the growth and acidification of the incubation medium by *Zea mays* L. coleoptile segments.

Key words: *manganese, auxin, elongation growth, acidification of the medium*

INTRODUCTION

The effect of manganese on the growth processes stimulated by IAA (indole-3-acetic acid) has been recognized for a relatively long time. For example, Bonner (1949), Cooil (1952) and Thimann (1956) found that the bivalent manganese ion (Mn^{+2}) stimulates the IAA-induced elongation growth of oat coleoptile segments. It was also found that the degradation of IAA, mainly by enzymic oxidation, is controlled by manganese (Wagenknecht and Burris 1950, Maclachlan and Waygood 1956). Tomaszewski and Thimann (1966), in turn, have shown that polyphenols act synergetically with IAA in stimulating growth by counteracting the decarboxylation of IAA. Monophenols, however, inhibit growth and stimulate the decarboxylation of IAA, and this effect is greater in the presence of Mn^{+2} ions.

Interest in the role of manganese in growth processes increased considerably in the 1970's in connection with the paramagnetic properties of the Mn^{+2} ion. In studies on cucumber cotyledons, Bharti et al. (1978) found that manganese has a characteristic ESR (electron spin resonance) signal that is proportional to the concentration of Mn^{+2} in the tissue. A decrease in the paramagnetic properties of manganese, as suggested by the authors, can be related to the participation of manganese in forming complexes (e.g. with EDTA) or with changes in its redox state. It was also found that a drop in the Mn^{+2} content of cells is accompanied by inhibition of growth stimulation by IAA (Mohan Krishna and Bharti 1983). In their opinion, this fact confirms previous suppositions, according to which growth of plant tissues depends on the ratio of free to conjugated or oxidized manganese (Bharti and Laloraya 1981).

The currently popular "acid growth theory" of auxin action (Hager et al. 1971, Rayle and Cleland 1972) assumes that auxin causes growth enhancement by stimulating a plasma membrane-H^+-ATPase (proton pump) which transports protons from the protoplast to the cell wall space. This apoplastic acidification, which attains pH values of 4.5-5.0, leads to the loosening of the cell wall and, consequently, to increasing the cell volume (Rayle and Cleland 1977, Cleland 1980).

It seemed interesting to study the effect of manganese, a metal with a high redox potential, on the electrogenic activity of the proton pump stimulated by IAA in cells of *Zea mays* L. coleoptiles.

MATERIAL AND METHODS

The experiments were conducted on maize coleoptiles (*Zea mays* L.) of the "Koński Ząb" variety obtained from the Experimental Plant Breeding and Acclimatization Station in Smolice. The seedlings were cultured in the dark at a temperature of 27°C for 5 days. For the experiments, 10 mm long segments were used that were excised from 40-50 mm high coleptiles which were cut off at their bases and decapitated 4 mm from their apex. Similar methods of preparing coleoptile segments for growth and proton pump activity studies can be found in papers by other authors, e.g. Lado et al. (1972a, b), Marrè et al. (1973a, b). Coleoptile segments prepared in this way were preincubated in a solution labeled APW of the folowing composition: 1.0 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$ for 2 hours in darkness with constant aeration. The aim of the preincubation was to attain a constant value of the pH of the incubation medium (Vanderhoef et al. 1977), and maximum sensitivity to exogenous growth regulators (Evans and Vesper 1980). After preincubation, the coleoptiles were placed in an experimental chamber in which the volume of the incubation medium was adjusted according to the ratio recommended by Marrè et al. (1972) of 0.5 cm^3 per segment. The construction of the experimental chamber was such that simultaneous measurement of coleoptile

segment growth rates and changes in the pH of the medium could be made. In the growth experiments, IAA at a final concentration of 10^{-5} M and manganese ($MnCl_2$ $4H_2O$) at 10^{-2}-10^{-4} M were used.

The pH was initially set at 6.5. Growth and pH were recorded every 10 min for the first 30 minutes, then every 30 min for 3 hours. For the sake of clarity, on the growth curves changes in growth are shown every 30 min. Changes in pH were calculated by the difference between the pH value at a given time in the experiment and the initial pH, with the "−" sign used to underscore the acidic nature of the changes. In turn, the $-\Delta$pH was calculated per gram fresh weight of the coleoptiles ($-\Delta$pH g^{-1} f.w.). The pH was measured using a N-517 digital pH-meter with a OSH-10-10 electrode.

