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The effect of 2,4-D and ABA on respiration of isolated mitochondria from maize coleoptiles

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Abstract

The susceptibility of isolated maize mitochondria to the growth regulators: 2,4-dichlorophenoxyacetic acid (2,4-D) and abscisic acid (ABA) was studied. It was found that 2,4-D (a herbicide) inhibits respiration in mitochondria, as do other herbicides or phenoxy-acids. In the entire range of concentrations used (10^{-3}-10^{-9} M), 2,4-D introduced into the medium before the respiration reaction was begun, or during it, limited the intensity of succinate oxidation. It did not, however, markedly change phosphorylation properties. Uncoupling of oxidative phosphorylation took place only after preincubation of mitochondria with 2,4-D and was the result of the destruction of mitochondrial membranes. ABA (a growth inhibitor of plants) caused a similar response in maize mitochondria. Preincubation of mitochondria with ABA lead to the uncoupling of oxidative phosphorylation. Whereas ABA introduced during respiration (state 4 respiration) or before its onset, lowered the oxidative potential of mitochondria, it also changed the pattern of state 4-3-4 transition after addition of ADP (it was especially visible at high concentrations), which indicates that the coupling of oxidative phosphorylation with the respiratory chain has faltered. It seems that this negative effect of 2,4-D and ABA on respiration of isolated maize mitochondria is connected with the inhibitory effect of these growth regulators on the growth of maize coleoptiles. Interference in the organization mitochondrial membranes results in a lowered supply of ATP — a source of energy needed in elongation processes.

Key word: mitochondria, maize, respiration, 2,4-D, ABA

INTRODUCTION

Reports on the effect of a synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) a known herbicide, on respiration of isolated plant mitochondria date back from the 1960's and 70's (Baxter and Hanson 1968, Gamburg

1976). It can be seen from these reports that 2,4-D changes the respiratory activity of plant mitochondria. However, similar studies with abscisic acid are not found, even though it inhibits elongation of plant cells (Hartwig et al. 1980) as does 2,4-D in defined concentrations (Kennedy and Stewart 1980, Zientara 1982). Mentioned was found, however, that ABA lowers both the consumption of oxygen by germinating beans and the ATP/ /ADP ratio and also lowers the photosynthetic activity of plants (Walbot et al. 1975). In addition, it is also known that stimulation of plant growth by natural auxin (IAA) and a model growth substance (FC) takes place in the presence of oxygen and a normally functioning oxidation-phosphorylation mechanism (Marrè 1979). This effect is cancelled by the presence of inhibitors of electron transport in the respiratory chain and by oxidative phosphorylation uncoupling agents (Lado et al. 1973). The sensitivity of mitochondria from higher plants to natural auxins is also known (Sarkissian and Mc Daniel 1966, Raczek 1983a, b). At low concentrations, IAA is a promotor of respiration of isolated maize mitochondria (may be by allosteric effect on the enzymes of the respiratory chain in the mitochondrial membrane or matrix). Whereas at high concentrations, it destroys the organization of mitochondrial membranes (Raczek 1983a, b).

It would then seem necessary to check if maize mitochondria are sensitive to 2,4-D and ABA, how these growth regulators affect respiration of isolated maize mitochondria and if this effect can have any meaning in explaining the inhibitory role of 2,4-D and ABA in the hormonal regulation of plant growth. These were the aims of the study presented here.

MATERIAL AND METHODS

The experiments were done on mitochondria isolated from four-day-old, etiolated coleoptiles of maize, *Zea mays* L. (double hybrid, grains of the tooth-shaped type) from the Ośrodek Hodowli i Aklimatyzacji Roślin in Smolice. The maize was grown at a temperature of 27°C.

2,4-D and ABA were obtained from BDH Chemicals Ltd. and Sigma Chemical Comp., respectively.

Mitochondria were isolated using the method of Day and Hanson (1977a, b), substituting 5 mM EDTA for 5 mM EGTA (Hanson et al. 1965). Mitochondrial protein was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as a standard.

Respiration in isolated mitochondria was followed using: 1) a Clark's oxygen electrode (oxidative-phosphorylative properties), 2) a Carl Zeiss Jena spectrophotometer (swollen and contracted states), 3) a N-517 pH-meter aligned with a EZ-10 linear recorder. Respiration was measured at a tem-

perature of 25°C (TB25 thermostat) with constant mixing. Sodium succinate (10 mM) was used as the substrate for oxidation (Pomeroy 1974) and $1.8 \cdot 10^{-4}$ M ADP as the substrate for phosphorylation (Bonner 1974). The composition and pH of the respiration mixture was as given by Pomeroy (1974), substituting 0.3 M sucrose for 0.3 M mannitol (Douce et al. 1972) and 0.1% BSA for 0.75% BSA (Day and Hanson 1977a). Transition between swollen and contracted states was measured in an osmoticum of the composition proposed by Day and Hanson (1977b) at a wave length of $\lambda = 520$ nm. Spontaneous swelling was carried out for 10 minutes, contraction was initiated by 10 mM sodium succinate and followed for a further 10 minutes.

Several experimental variants were used in the polarographic studies: 1) growth regulators were introduced at state 4 respiration (according to Chance, this is a state of respiratory control characterized by oxygen uptake by mitochondria in the presence of the oxidation substrate and absence of a phosphorylation substrate — Bonner 1974); 2) growth substances were added before initiating respiration; 3) mitochondria were preincubated for 3 min with growth regulators and next, their oxidative-phosphorylative properties were studied.

