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Growth of maize coleoptiles in the presence of natural and synthetic growth regulators. Growth correlations

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Abstract

The effect of natural (IAA, FC, ABA) and synthetic (2,4-D) growth substances on the increase of the fresh weight of maize coleoptile segments and change of the pH of the incubation medium, accepted here as criteria of maize coleoptile growth, was studied. The growth of maize coleoptiles depended on the concentration of the growth substances, as well as, on the composition of the incubation medium. The highest stimulation of coleoptile growth was seen with FC at a concentration of 10^{-4} M, whereas ABA at 10^{-3} M gave the highest inhibition of maize coleoptile fresh weight increase and caused alkalization of the medium. The presence of K^+ ions in the incubation medium enhanced the stimulatory effect of IAA and FC on the increase of the coleoptile fresh weight, whereas the presence of these ions and phosphate buffer abolished the growth-promoting effect of IAA and FC. The best correlation of the "fresh weight" and "pH" effects was found in the case of the growth of maize coleoptiles in the presence of FC ($r_{xy} = 0.67$). The inhibition of maize coleoptile growth in the presence of high concentrations of IAA can be explained by the destructive effect of natural auxin at these concentrations on the integrity of mitochondrial membranes, and therefore on the normal functioning of mitochondria.

Key words: maize, coleoptiles, growth substances, growth correlations

INTRODUCTION

A review of literature from the last 10 years gives a picture of hormonal growth regulation in plants (Marrè 1979, Vanderhoef 1980). The studies of Cleland (1975), Cocucci et al. (1976), Cocucci and Cocucci (1977) and Böcher et al. (1980) indicate that the reactions accompanying the elongation of plant cells, induced by phytohormones are: acidification of the incubation medium, change (hyperpolarization or depolarization) of the transmembrane electric potential difference (PD), stimulation of H^+/K^+ exchange in the plasmalemma and increased transport of cations, anions, amino acids and sugars into the cell. The most specific indicator of elongation (growth) of plant organs, induced by growth regulators, seems to be, aside from fresh weight, a change in the pH of the incubation medium. The interdependence of these growth criteria has been documented for many plant species and for different plant organs, which confirms the tissue-specific nature of individual hormones (Lado et al. 1973, Marrè et al. 1973a, 1974). Gabella and Pilet (1979) proposed a statistical interpretation of the results achieved, showing that in the case of the growth of maize roots with FC (fusicoccin) in the medium, positive correlations between changes in the fresh weight of maize roots and in the pH of the growth medium became evident.

That is why it seemed purposeful to look for a dependence between the increase in the fresh weight of maize coleoptiles and a change in the pH of their growth medium, induced by natural and synthetic growth substances at a concentration of 10^{-8} to 10^{-3} M. In addition, it seemed intersting to find such an interpretation of growth curves (curves showing the "growth effect" as a function of the concentration of the growth regulator) which would lead to showing the correlation between the "fresh weight effect" and the "pH effect", caused by a defined concentration of a given growth substance. This kind of interpretation of the results was chosen because of the very few presentations of growth experiments in the form of growth curves (Lado et al. 1973, Gabella and Pilet 1979).

MATERIAL AND METHODS

The experiments were carried out on four-day-old, etiolated maize coleoptiles having the primary leaf inside (referred to as coleoptiles) growing at a temperature of 27°C. The age of the plants was accepted as given by Cerana et al. (1976). Grains of maize, *Zea mays* L. (double hybrid, tooth-shaped type grains) were obtained from the Ośrodek Hodowli i Aklimatyzacji Roślin in Smolice. The following substances were used:

— indole-3-acetic acid (IAA) — a natural auxin (Fluka A. G. Buchs S. G.),
— fusicoccin (FC) — a model growth substance (Montedison, Milan),
— 2,4-dichlorophenoxyacetic acid (2,4-D) — a synthetic auxin (BDH Chemicals Ltd.),
— abscisic acid (ABA) — a natural growth inhibitor (Sigma Chemical Co.).