Growth was measured by the shadow-graphic method, using an optical system giving a magnification of 18x. Growth measurements were made at 23°C in green light.

RESULTS

The presence of 10^{-5} M Mn^{2+} in the incubation medium clearly stimulated elongation growth in maize coleoptile segments (over 30% in comparison with growth in APW measured on the third hour of growth), while concentrations of 10^{-2}-10^{-4} clearly inhibited it. In the case of manganese present at a concentration of 10^{-2} M, contraction of the segments was observed after 2.5 hrs, while at a concentration of 10^{-6} M no significant effect of manganese on growth was noted (Fig. 1).

IAA present at a concentration of 10^{-5} M (Fig. 2) reversed the inhibitory effect of manganese (10^{-3}-10^{-4} M) with simultaneous, evident stimulation of elongation growth of the segments. Growth stimulation was, however, distinctly lower than that caused by auxin alone (APW+IAA). Growth of segments incubated with 10^{-2} M manganese also was modified. In this case, growth during the first phase was significantly intensified while the contraction phase of the segments was significantly extended. No synergetic effect of manganese (10^{-5} M) and IAA (10^{-5} M) was found. The measurements of acidification of the medium which were conducted concomitantly with the growth measurements showed that (Fig. 3) Mn^{+2} at concentrations of 10^{-2}-10^{-6} M significantly inhibited (in comparison with the APW control) the acidification of the medium, especially in the initial phase of the experiment (that is, during the first hour). Inhibition of acidification caused by 10^{-2}-10^{-3} M manganese decreased considerably during the next 2 hours of incubation, while manganese at concentrations of 10^{-4}-10^{-5} M inhibited the acidification of the medium after about 60 minutes of incubation.

At a concentration of 10^{-5} M (Fig. 4), auxin completely reversed the manganese-inhibited (10^{-3}-10^{-6} M) acidification of the incubation medium. The level of acidification in the Mn^{2+}-containing incubation medium, within the 10^{-4}-10^{-6} M range, in the presence of IAA (10^{-5}) M was approximately equal to that induced by auxin alone. In contrast, considerable enhancement of

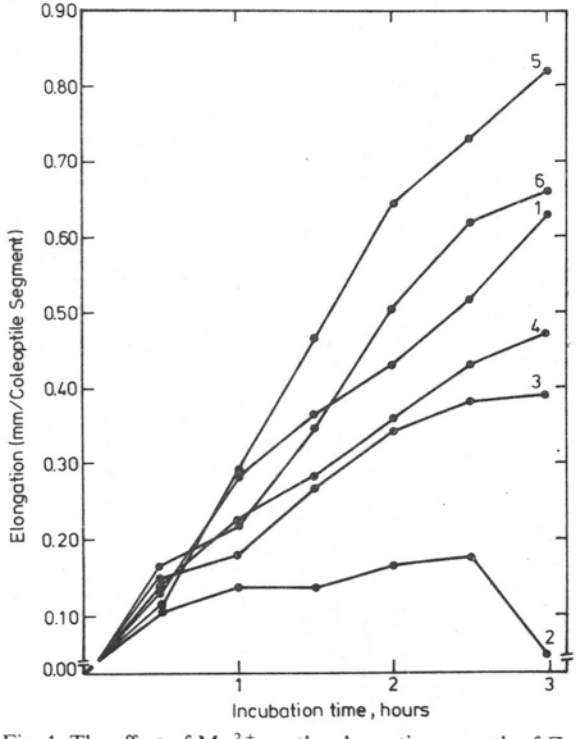

Fig. 1. The effect of Mn^{2+} on the elongation growth of *Zea mays* L. coleoptile segments. 1 – APW (control), 2 – APW + Mn^{2+} (10^{-2} M), 3 – APW + Mn^{2+} (10^{-3} M), 4 – APW + Mn^{2+} (10^{-4} M), 5 – APW + Mn^{2+} (10^{-5}) M, 6 – APW + Mn^{2+} (10^{-6} M). The given values are averages obtained from 7 experiments (the lengths of 7-10 coleoptiles were measured in each experiment). The standard error did not exceed 8%