In the experiments with an inhibitor of electron transport in the respiratory chain — antimycin A ($2 \cdot 10^{-6}$ M) and an uncoupler of oxidative phosphorylation — DNP ($5 \cdot 10^{-5}$ M), the first experimental variant was used. 2,4-D and ABA were used in a concentration range of 10^{-9}-10^{-3} M. The concentration of ADP was determined spectrophotometrically (Specord UV VIS) at $\lambda = 260$ nm, using a molar extinction coefficient = 15.4. The respiration of isolated maize mitochondria was calculated according to Bonner (1974) using: RC, ADP:O ratio, intensity of oxygen uptake expressed in nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein (mp) $\cdot min^{-1}$. The osmotic properties of mitochondria were expressed in $\Delta A_{520} \cdot mg^{-1}$ mp $\cdot min^{-1}$. Changes in pH accompanying the respiration of isolated maize mitochondria were expressed as-$\Delta pH \cdot mg^{-1}$ mp.

RESULTS AND DISCUSSION

The experiments with 2,4-D done in this study show that at all of the concentrations used (Table 1, Figs. 1 and 2), this synthetic auxin inhibits the oxidative properties of isolated maize mitochondria, whereas it affects the phosphorylative properties only after preincubation of mitochondria with it (Fig. 4). The highly changed patterns of the swollen and contracted states obtained with 10^{-7} as well as 10^{-3} M 2,4-D (Fig. 7) confirm the destructive effect of 2,4-D on mitochondrial membranes. Swelling in the presence of isotonic KCl is a result of osmotic water flow which accom-

