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The role of Na_2-EDTA and metal ions in the induction of adventitious roots

J. BUCZEK

INTRODUCTION

Bennet-Clark (1956) and Heath and Clark (1956, 1960) demonstrated that EDTA and IAA exert a similar effect on the elongation of the coleoptile in oat and wheat. In willow twigs (Buczek 1965) the formation of adventitious roots is induced both by EDTA and by IAA. It is well known that in cuttings the formation of adventitious roots begins by an intensive multiplication of tissue cells. The divisions of cells lead to the formation of adventitious root primordia and further to the development of adventitious roots. Thus the function of factors stimulating the rooting would lie in inducing some processes by which the given developmental trend of the tissue producing adventitious roots primordia is conditioned. These processes, in turn, are closely related with nucleoproteides metabolism (Fellenberg 1963, 1965). It is very likely that EDTA as a chelating agent, possessing a high ability of forming chelating compounds in plants (Weinstein et al. 1956; Brown et al. 1960; Burström 1963) may affect the metabolism of nuclear (Mazia 1953) or cytoplasmic (Ts'a 1958, Hanson 1960) nucleoproteides.

In view of the chelating properties of EDTA and its ability to stimulate the formation of adventitious roots in willow twigs, the effect of this agent on the rooting of leaves and shoots in tomato, and on leaves in bean was compared with the effect of IAA. The investigations were based on the series of metal ions estabilished by Martell and Calvin (1952).

MATERIAL AND METHODS

The experiment was carried out on leaves and shoots of *Lycopersicum aesculentum* Mill. variety 'Best of all' and on leaves of *Phaseolus vulgaris* L. variety 'Saxa'. The plant material for study and the method of its preparation were the same as described by Buczek (1966). The experimental shoots were obtained by cutting off the two-week-old seedlings at the level of their root neck.

The cuttings were first placed in medium I for 24 hours, then they were throughly washed in bidistilled water and placed in dark-glass jars filled with medium II.

Medium I — either redistilled water (control) ar a IAA or EDTA solution with or without metal ions.

Medium II — KNO_3 — 0,001 M, H_3BO_3 — 3,1 mg/l. and H_3MoO_3 — 0,5 mg/l. with or without the investigated ions.

The composition of the solution was the same as in earlier investigations (Buczek 1966), with due consideration of the role of potassium in the metabolism of RNA (Hanson 1960). The cuttings were placed in medium II 1 cm deep. The jars were then transferred to a thermostat with constant temperature of 25°, maximum air humidity and continuous electric illumination (5000 lux). The possible loss in the volume of the solution was compensated by adding redistilled water every day. Ten days later the number of adventitious roots was counted, results being given for one leaf or shoot. Each combination consisted of 12 replications and each experiment was repeated at least three times.

The following substances were used: Indole-3 acetic acid — IAA (Chemapot, Czechoslovakia), disodium salt of ethylenediamine tetraacetic acid — EDTA (Ciech, Poland) three times recrystallized, and the solution of the particular (three times recrystallized) salts: $Fe_2(SO_4)_3$; $CuSO_4 \cdot 5H_2O$, $NiSO_4 \cdot 7H_2O$, $CoSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 7H_2O$, $CaSO_4$ $2H_2O$, $MgSO_4 \cdot 7H_2O$. All solution were prepared with redistilled water.

RESULTS

Effect of EDTA and Fe^{3+}-ions on the formation of adventitious roots

Results referring to the effect of various EDTA concentrations on the formation of adventitious roots in bean and tomato cuttings are given in Table 1. In both these species the best rooting effects were observed when EDTA was applied in a concentration of 5×10^{-4} M. Optimum dosage of EDTA increased the number of roots in bean and tomato by about 25% and 87%, respectively. At lower EDTA concentrations no differences in the number of roots were observed. The stimulative effect of EDTA did not appear if the leaves had been incubated in EDTA solutions supplemented with an equimolar quantity of Fe^{3+}-ions for 24 hours. This influence was particularly pronounced at optimum EDTA concentrations, whereas at lower concentrations the effect of iron was markedly weaker.

It follows from the data in Table 1 that the best rooting effects due to EDTA are obtained if the cuttings incubated in EDTA for 24 hours (medium I) were placed in medium II supplemented with Fe^{3+}-ions in a concentration equimolar in respect to the former dose of EDTA. In all the plants investigated this relation was very distinctly manifested at optimum concentrations of EDTA being also observed in concentrations lower than optimum. On the other hand, medium II supplemented with Fe^{3+}-ions in concentrations lower than equimolar in respect to the previous dose of EDTA was found to considerably reduce the rooting effect. The above relation may be observed in the case of bean (Table 1).

Effect of EDTA and IAA

The data in Table 2 show that the effect of IAA was several times stronger, though the rooting effect due to EDTA was very distinct. Like in former experiments, 24-hour incubation of leaves in the solution supplemented with an equimolar concentration of Fe^{3+}-ions entirely abolished the effect of EDTA, causing even an inhibition of the rooting process. No similar phenomenon was observed in the case of IAA. The effect of IAA suplemented with an equimolar concentration of Fe^{3+}-ions only slightly differed from that observed for the pure solution.