Modifications of the growth experiments of Lado et al. (1972a, b) and Marrè et al. (1972, 1973a, b) were used. Coleoptiles were decapitated 2 mm from the growth apex, after which two 10-mm segments were cut from them. Twenty segments were shaken (using a LMiM model LE 20311 shaker) for 2 h in 6 cm^3 of distilled water in the dark at 27°C. Preincubation in distilled water was done so that the coleoptiles had the highest sensitivity to exogenous growth regulators and could establish the appropriate pH of the incubation medium for themselves (Abu-Tabikh and Vanderhoef 1979, Evans and Vesper 1980). Next, the segments of maize coleoptiles were incubated for 3 h with growth substances. Distilled water was used as the control medium. The remaining experimental conditions (temperature, darkness, agitation) were as during preincubation. The dependence of the growth of maize coleoptile segments on IAA or FC (10^{-6} M) and the composition of the incubation medium was studied in the following experimental systems: 1) KCl (10^{-2} M), pH = 5.90 ± 0.05; 2) KCl (10^{-2} M)+potassium-phosphate buffer ($6.7 \cdot 10^{-2}$ M Na_2HPO_4-KH_2PO_4), pH = 6.50 ± 0.05 (Polevoy and Salamatova 1975, Cerana et al. 1976).

Growth of maize coleoptiles was measured by means of: 1) increase of fresh weight expressed in Δ $g \cdot g^{-1}$ fresh weight $\cdot 5\ h^{-1}$ and 2) change in the pH of the incubation medium calculated per gram fresh weight ($-\Delta pH \cdot g^{-1}$ f.w. $\cdot 3\ h^{-1}$), given the "–" sign to underscore its acidic character. Increase in fresh weight can be the appropriate growth criterion (Lado et al. 1972a, b). The initial pH (6.50 ± 0.05) was measured immediately after introducing the growth factor, the final pH, after 3 h incubation (Lado et al. 1972a).

In the statistical analysis of the results, the average standard deviation, correlation coefficient (r_{xy}) and regression curves were calculated (Oktaba 1974).

RESULTS AND DISCUSSION

The growth curves for maize coleoptiles growing in the presence of IAA and FC obtained in this study (Fig. 1) are bell-shaped, which was also found earlier by Lado et al. (1973) for pea internodes. The graph of the changes in the fresh weight as a function of the concentration of 2,4-D (Fig. 1) also has a similar pattern. It can be seen from it that 2,4-D at a concentration of 10^{-6} and 10^{-5} M acts similarly to auxin whereas at the remaining concentrations it has a herbicidal nature. This duality in the effect of 2,4-D on the elongation of plant cells and associated reactions has already been emphasized by Kennedy and Stewart (1980) and Zientara (1982). ABA exhibited properties typical for a plant growth inhibitor at all concentrations used (Fig. 1), which is confirmed by Walbot et al. (1975).