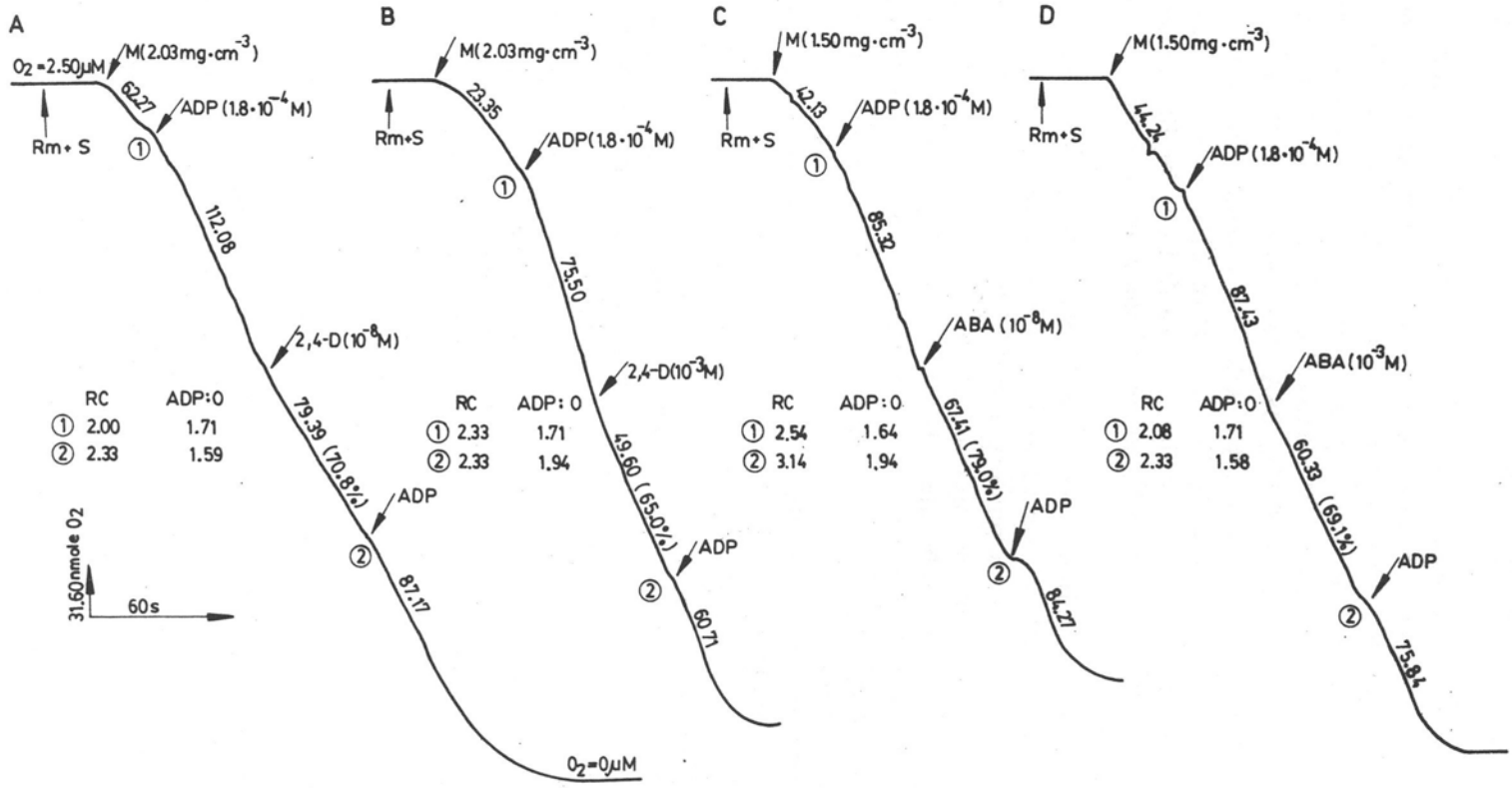

Fig. 1. Oxidative-phsphorylative activity of isolated maize mitochondria during state 4 respiration after adding: 2,4-D (A — 10^{-8} M, B — 10^{-3} M) and ABA (C — 10^{-8} M and D – 10^{-3} M). Respiration of mitochondria was in a reaction medium (Rm) composed of: 3.0 M sucrose, 10 mM KCL, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 10 mM Tris, 0.1% BSA and pH = 7.2(HCl). Oxidation substrate — 10 mM sodium succinate (S), phosphorylation substrate — $1.8 \cdot 10^{-4}$ M ADP. The numbers by the oxygrams are the values of respiratory intensity in nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein $\cdot min^{-1}$. The values of the respiratory control (RC) and ADP:O ration are given to the oxygrams (after 1 and 2 addition of ADP). The number near M (mitochondria) denote the amount of mitochondrial protein in the reaction medium

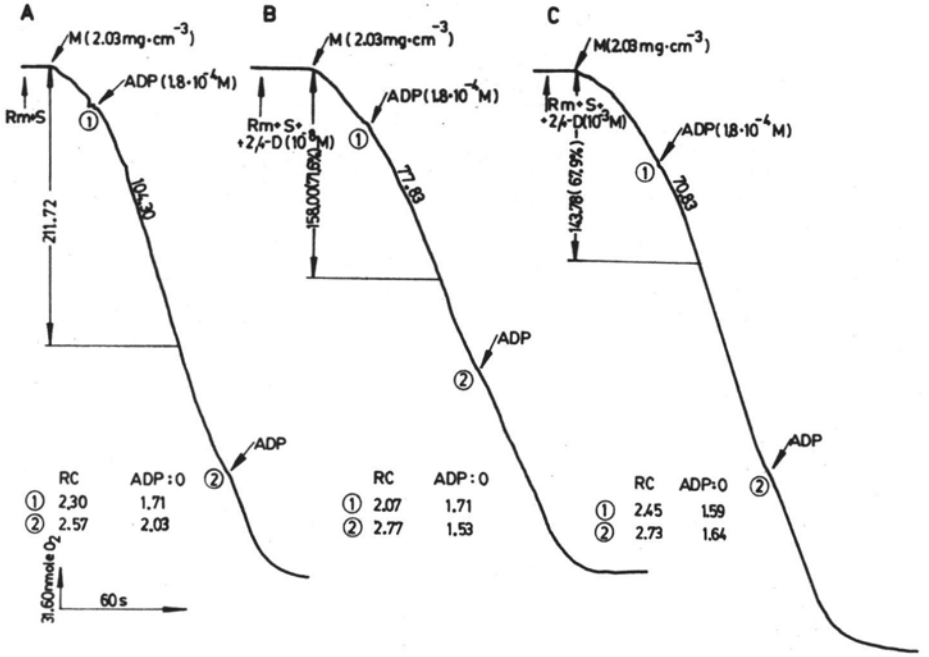

Fig. 2. The oxidative-phosphorylative activity of isolated maize mitochondria in media without 2,4-D (A), with 2,4-D at concentrations of 10^{-8} M (B) and 10^{-3} M (C). The figures next to the arrows denote the intensity of O_2 uptake in nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein $\cdot min^{-1}$. The values in parentheses denote the oxygen uptake by mitochondria during the first minute of respiration in the presence of 2,4-D, expressed as a % of the oxygen uptake in the medium without 2,4-D. The remaining legends and experimental conditions as in Fig. 1

panies the diffusion of KCl into the mitochondria. Cl^- diffuses in accordance with the concentration gradient, whereas the influx K^+, in order to uphold electroneutrality, limits the rate of swelling. The increase in swelling in the presence of 2,4-D is a result of lowering the membranes resistance to K^+ by that herbicide, which may be connected with the entry of the synthetic auxin into the membrane itself and the resulting change in its conformation and highly ordered state (redox carriers, mitochondrial ATPase). In respect to mitochondrial respiration, 2,4-D exhibits marked herbicidal properties, which agrees with the role of phenoxy-acids proposed by Matlib et al. (1972) and with the findings of Moreland and Huber (1979), that herbicides inhibit the respiration of bean mitochondria.

