Comparative investigations on the effect of IAA and EDTA on water uptake and respiration of potato discs during and after the resting period

J. BUCZEK

INTRODUCTION

In the former paper (Buczek 1965 a) we found a significant difference in the respiratory metabolism of potato discs incubated in various respiratory substrates during and after the resting period. The respiratory substrates which appeared to be inactive during the resting period, stimulated both respiration and increase in weight if given together with IAA.

Since some processes appeared to be analogously induced by IAA and EDTA (Bennet-Clark 1956; Heath and Clark 1956, 1960; Buczek 1965 b), the author conducted some comparative investigations on the effects of IAA and EDTA on the absorption of water and respiration in potato discs during and after the resting period.

METHODS

The experiments were carried out on the potato varieties 'Dar' and 'Epoka'. The preparation of discs as well as other methodical details concerning incubation and measurements were performed by methods described in a former paper (Buczek 1965 a). The experiments were conducted in redistilled water, pH 6.0—6.2. The calcium content in the incubation solution was determined by the Gauthier-Pignard method (1958) with murexide as indicator.

The following substances were used: Indole-3 acetic acid — IAA (Chemapol, Czechoslovakia), ethylenediaminetetraacetic acid disodium salt — EDTA (Ciech, Poland). The solution of Fe^{3+} , Cu^{2+} and Ca^{2+} cations given in the form of sulphates were prepared from three times recrystallized salts.

RESULTS

Effect of IAA and EDTA on resting tissues

Results of the experiments conducted on dormant potato tubers in first week of December are given in Table 1. Oxygen uptake is expressed

Table 1

The effect of IAA and EDTA on respiration and water uptake by potato discs during the resting period

Variety		Day of measurements						
		0	2		4		6	
		Oxygen uptake	Oxygen uptake	Increase in weight %	Oxygen uptake	Increase in weight %	Oxygen uptake	Increase in weight %
Dar	Water (control)	19.5	48.5	3.5	51.5	8.3	46.7	9.1
	IAA		± 3.12	± 0.52	± 2.87	± 0.60	± 3.01	± 0.71
	$\times 10^{-5}$ M	19.5	46.9	4.7	65.1	11.8	70.6	17.9
			± 2.11	± 0.48	± 3.25	± 0.43	± 5.18	± 0.82
Dar	Water (control)	14.7	49.5	2.1	55.5	6.3	42.9	6.2
	EDTA		± 2.55	± 0.73	± 4.10	± 0.87	± 5.12	± 0.47
	$\times 10^{-5}$ M	14.7	44.0	1.0	57.6	4.8	45.5	5.6
			± 3.18	± 0.48	± 5.18	± 0.53	± 3.29	± 0.58
Epoka	Water (control)	21.2	66.5	4.3	63.9	13.2	61.8	16.9
	EDTA		± 4.12	± 0.37	± 5.10	± 0.41	± 6.13	± 0.39
	$\times 10^{-5}$ M	21.2	60.6	4.3	61.8	13.3	59.4	16.9
			± 3.87	± 0.22	± 6.25	± 0.25	± 4.27	± 0.41

$\pm$ S. D. E.

Table 2

The effect of IAA and EDTA on the increase in fresh weight of dormant tissues and the amount of Ca ions passing to incubation solution

	Day of measurements					
	2		4		6	
	Increase in weight %	mg Ca per 660 mg of tissue	Increase in weight %	mg Ca per 660 mg of tissue	Increase in weight %	mg Ca per 660 mg of tissue
Water (control)	8.7	0.034	15.0	0.013	15.7	0.009
IAA	± 0.33		± 0.52		± 0.31	
$\times 10^{-4}$ M	9.7	0.071	19.8	0.011	24.7	0.009
IAA	± 0.48		± 0.49		± 0.62	
$\times 10^{-5}$ M	9.9	0.039	17.8	0.015	21.6	0.014
EDTA	± 0.37		± 0.33		± 0.90	
$\times 10^{-4}$ M	9.0	0.158	15.5	0.037	16.2	0.023
EDTA	± 0.44		± 0.71		± 0.57	
$\times 10^{-5}$ M	7.2	0.063	14.2	0.026	16.1	0.019
	± 0.41		± 0.60		± 0.48	

as $\mu\text{l O}_2/5\text{discs}/30\text{ min}$. Water absorption was determined as increase in weight. The results are mean values from 10 determinations. Both water absorption and respiration were enhanced in all combinations with IAA, and did not show any significant differences when treated with EDTA.

Table 2 contains the data concerning the increase in fresh weight of resting discs, and the amount of calcium that passed to the incubation solution. Water uptake was expressed as percentage of the increment in fresh weight, the amounts of calcium being given in milligrams per 660 mgr of tissue, for each day of measurements. It appears that the higher the concentration of the external IAA or EDTA solution the greater amounts of calcium cations pass to the external solution, especially on the second day of the measurements. We could not observe, however, any correlation between the increase in fresh weight and the loss of calcium either for IAA or EDTA combinations.