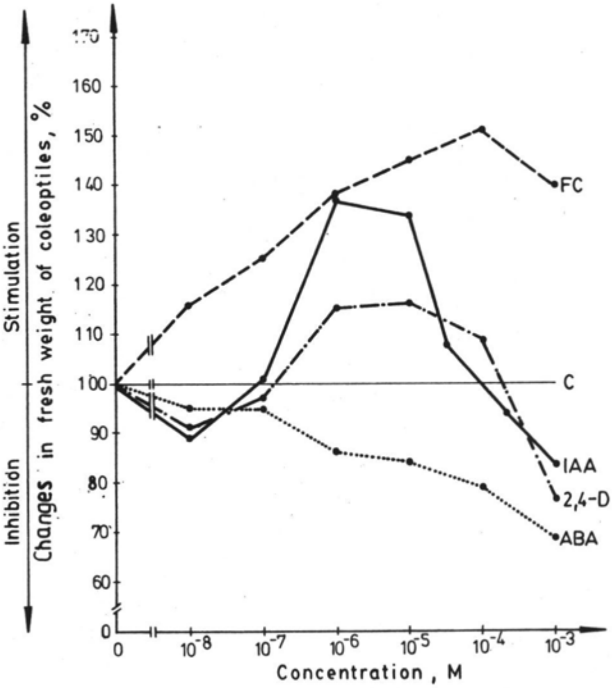

Fig. 1. The effect of IAA (——), FC (– – –), 2,4-D (–·–·–) and ABA (.....) on the increase in fresh weight of coleoptiles incubated with growth regulators, calculated as a % of the increase of fresh weight of control coleoptiles (C)

Similarly, the curves expressing the change in pH as a function of growth regulator concentration for maize coleoptiles growing in the presence of IAA, FC, 2,4-D have a different shape than the pH curve for coleoptiles growing in the presence of ABA (Fig. 2). When maize coleoptiles grew in a medium with ABA (also in media with high IAA and 2,4-D concentrations), alkalization of the incubation medium was noted. The reason for this alkalization in the presence of growth inhibitors may be the increased capability of plant cells to buffer the pH of the cell wall, or the resorption of protons (H^+) by the coleoptiles, caused by a change in the permeability of the plasmalemma (Marrè et al. 1973b).

Comparison of the fresh weight and pH curves for each growth regulator (Fig. 3) shows that the change in fresh weight better characterizes the growth of maize coleoptiles, which has been observed earlier for segments of pea stems by Lado et al. (1972b). This is why in the experiments on growth in the presence of K^+ ions in the incubation medium, only the increase of fresh weight was used as a criterion of maize coleoptile growth. It can be clearly seen (Table 2) that the presence of K^+ in the incubation medium ($pH = 5.90 \pm 0.05$) has a synergic effect on growth stimulation by both IAA and FC. The presence of K^+ and phosphate buffer ($pH = 6.50 \pm 0.05$) however, cancels the promoting effect of IAA and

FC. Potassium ions, in the incubation medium seem to be necessary in the electrogenic H^+/K^+ exchange and for the accumulation of potassium malate in the vacuoles (Marrè 1979).

Superimposition of the fresh weight and pH curves for IAA, FC and 2,4-D showed the similar pattern of each pair and allowed the calculation of growth correlations (Fig. 3). The correlation coefficients and regression curves calculated using the method of the least squares for IAA, FC, 2,4-D and ABA confirmed the supposition about the coincidence of the effect of the first three regulators on the fresh weight and pH, accepted here as criteria of maize coleoptile growth (Fig. 4). There is a lack of correlation in the case of ABA ($r_{xy} = -0.13$). The best correlation of growth effects was obtained with FC ($r_{xy} = 0.67$).

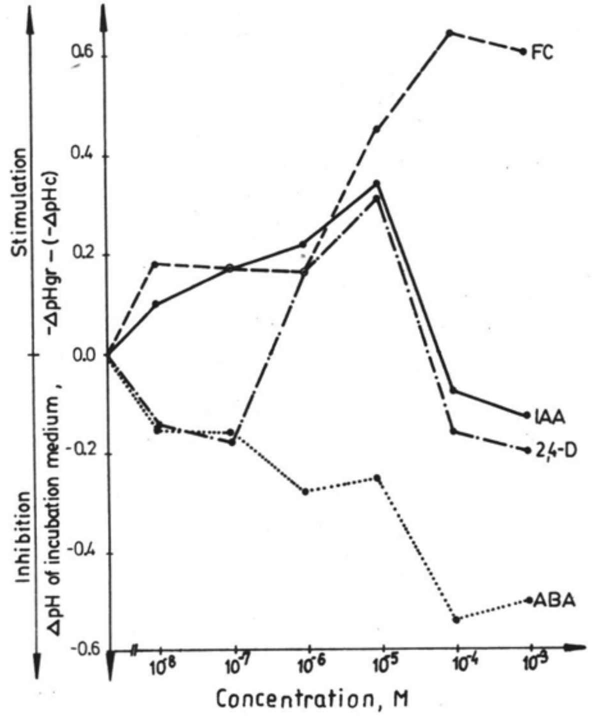

Fig. 2. The effect of IAA (———), FC (– – –), 2,4-D (–·–·–) and ABA (.....) on the change of the pH of the incubation medium of growing maize coleoptiles. The values are average changes from at least 12 measurements which did not differ from each other by more than 0.05 pH units