In the case of respiration in maize mitochondria in the presence of abscisic acid (a plant growth inhibitor), inhibition of oxidative-phosphorylative activity of mitochondria was found (Table 2, Figs. 1 and 3). ABA affected the phosphorylative properties through its presence in the respiration medium (Fig. 3) or by preincubation (Fig. 4), showing a clear increase of this effect with increase in concentration. Walbot et al. (1975) found

Table 1

The effect of 2,4-D on the intensity of oxygen uptake by isolated maize mitochondria

Concentration of 2,4-D, M	Intensity of oxygen uptake, nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein $\cdot min^{-1}$		
	before the addition of 2,4-D	after the addition of 2,4-D	
10^{-3}	90.88 ± 8.45	58.13 ± 5.25	(62.9%)
10^{-4}	82.70 ± 4.47	56.81 ± 2.53	(68.7%)
10^{-5}	128.44 ± 6.59	87.15 ± 8.77	(67.9%)
10^{-6}	92.57 ± 4.07	59.85 ± 4.51	(64.7%)
10^{-7}	116.64 ± 6.02	81.01 ± 4.71	(69.5%)
10^{-8}	106.09 ± 6.60	78.55 ± 5.88	(74.0%)
10^{-9}	134.92 ± 4.84	109.71 ± 5.32	(81.3%)

Oxygen uptake was measured in a reaction medium composed of: 0.3 M sucrose, 10 mM KCl, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 10 mM Tris, 0.1% BSA and pH = 7.2 (HCl). The oxidation substrate was 10 mM sodium succinate, the phosphorylation substrate — $1.8 \cdot 10^{-4}$ M ADP. The results given are averages of at least 12 measurements, ± — standard error of the mean, the figures in parentheses express the intensity of oxygen uptake (nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein (mp) $\cdot min^{-1}$) after the addition of 2,4-D in % of the intensity of oxygen uptake before the addition of a growth regulator. The vitality of the isolated mitochondria is characterized by: RC = 2.66 ± 0.08, ADP:O ratio = 1.75 ± 0.03.

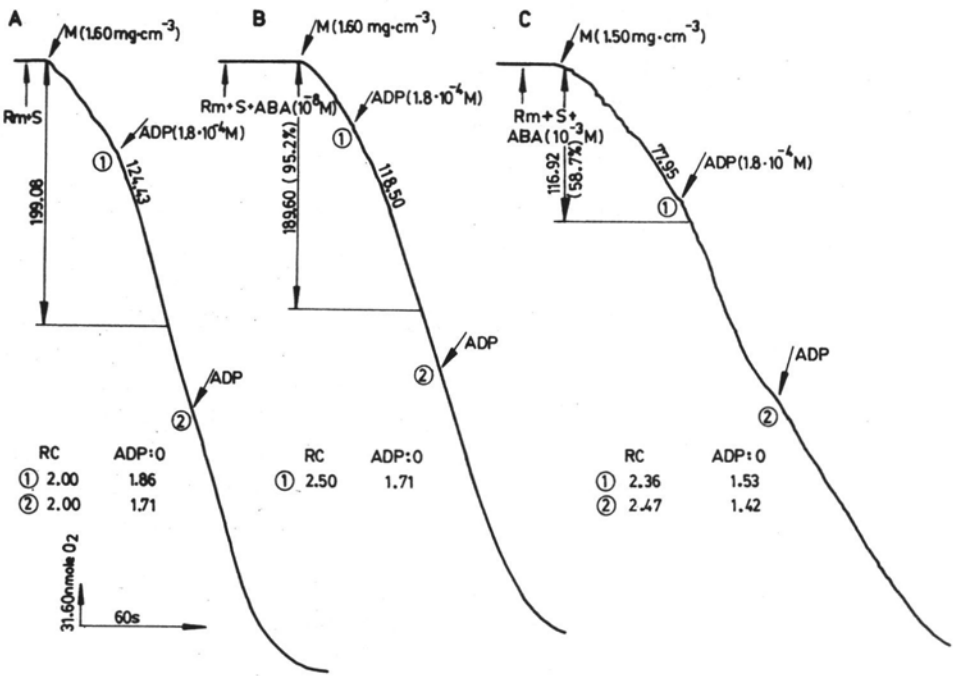

Fig. 3. The oxidative-phosphorylative activity of isolated maize mitochondria in media without ABA (A), with ABA at a concentration of 10^{-8} M (B) and 10^{-3} M (C). Experimental conditions and legends as in Fig. 2.