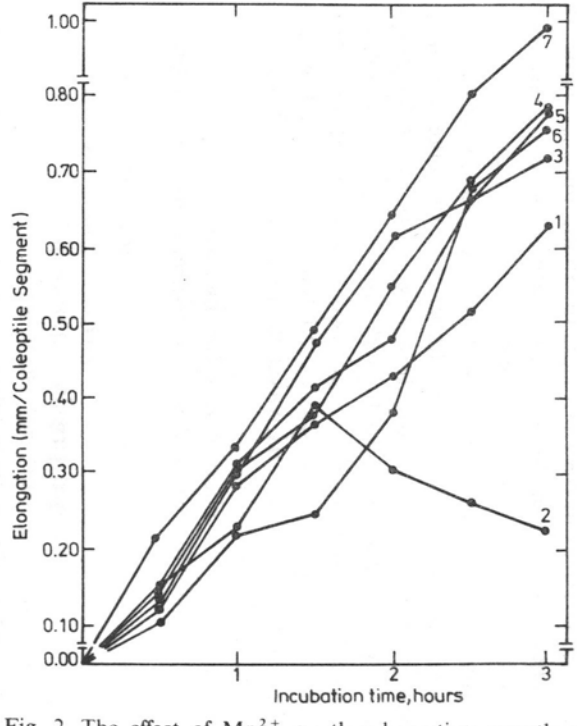

Fig. 2. The effect of Mn^{2+} on the elongation growth of coleoptile segments of *Zea mays* L. in the presence of IAA (10^{-5} M). 1 – APW (control), 2 – APW + Mn^{2+} (10^{-2} M) + IAA, 3 – APW + Mn^{2+} (10^{-3} M) + IAA, 4 – APW + Mn^{2+} (10^{-4} M) + IAA, 5 – APW + Mn^{2+} (10^{-5} M) + IAA, 6 – APW + Mn^{2+} (10^{-6} M) + IAA, 7 – APW + IAA. The presented values are averages obtained from 7 experiments (in each experiment the lengths of 7-10 coleoptiles were measured). The standard error did not exceed 8%

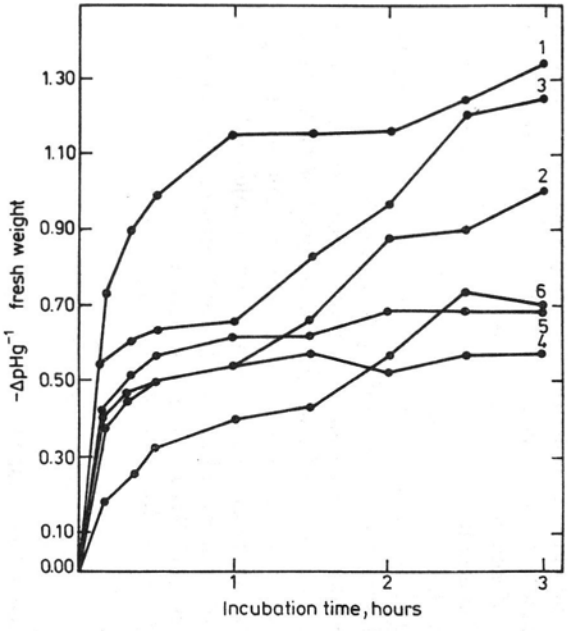

Fig. 3. The effect of Mn^{2+} on changes in the pH of the incubation medium of segments of *Zea mays* L. coleoptiles (symbols as in Fig. 1) The values are the averages of 7 measurements of the pH of the incubation medium made simultaneously with growth measurements. The standard error did not exceed 10%