Table 3

Interaction of EDTA and IAA on the water uptake by potato discs during the resting period

Solution		Day of measurements		
IAA in M	EDTA in M	2	4	6
0	0	1.3	3.3	5.5
10^{-5}	0	2.1	8.6	11.3
10^{-5}	10^{-5}	1.9	6.8	8.8

Table 3 does not indicate any synergistic action between EDTA and auxin in the increase in fresh weight of dormant tissues. It appeared namely, that the addition of EDTA to the solution of IAA reduced the effectiveness of auxine.

Effects of EDTA and IAA on tissues after the resting period

Results of the experiments conducted on potato discs after the resting period are presented in Table 4. The experiment was carried out in the last week of April. It appears that the effect of IAA on the absorption of water and respiration is similar to that in dormant tissues. One observes, namely, that both processes are stimulated, whereas EDTA stimulates only the increase in fresh weight, exerting no effect on respiration. The slight differences observed in respiration appeared to be statistically insignificant.

It is known that storage tissues in plant are able to take up water from solutions hypertonic with respect to the cell contents (Bonner et al., 1953) in presence of auxins. Fig. 1 illustrates the results of comparative studies on potato discs and their ability to absorb water from a mannitol solution in presence of IAA or EDTA. The discs were kept under aerobic conditions in IAA or EDTA solutions for 3 days. Then one part of the discs were placed in the same solutions and the

Table 4

The effect of IAA and EDTA on respiration and water uptake by potato disc after the resting period

		Day of measurements						
		0	2		4		6	
Variety		Oxygen uptake	Oxygen uptake	Increase in weight %	Oxygen uptake	Increase in weight %	Oxygen uptake	Increase in weight %
Dar	Water (control)	14.5	45.0 ±2.91	2.1 ±0.12	47.2 ±6.8	5.9 ±0.37	36.6 ±4.12	5.2 ±0.25
	IAA ×10 ⁻⁵ M	14.5	54.8 ±3.18	2.6 ±0.15	60.5 ±4.12	10.0 ±0.42	48.1 ±4.85	11.8 ±0.57
	EDTA ×10 ⁻⁵	14.5	49.3 ±3.55	1.9 ±0.25	50.6 ±5.10	7.6 ±0.44	34.5 ±3.55	8.1 ±0.32
	Water (control)	20.1	48.4 ±3.10	6.9 ±0.55	50.1 ±3.55	14.0 ±0.38	39.2 ±6.10	17.1 ±0.44
	IAA ×10 ⁻⁵ M	20.1	53.0 ±2.19	8.5 ±0.42	75.7 ±5.14	22.8 ±0.62	51.4 ±4.82	27.0 ±0.51
Epoka	EDTA ×10 ⁻⁵ M	20.1	50.0 ±2.55	7.8 ±0.33	52.6 ±4.10	21.1 ±0.55	40.1 ±3.25	22.7 ±0.68

Table 5

Effect of 10⁻⁵ M EDTA, 10⁻⁵ M metal cations and equimolar solutions of EDTA+metal cations (10⁻⁵ M) on the absorption of water by potato discs, after the resting period

	Day of measurement		
	2	4	6
	Increase in weight %	Increase in weight %	Increase in weight %
Water	9.2±0.53	12.8±0.33	17.1±0.81
Fe ³⁺	9.9±0.48	15.6±0.48	18.5±0.32
Cu ²⁺	3.7±0.72	2.2±0.50	1.5±0.44
Ca ²⁺	6.0±0.61	13.6±0.67	18.7±0.71
EDTA	10.3±0.31	18.2±0.48	22.8±0.33
EDTA+Fe ³⁺	9.2±0.25	15.2±0.53	18.4±0.44
EDTA+Cu ²⁺	7.5±0.47	13.1±0.71	17.7±0.52
EDTA+Ca ²⁺	7.9±0.52	15.1±0.62	18.1±0.61

remaining ones in solution of mannitol. After 24 hours the discs were weighed. Fresh weight was calculated from the differences between the initial and final weights. It was found that starting with 0,1 M mannitol the control discs absorbed considerably less water, whereas IAA or EDTA treated discs distinctly augmented their weight. Mannitol 0,4 M solution

Fig. 1. Effect of IAA and EDTA on water absorption from hypertonic solutions of mannitol.

1 (—) control; 2 (—○—) IAA $\times 10^{-5}$ M; 3 (—x—) EDTA $\times 10^{-5}$ M

caused a smaller increment in fresh weight of the control discs, while the discs previously incubated in IAA or EDTA solutions were still able to absorb a certain amount of water.

Further experiments (Table 5) showed the effect exerted by: Fe^{3+} , Cu^{2+} , Ca^{2+} ions, EDTA, and equimolar solutions of EDTA with the above mentioned ions, on water uptake by potato discs after the resting period. It appeared that the cations in question did not exerted any distinct effect on the increase in fresh weight, except for Cu^{2+} which remarkably inhibited this process. Equimolar solutions of cations given together with EDTA inhibited water uptake as compared with pure EDTA. The increment in fresh weight observed for the above combinations

was similar to that of the control. If, however, we compare the effect of given together with EDTA with the effect of single ions, it follows that EDTA compensates the inhibitory effect of Cu ions, being indifferent with respect to the remaining ions.