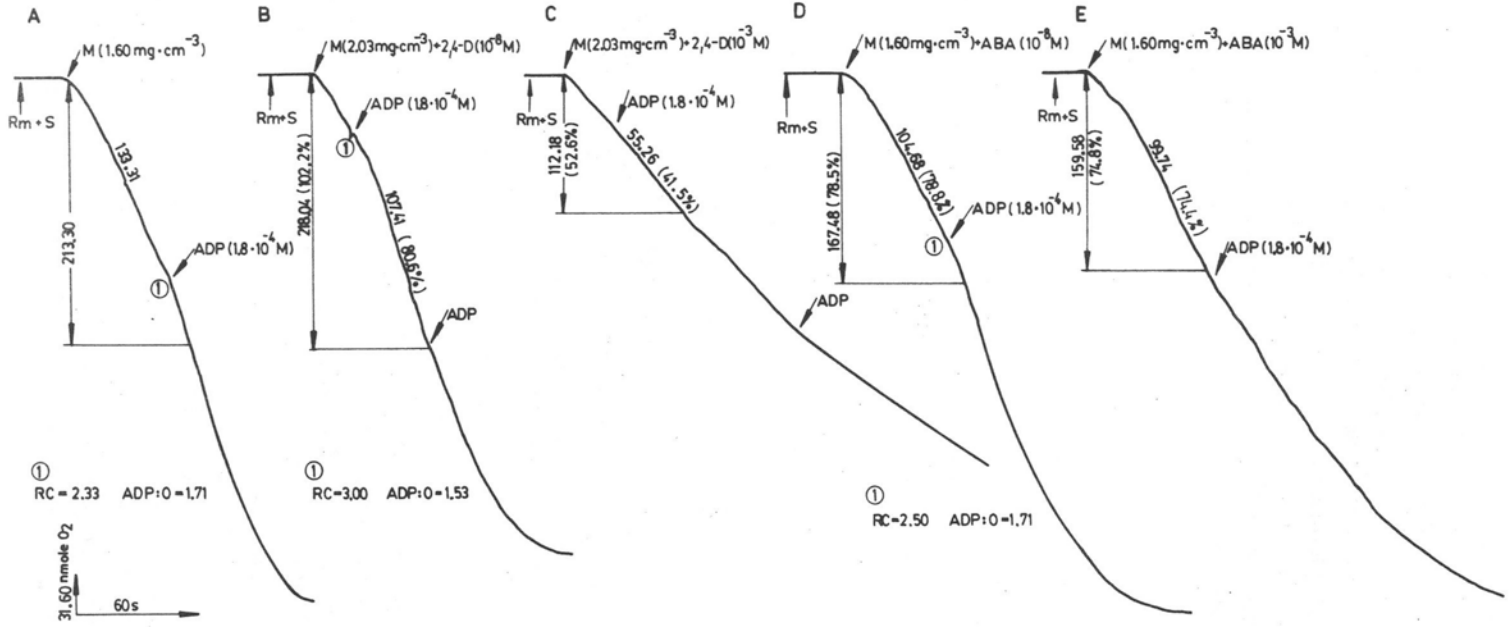

Fig. 4. The oxidative-phosphorylative activity of isolated maize mitochondria after preincubation in a media without growth regulators (A) and with 2,4-D at a concentration of 10^{-8} M (B) and 10^{-3} M (C) and ABA (10^{-8} M — D, 10^{-3} M —E). Experimental conditions and legends as in Fig. 2

that ABA in experiments on whole plants organisms also shows a close dependence of its inhibitory effect on QO_2 and the ATP/ADP ratio on its concentration. It can then be accepted on the basis of the experiments presented here, that ABA is a destroyer of mitochondrial membranes (Fig. 7). From the spectrophotometric studies it can be seen that ABA stimulates swelling, changes the picture of transition to the contracted state (10^{-7} M) or prevents the contraction of mitochondria (10^{-3} M). Swelling in the presence of high concentrations of the growth regulators used here is similar to the swelling of mitochondria in the presence of destroyers of respiratory

Table 2

The effect of ABA on the intensity of oxygen uptake by isolated maize mitochondria

Concentration of ABA, M	Intensity of oxygen uptake, nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein $\cdot min^{-1}$		
	before the addition of ABA	after the addition of ABA	
10^{-3}	102.31 ±0.00	70.71 ±0.00	(69.1%)
10^{-4}	118.90 ±14.00	78.21 ±7.40	(65.8%)
10^{-5}	110.31 ±13.12	76.55 ±11.02	(69.4%)
10^{-6}	75.84 ±0.00	55.10 ±0.00	(72.7%)
10^{-7}	74.71 ±0.00	54.83 ±0.00	(73.4%)
10^{-8}	85.63 ±0.00	66.87 ±0.00	(78.1%)
10^{-9}	77.21 ±6.32	68.15 ±3.63	(88.3%)

The oxygen uptake was measured in a reaction medium composed of: 0.3 M sucrose, 10 mM KCl, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 10 mM Tris, 0.1% BSA and pH = 7.2 (HCl). The oxidation substrate was 10 mM sodium succinate, the phosphorylation substrate — $1.8 \cdot 10^{-4}$ M ADP. The figures given are averages of at least 12 measurements, ± — standard error of the mean, the figures in parentheses express the intensity of oxygen uptake (nmoles $O_2 \cdot mg^{-1}$ mitochondrial protein ($mp \cdot min^{-1}$) after the addition of ABA in % of the intensity of oxygen uptake before the addition of the growth regulator.

processes — antimycin A and DNP (results not shown indicate that in the presence of 2,4-D, swelling attains 184% of the swelling of control mitochondria, whereas in the presence of ABA, antimycin A and DNP, 192, 264 and 498%, respectively). On the basis of the spectrophotometric studies it can be said that 2,4-D and ABA in high doses act destructively on the integrity of mitochondrial membranes and the normal functioning of mitochondria. Figures 5 and 6 show the respiratory activity of isolated mitochondria in the presence of growth regulators and antimycin A or DNP.

The experiments in which the pH was measured during respiration of mitochondria in the presence of growth regulators (Figs. 8 and 9) were intended to supplement the polarographic and spectrophotometric experiments.