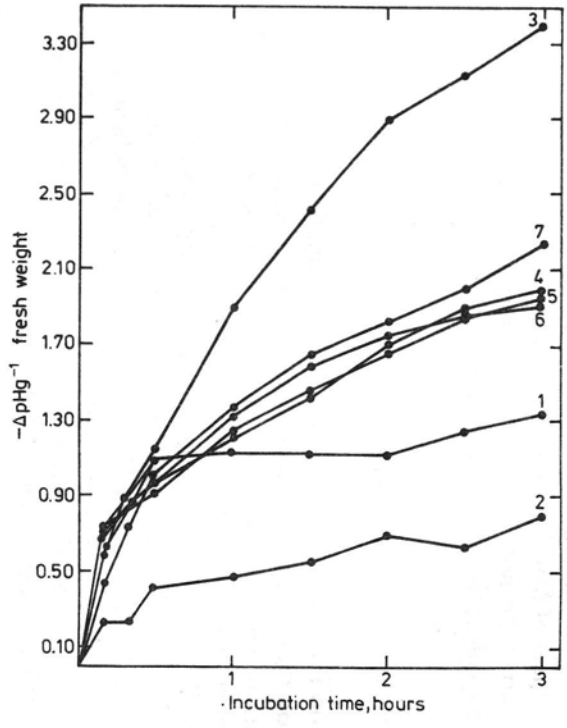

Fig. 4. The effect of Mn^{2+} on changes of the pH of the incubation medium of *Zea mays* L. coleoptile segments in the presence of IAA (10^{-5} M) (symbols as in Fig. 2). The values are the averages of 7 measurements of the pH of the incubation medium made at the same time as growth determinations. The standard error did not exceed 10%

acidification was observed in the action of auxin with 10^{-3} M manganese. In this case, a synergetic effect of Mn^{2+} ions and IAA was seen. In the presence of 10^{-2} M manganese, auxin did not, for all practical purposes, change the level of acidification of the coleoptile segment medium.

DISCUSSION

In the literature on this subject, there is a lack of systematic studies on the effect of manganese on the elongation growth and simultaneous changes in the pH of the incubation medium. In the paper by Tomaszewski and Thimann (1966), cited in the introduction, there is mention of the fact that manganese increased the growth of oat coleoptiles induced by IAA at relatively high concentrations (3×10^{-3} M), and also caused a 40% increase in growth in the absence of IAA. Shibaoka and Hurusawa (1964) also indicated the stimulation of growth activity of oat coleoptiles in the presence of 10^{-3} M manganese. These authors found that manganese (10^{-3} M) partially restored the stimulatory effect of IAA on the growth of oat coleoptiles in the presence of the following acids: L-malic, ketoglutaric, succinic and fumaric, which in a medium not containing magnesium chloride, inhibit auxin-induced growth. In this study we have demonstrated the stimulatory effect of manganese at a concentration of 10^{-5} M on the growth of *Zea mays* L. coleoptile segments (Fig. 1). However, we have not found the synergetic effect of manganese and IAA on the above-mentioned process, which was shown in, e.g., the paper by Tomaszewski and Thimann (1966). This discrepancy can result from the fact that in the cited work, growth was only measured after 24 hours. The fact demonstrated by us, that IAA completely reverses the inhibitory effect of manganese (with the exception of 10^{-2} M) on the growth and acidification of the incubation medium by *Zea mays* L. coleoptile segments seems interesting, as does the fact that manganese at a concentration of 10^{-3} M significantly stimulates the acidification of the medium induced by IAA.

In deliberating the role of manganese in the growth process induced by IAA in light of the "acid growth theory", the evident lack of correlation between growth and acidification of the medium in the presence of 10^{-3} M manganese (stimulation of IAA-induced acidification by 50% with a simultaneous lack of growth stimulation) must be taken into account. From this point of view, these data provide more evidence against the "acid growth theory" of IAA action (Kutschera and Schopfer 1985), and can as well support (due to the high redox potential of manganese) the hypothesis which assigns a role to the redox processes occurring in the plasma membranes of plant cells (Böttger and Lüthen 1986, Rubinstein and Stern 1986) in growth and the accompanying changes in the pH of the incubation medium.

Acknowledgment

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Krótkotrwały wpływ Mn^{2+} na wzrost wydłużeniowy i usuwanie jonów H^+ w segmentach koleoptyli Zea mays L.

Streszczenie

Zbadano wpływ Mn^{2+} w stężeniach 10^{-2}-10^{-6} M na wzrost i zakwaszanie środowiska inkubacyjnego segmentów koleoptyli *Zea mays* L., Wykazano, że mangan w stężeniu 10^{-5} M stymulował wzrost (w odniesieniu do kontroli bez Mn^{2+}), podczas gdy w stężeniach 10^{-2}-10^{-4} M wyraźnie go hamował. Stwierdzono również, że mangan w badanym zakresie stężeń znacznie hamował zakwaszsanie środowiska inkubacyjnego. Auksyna w stężeniu 10^{-5} M znosiła hamujące działanie Mn^{2+} (z wyjątkiem stężenia 10^{-2} M) na wzrost i zakwaszanie środowiska inkubacyjnego segmentów koleoptyli *Zea mays* L.