Comparison of the shape of the fresh weight variability curves for IAA and FC shows an interesting difference in the growth response of maize coleoptiles to high concentrations of these growth regulators. A clear inhibitory effect of IAA is visible (a steep fall in the growth curve in the high concentration range). FC, however, promotes growth

Table 1

The effect of IAA, FC, 2,4-D and ABA on the increase in maize coleoptile fresh weight $(\Delta g \cdot g^{-1}$ fresh weight $\cdot 5\ h^{-1})$

Growth regulator concentration, M	IAA	FC	2,4-D	ABA
Control (distilled water) 10^{-3}	0.2008 ± 0.0171 0.1674 ± 0.0050 (83.4%)[x]	0.2825 ± 0.0 0.3952 ± 0.0 (139.9%)	0.2651 ± 0.0202 0.2030 ± 0.0185 (76.6%)	0.2908 ± 0.0 0.2003 ± 0.0149 (68.9%)
Control 10^{-4}	0.2109 ± 0.0100 0.1985 ± 0.0048 (94.1%)	0.3259 ± 0.0219 0.4935 ± 0.0372 (151.4%)	0.2743 ± 0.0116 0.2979 ± 0.0096 (108.6%)	0.2647 ± 0.0196 0.2093 ± 0.0117 (79.1%)
Control 10^{-5}	0.1719 ± 0.0269 0.2295 ± 0.0203 (133.5%)	0.2738 ± 0.0126 0.3973 ± 0.0155 (145.1%)	0.3285 ± 0.0 0.3824 ± 0.0177 (116.4%)	0.2025 ± 0.0 0.1695 ± 0.0028 (83.7%)
Control 10^{-6}	0.2035 ± 0.0033 0.2796 ± 0.0250 (137.4%)	0.2293 ± 0.0177 0.3168 ± 0.0156 (138.2%)	0.1828 ± 0.0160 0.2110 ± 0.0109 (115.4%)	0.1828 ± 0.0160 0.1574 ± 0.0089 (86.1%)
Control 10^{-7}	0.1720 ± 0.0223 0.1726 ± 0.0107 (100.3%)	0.1284 ± 0.0 0.1606 ± 0.0064 (125.1%)	0.1951 ± 0.0127 0.1894 ± 0.0084 (97.1%)	0.2200 ± 0.0 0.2084 ± 0.0071 (94.7%)
Control 10^{-8}	0.1632 ± 0.0107 0.1455 ± 0.0026 (89.2%)	0.1391 ± 0.0 0.1607 ± 0.0065 (115.5%)	0.2130 ± 0.0132 0.1936 ± 0.0072 (90.9%)	0.2109 ± 0.0125 0.1999 ± 0.0062 (94.8%)

The figures given are the averages from 12 replicates. ± — standard error of the mean. * The values in parentheses are the increase in the fresh weight of coleoptiles incubated with growth regulators calculated as a per cent of the fresh weight of control coleoptiles.