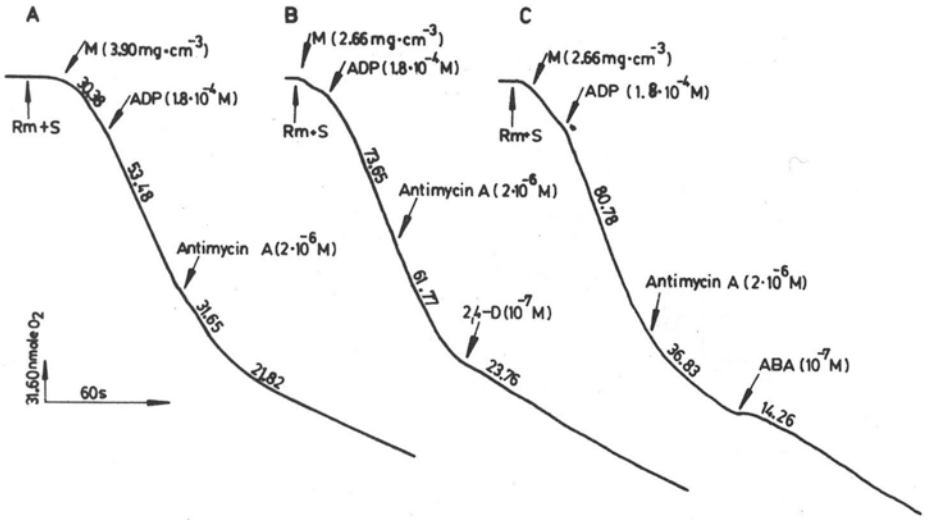

Fig. 5. Oxidative and phosphorylative properties of isolated maize mitochondria in a medium with a respiration inhibitor — antimycin A (A) and in a medium with antimycin A and growth regulators: 2,4-D (B) and ABA (C). Experimental conditions and legends as in Fig. 1

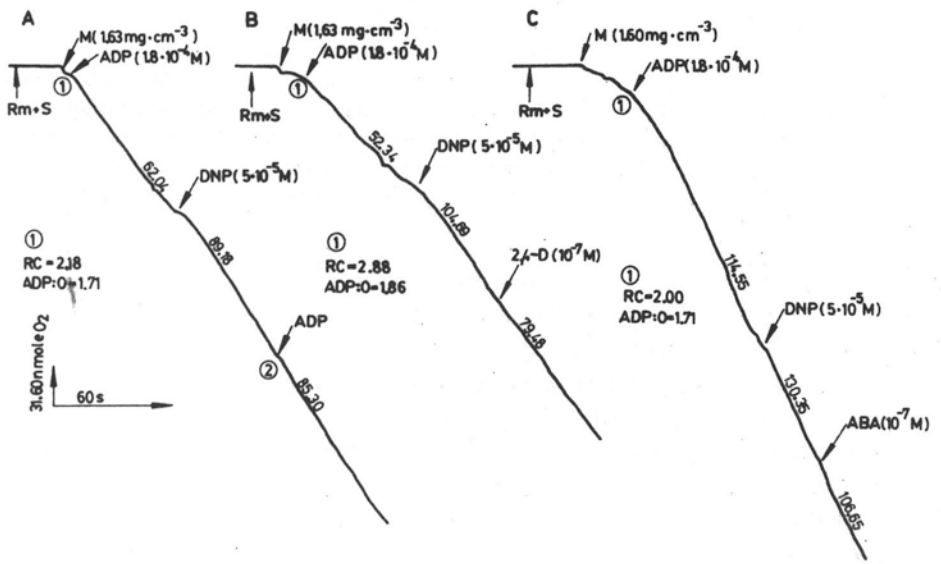

Fig. 6. Oxidative-phosphorylative properties of isolated maize mitochondria in a medium with a phosphorylation uncoupler — DNP (A) and in a medium with the uncoupler and growth regulators: 2,4-D (B) and ABA (C). Experimental conditions and legends as in Fig. 1

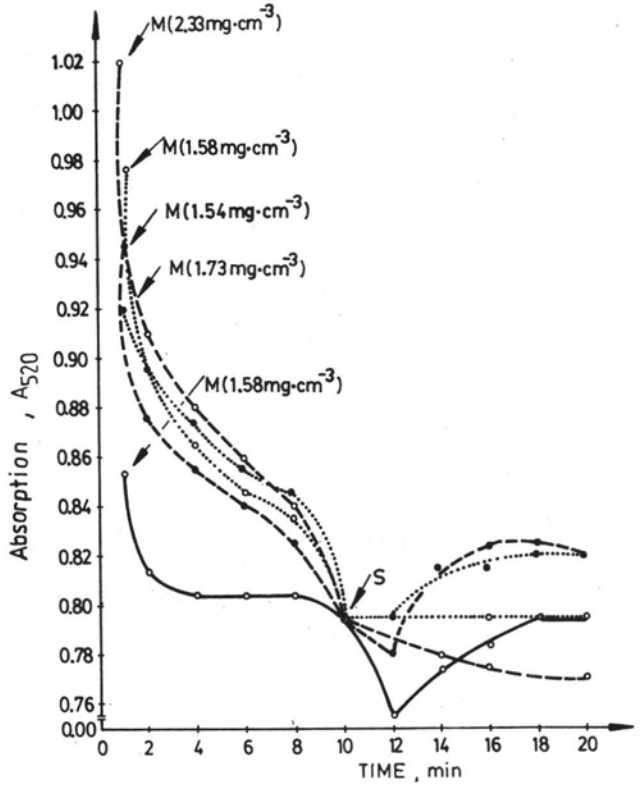

Fig. 7. Swelling and contraction of isolated maize mitochondria in a medium with 2,4-D (– –,–) and ABA (.....) at concentrations of 10^{-7} (●) and 10^{-3} M (O). Swelling was carried out in a reaction medium of the following composition: 150 mM KCl, 20 mM Tris, 1 mg·cm^{-3} BSA at pH = 7.5. Contraction was induced with 10 mM sodium succinate (S). The continuous line shows swelling and contraction in an osmoticum without growth regulators. Numbers next to M (mitochondria) show the amount of mitochondrial protein in mg·cm^{-3} in the reaction medium