DISCUSSION

Analysis of the experiments shows that IAA and EDTA exert similar effect on some auxin-dependent processes. It appears namely, that the auxin-induced increase in fresh weight, as well as the absorption of water in hypertonic solutions of mannitol are also stimulated in presence of EDTA. In spite of this analogy, one observes the following differences in the effectiveness of both substances: 1. IAA affects the water absorption by potato discs both during and after the resting period, while EDTA manifested this effect only in tissues whose dormancy was terminated. 2. IAA stimulates the respiration and increment in fresh weight, whereas EDTA induces solely an increase in fresh weight. 3. Addition of EDTA to IAA solution reduces the effectiveness of auxine.

These observations suggest the following remarks: 1° The effects of EDTA and IAA on the absorption of water by potato discs differ in type. 2° Synergy between EDTA and IAA should be excluded. 3° EDTA plays a specific role in induction of water uptake by storage tissues.

The role of EDTA considered as an agent which affects some processes in a way similar to IAA has been widely discussed. According to Bennet-Clark (1956), Heath and Clark (1956) and Weinstein (1956) elongation growth is induced by EDTA by way of removal of Ca ions from the cell walls or, (Thimann and Takahashi 1963) owing to synergy with EDTA, whereas, according to Carr and Ng (1959) and Ng and Carr (1959) and Cleland (1960) it appears that these chelating agents promoting growth do not remove calcium from cell walls.

It is obvious that in our experiment the effect of EDTA is reduced to chelation of some ions which might play a certain part in the process of water absorption by potato discs. This supposition has been confirmed by our experiments (Table 5). It follows namely, that only pure EDTA solutions are able to induce the increase in fresh weight, since the solutions of EDTA supplemented with an equimolar quantity of Fe^{3+} , Cu^{2+} and Ca^{2+} ions did not manifest this effect. This phenomenon is likely to be caused by formation of certain exogenic chelates which (as follows from our experiments) do not exert any effect on the increase in fresh weight. It seems that the Ca ions cannot be responsible for the formation of chelates, although in presence of EDTA, large quantities

of these ions pass to the external solution. This process, however, does not stand in relation with the increase in fresh weight.

It is well known that in storage tissues incubated in water the increase in fresh weight depends on oxygen metabolism (Bonner et al., 1953) and that it may be induced by respiratory stimulators (Buczek 1965 a). It seems, moreover, that the effect of IAA on the increase in fresh weight is at least partially, related with the increased intensity of respiration. As in the case of EDTA this relations has not been established and since this agent shows a great capacity to form endogenic chelates (Brown et al., 1960), it seems probable that the process of water absorption by storage tissues is additionally regulated by some other agents (metal ions).

The addition of EDTA either eliminates or activates a suitable ions, which consequently causes increased water absorption. The fact that EDTA stimulates the absorption of water only in tissues that have emerged from dormancy (i.e. in tissues manifesting intensive metabolic processes) allows to suppose that the effect of EDTA on the absorption of water is conditioned by metabolic processes.

SUMMARY

Comparative studies on the effect of IAA and EDTA on the increase in fresh weight and respiration in potato discs during and after dormancy were based on the analogy found in the effect of IAA and EDTA on some auxin-induced processes. The experiment was performed with the varieties "Dar" and "Epoka" in December and April.

It was found that 1° EDTA induces an increase in fresh weight only in tissues which have emerged from dormancy, whereas the effect of IAA is not dependent on this period. 2° EDTA does not affect the changes in the intensity of respiration whereas IAA stimulates this process.

Comparison of the effects of EDTA and IAA suggests that the versenate affects the increase in fresh weight in a manner different than does IAA as it probably activates some ions which take part in the metabolism of potato tissues after the resting period.

*Department of Plant Physiology,
University, Wrocław,
Kanonka 6/8.*

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Streszczenie

W oparciu o dane odnośnie podobieństwa w działaniu IAA oraz EDTA na niektóre procesy indukowane auksyną przeprowadzono porównawcze badania nad wpływem IAA oraz EDTA na przyrost świeżej masy i oddychanie krążków ziemniaka przed i po zakończeniu okresu spoczynku. Doświadczenia przeprowadzono na ziemniakach odmiany „Dar” i „Epoka” w miesiącach grudniu i kwietniu.

Stwierdzono, że: 1. EDTA indukuje przyrost świeżej masy tylko u tkanek, które zakończyły okres spoczynku. Wpływ IAA natomiast jest niezależny od tego czy tkanki znajdują się w stanie spoczynku czy po jego zakończeniu. 2. EDTA nie wpływa na zmiany w intensywności oddychania, podczas gdy IAA stymuluje oddychanie.

Porównując działanie IAA i EDTA wysunięto przypuszczenie, że wersenian wpływa na przyrost świeżej masy na innej drodze niż IAA aktywując prawdopodobnie jakieś kationy czynne w metabolizmie tkanek ziemniaka po zakończonym okresie spoczynku.