Table 2

The effect of IAA (10^{-6}M) and FC (10^{-6}M) on the increase in fresh weight of maize coleoptiles in incubation media with K^+ ions (KCl — 10^{-2}M, pH = 5.90 ± ± 0.05) and K^+ ions and potassium-phosphate buffer ($6.7 \cdot 10^{-2}$M potassium-phosphate buffer — bK-P, pH = 6.50 ± 0.05)

Incubation medium	Increase of fresh weight, $\Delta g \cdot g^{-1}$f.w.$\cdot 5\ h^{-1}$	
Distilled water	0.2211 ± 0.0198	
IAA	0.2796 ± 0.0250	(126.5%)*
FC	0.3168 ± 0.0156	(143.3%)
KCl	0.0916 ± 0.0	
KCL + IAA	0.1575 ± 0.0135	(172.0%)
KCl + FC	0.1974 ± 0.0244	(215.5%)
KCl + bK-P	0.1154 ± 0.0	
KCl + bK-P + IAA	0.1348 ± 0.0056	(116.8%)
KCl + bK-P + FC	0.1535 ± 0.0047	(133.0%)

The figures given are averages of 9 measurements, ± — standard error of the mean. * The values in parentheses are the increase in fresh weight of coleptiles incubated in the presence of growth regulators or growth regulators and KCl, calculated as a per cent of the fresh weight of control coleoptiles.

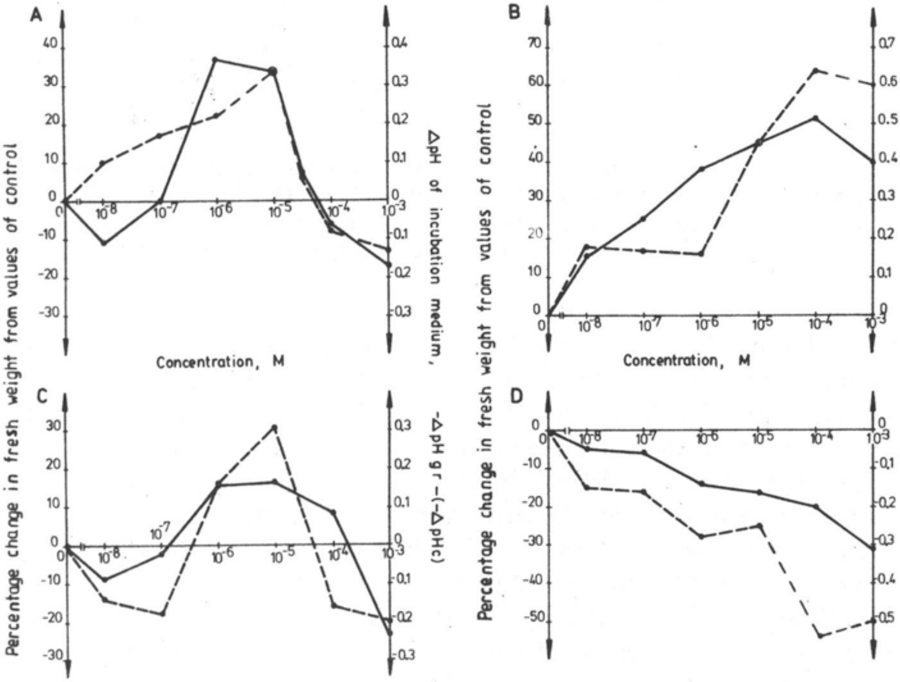

Fig. 3. Correlation of changes in fresh weight (———) and pH (– –) caused by the growth of maize coleoptiles in IAA (A), FC (B), 2,4-D (C) and ABA (D)