In fact, 2,4-D and ABA at a concentration of 10^{-3} M, ($-\Delta pH_{2,4\text{-}D} = 0.07 \cdot \cdot mg^{-1}$ mitochondrial protein (mp), $-\Delta pH_{ABA} = 0.12 \cdot mg^{-1}$ mp) stimulated acidification of the medium of the respiring mitochondria compared with the control ($-\Delta pH_{Rm} = 0.021 \cdot mg^{-1}$ mp) or with 2,4-D and ABA at a concentration of 10^{-7} M ($-\Delta pH_{2,4\text{-}D} = 0.020 \cdot mg^{-1}$ mp, $-\Delta pH_{ABA} = 0.029 \cdot mg^{-1}$ mp). This proves that the destruction of mitochondrial membranes changes the buffering properties and does not allow the isolated mitochondria to function normally.

Comparing these results with those of Raczek (1983a, b) on the effect of natural auxin (IAA) on respiration of isolated mitochondria, it can be stated that the destructive effect of growth regulators (IAA, 2,4-D and ABA) on mitochondrial membranes can be the reason for their inhibition of

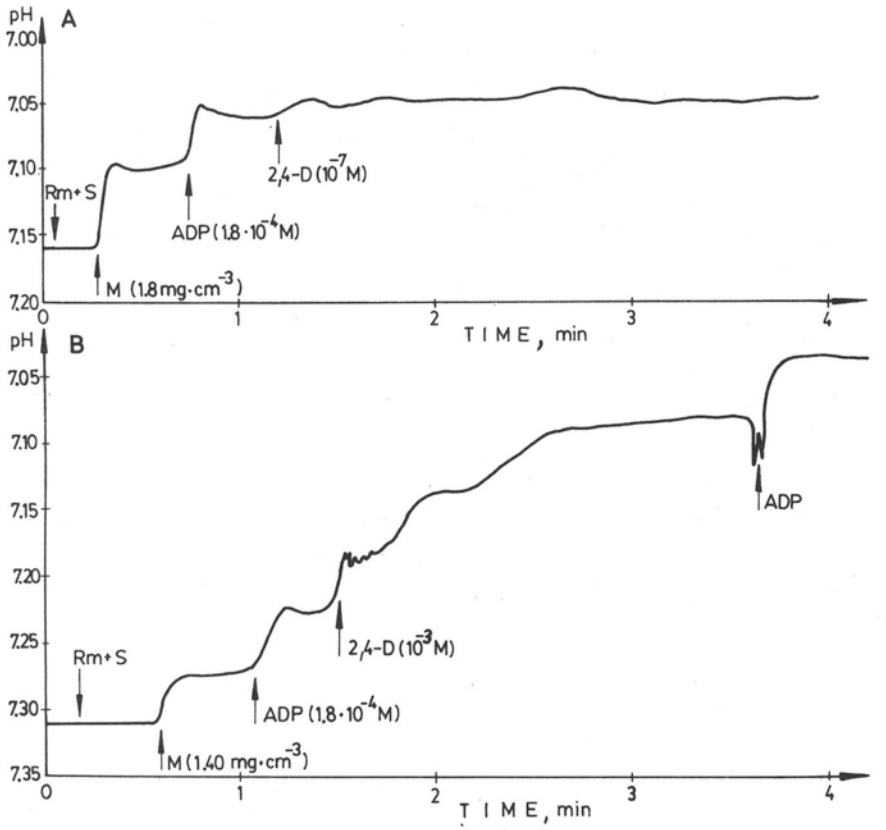

Fig. 8. Changes in the pH of the reaction medium during respiration of isolated maize mitochondria in the presence of 2,4-D at concentrations of 10^{-7} M (A) and 10^{-3} M (B). Respiration of the mitochondria took place in a reaction medium (Rm) of the following composition: 0.3 M sucrose, 10 mM KCl, 5 mM $MgCl_2$, 10 mM KH_2PO_4, 10 mM Tris, 0.1% BSA and pH = 7.2±0.1 (HCl). Oxidation substrate — 10 mM sodium succinate (S), phosphorylation substrate — $1.8 \cdot 10^{-4}$ M ADP. The numbers in parentheses next to M (mitochondria) express the amount of mitochondrial protein in mg·cm^{-3} in the reaction medium

plant cell elongation. Miller (1980, 1982), too, equates the inhibitory effect of cytokinins (6-benzylaminopurine, kinetin, zeatin and its esters) on the respiratory activity of isolated mitochondria and submitochondrial particles with the capability of these growth regulators to bind to and interact with mitochondrial membranes. He attempts to explain some important processes in the life of the plant, such as the production of ethylene or the initiation of callus, by the action of cytokinins on plant mitochondria. Whereas, this paper could explain the dependence of the plant growth reaction induced by growth regulators on the response of plant

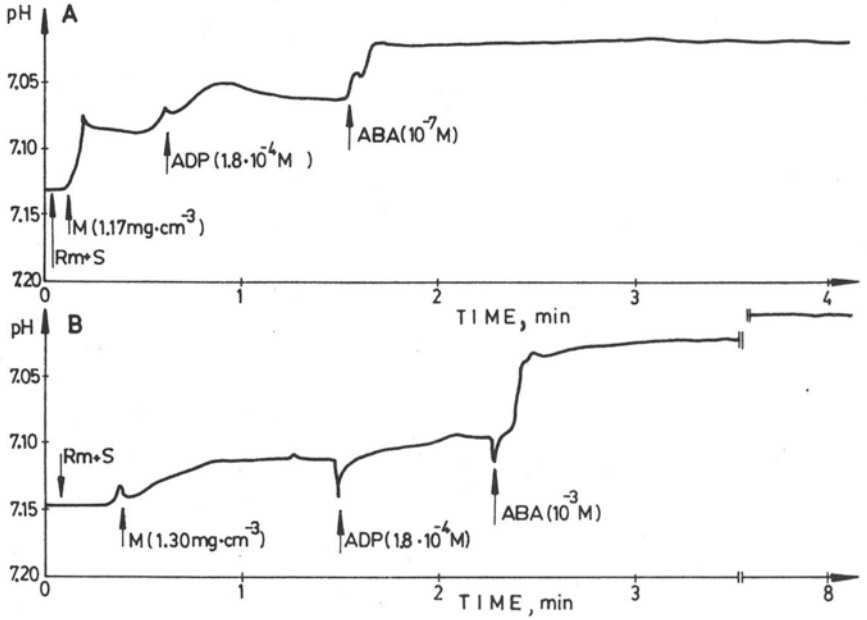

Fig. 9. Changes in the pH of the reaction medium during respiration of isolated maize mitochondria in the presence of ABA at a concentration of: 10^{-7} M (A) and 10^{-3} M (B). Experimental conditions and legends as in Fig. 8

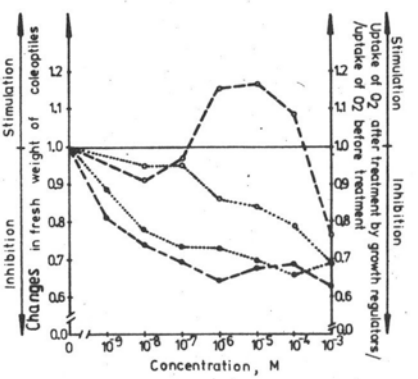

Fig. 10. Comparison of the effect of 2,4-D (–––––) and ABA (.....) on the growth of maize coleoptiles (O) and on respiration of isolated maize mitochondria (●). Growth of maize coleoptiles is expressed as the ratio of the increase of fresh weight of coleoptiles in the presence of growth regulators to the increase of fresh weight in the control medium. The results showing the effect of 2,4-D and ABA on the growth of maize coleoptiles are from the paper by Raczek (1983b)

mitochondria to these regulators. In the case of 2,4-D it would explain its herbicidal effect on the elongation of plants, in respect to ABA — its concentration-dependent inhibitory action (Fig. 10). In addition, the results of the experiments presented here can indirectly point to the participation of mitochondria in hormonal regulation of plant growth, not only through the coupling of energetic and elongation processes, but also through the sensitivity of the microstructures of these organelles to natural and synthetic growth regulators.

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Oddziaływanie 2,4-D i ABA na aktywność oddechową izolowanych mitochondriów koleoptyli kukurydzy

Streszczenie

Badano wrażliwość izolowanych mitochondriów kukurydzy na regulatory wzrostu: kwas 2,4-dwuchlorofenoksyoctowy (2,4-D) i kwas abscysynowy (ABA). Stwierdzono, że 2,4-D (herbicyd) inhibował aktywność oddechową mitochondriów, podobnie jak inne herbicydy czy fenoksy-kwasy. 2,4-D w całym zakresie stosowanych stężeń (10^{-3}-10^{-9} M) — wprowadzony przed rozpoczęciem reakcji oddechowej lub w czasie jej trwania, hamował intensywność utleniania bursztynianu. Nie zmieniał jednak wyraźnie własności fosforylacyjnych. Rozprzężenie fosforylacji oksydacyjnej następowało dopiero w wyniku preinkubacji mitochondriów z 2,4-D i było konsekwencją destrukcji błon mitochondrialnych. ABA (inhibitor wzrostu roślin) wywoływał podobną odpowiedź oddechową mitochondriów kukurydzy. Preinkubacja mitochondriów z ABA prowadziła do rozprzężenia fosforylacji oksydacyjnej. Natomiast ABA dodany w trakcie oddychania (4 stan oddechowy) lub przed jego rozpoczęciem zmniejszał zdolności utleniające mitochondriów; zmieniał również obraz tranzycji stanów 4-3-4 po dodaniu ADP (szczególnie wyraźnie w dużych stężeniach), co świadczy o zachwianiu sprzężenia fosforylacji oksydacyjnej z łańcuchem oddechowym. Wydaje się, że ten ujemny wpływ 2,4-D i ABA na aktywność oddechową izolowanych mitochondriów kukurydzy ma związek z hamującym oddziaływaniem tych regulatorów wzrostu na wzrost koleoptyli kukurydzy. Ingerencja w organizację błon mitochondrialnych kończy się zmniejszoną dotacją ATP — źródła energii potrzebnej w procesach wydłużeniowych.