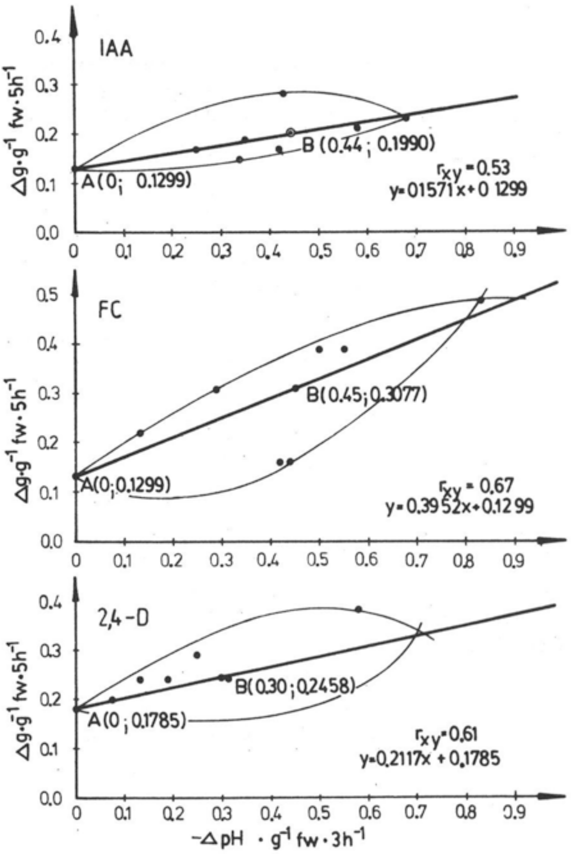

Fig. 4. Regression curves for IAA, FC, 2,4-D. The correlation coefficient and regression curves were plotted on at least 90 xy pairs ($x = -\Delta pH \cdot g^{-1}$ f.w.$\cdot 3\ h^{-1}$, $y = \Delta g \cdot g^{-1}$ f.w.$\cdot 5\ h^{-1}$). Points other than A and B on the figure are averages from the following series of measurements: IAA, FC, 2,4-D — O M, 10^{-8}-10^{-3} M

of maize coleoptiles at a concentration range of 10^{-8} to 10^{-3} M. These phenomena are explained by Lado et al. (1973) who claim that IAA at high doses stimulates the production of ethylene — a plant growth inhibitor, fusicoccin, however, does not posses this property of a natural auxin. From the reports of Raczek (1983a, b) it is known that high concentrations of IAA act destructively on the permeability and integrity of mitochondrial membranes and, therefore, on the coupling of oxidative phosphorylation with the mitochondrial electron transport system.

Therefore, the uncoupling of oxidative phosphorylation by high doses of IAA would be a new explanation (reason) for the inhibition of the growth of plants by natural auxin. This reasoning is confirmed by the experiments with FC, 2,4-D and ABA carried out on maize coleoptiles and mitochondria isolated from them. 2,4-D and ABA change the respiratory activity of isolated maize mitochondria, acting destructively of their membranes. They also inhibit the growth of maize coleoptiles. FC does not uncouple mitochondria and does not inhibit maize coleoptile growth (Raczek 1983b)

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Wzrost koleoptyli kukurydzy w obecności naturalnych i syntetycznych regulatorów wzrostu. Korelacje wzrostowe

Streszczenie

Badano wpływ substancji wzrostowych naturalnych (IAA, FC, ABA) i syntetycznych (2,4-D) na przyrost świeżej masy segmentów koleoptyli kukurydzy i zmiany pH środowiska inkubacji, przyjęte tu za kryteria wzrostu koleoptyli. Wzrost koleoptyli kukurydzy zależał od stężenia zastosowanej substancji wzrostowej, jak również od składu mieszaniny inkubacyjnej. Maksymalną stymulację wzrostu koleoptyli odnotowano w przypadku 10^{-4} M FC. Natomiast ABA w stężeniu 10^{-3} M hamował najsilniej przyrost świeżej masy koleoptyli i powodował alkalizację środowiska zewnętrznego. Obecność jonów K^+ w mieszaninie inkubacyjnej wzmagała stymulujące oddziaływanie IAA i FC na przyrost świeżej masy koleoptyli kukurydzy. Zaś obecność jonów K^+ i buforu fosforanowego znosiła promujący wpływ IAA i FC na ich wzrost. Najlepszą korelację "efektu świeżej masy" i "efektu pH" odnotowano w przypadku wzrostu koleoptyli w obecności FC ($r_{xy} = 0.67$). Hamowanie wzrostu koleoptyli kukurydzy w obecności dużych stężeń IAA można tłumaczyć destrukcyjnym wpływem naturalnej auksyny na integralność błon mitochondrialnych, prawidłowe funkcjonowanie mitochondriów.