Both IAA and EDTA appeared to exert the strongest effect on rooting if the plants, after a 24-hour incubation in medium I, were transferred to medium II supplemented with an equimolar concentration of Fe^{3+}-ions in respect to the previous doses of IAA or EDTA. In the case of IAA this treatment did not bring any distinct effect. In the combination with Fe^{3+}-ions the number of adventitious roots increased only by 13—28% as compared to that in the combination wihout iron. In the case of EDTA, however, the number of adventitious roots developed in the presence of Fe^{3+}-ions (added to medium II) was markedly greater (by about 42—51%) than in its absence.

It should be emphasized that EDTA caused a progressive chlorosis of leaves, but the plants recovered when the medium was supplemented with iron.

Effect of metals on the EDTA activity

It was found in former experiments that the activity of EDTA in stimulation of adventitious roots greatly increased if the leaves incubated in pure EDTA were subsequently transferred to medium II supplemented with an equimolar concentration of Fe^{3+}-ions. The purpose of the experiments described below, was to investigate the direct effect of metals of the Martell and Calvin's series on the process of rooting, as well as their influence on the effectiveness of EDTA as rooting stimulator.

The choice of the concentrations of particular metal ion was dictated by the results obtained in some preliminary experiments on the effect of

Table 1

Effect of EDTA and Fe^{3+}-ions concentration on the rooting of tomato and bean leaves

	EDTA = 5×10^{-4} M Fe^{3+} = 5×10^{-4} M			EDTA = 10^{-4} M Fe^{3+} = 10^{-4} M		
	Medium I 24 h	Medium II 10 days	Number of roots per cutting	Medium I 24 h	Medium II 10 days	Number of roots per cutting
Tomatoes	Water	—	23	Water	—	23
	Water	Fe^{3+}	19	Water	Fe^{3+}	21
	EDTA	—	43	EDTA	—	22
	EDTA	Fe^{3+}	52	EDTA	Fe^{3+}	33
	EDTA $+Fe^{3+}$	—	2	EDTA $+Fe^{3+}$	—	9
Mung bean	Water	—	16	Water	—	16
	Water	Fe^{3+}	17	Water	Fe^{3+}	18
	EDTA	—	20	EDTA	—	18
	EDTA	Fe^{3+}	29	EDTA	Fe^{3+}	21
	EDTA	Fe^{3+} $\times10^{-5}$ M	18	EDTA	Fe^{3+} $\times10^{-5}$ M	20
	EDTA $+Fe^{3+}$	—	7	EDTA $+Fe^{3+}$	—	12

Cuttings first placed in medium I for 24 h, then transferred to medium II for 10 days. Number of adventitious roots calculated per one leaf. Results are aritmetical means obtained from 10 replications.

Table 2

Effect of IAA, EDTA and Fe^{3+} ions on the rooting of tomato leaves and shoots
For explanation see Table 1

	IAA = 10^{-4} M Fe^{3+} = 10^{-4} M			EDTA = 5×10^{-4} M Fe^{3+} = 5×10^{-4} M		
	Medium I 24 h	Medium II 10 days	Number of roots per cutting	Medium I 24 h	Medium II 10 days	Number of roots per cutting
Leaves	Water	—	18	Water	—	18
	Water	Fe^{3+}	20	Water	Fe^{3+}	20
	IAA	—	94	EDTA	—	41
	IAA	Fe^{3+}	106	EDTA	Fe^{3+}	62
	IAA $+Fe^{3+}$	—	102	EDTA $+Fe^{3+}$	—	9
Shoots	Water	—	30	Water	—	30
	Water	Fe^{3+}	35	Water	Fe^{3+}	33
	IAA	—	115	EDTA	—	58
	IAA	Fe^{3+}	137	EDTA	Fe^{3+}	83
	IAA $+Fe^{3+}$	—	116	EDTA $+Fe^{3+}$	—	8

Table 3

Effect of 5×10^{-4} M of EDTA and particular ions given in form of sulphates on the rooting of tomato leaves

For other conditions see Table 1

Medium I 24 h	Medium II 10 days	Number of roots per cutting	Stimulation due to EDTA %
Water	—	18	
EDTA	—	43	139
Water	Fe^{3+} 5×10^{-4} M	38	
EDTA	Fe^{3+} 5×10^{-4} M	72	90
Water	Cu^{2+} 10^{-5} M	55	
EDTA	Cu^{2+} 10^{-5} M	72	30
Water	Ni^{2+} 10^{-6} M	49	
EDTA	Ni^{2+} 10^{-6} M	59	20
Water	Co^{2+} 10^{-6} M	47	
EDTA	Co^{2+} 10^{-6} M	59	26
Water	Mn^{2+} 10^{-4} M	34	
EDTA	Mn^{2+} 10^{-4} M	50	46
Water	Ca^{2+} 5×10^{-4} M	19	
EDTA	Ca^{2+} 5×10^{-4} M	56	195
Water	Mg^{2+} 5×10^{-4} M	28	
EDTA	Mg^{2+} 5×10^{-4} M	60	114

Table 4

Effect of equimolar EDTA solutions and particular ions on the rooting of tomato leaves

Medium I 24 h	Medium II 10 days	Number of roots per cutting	Stimulation + inhibition — %
EDTA	—	43	
EDTA+Fe^{3+}	—	3	—93
EDTA+Cu^{2+}	—	24	—44
EDTA+Ni^{2+}	—	42	— 2
EDTA+Co^{2+}	—	43	0
EDTA+Mn^{2+}	—	43	0
EDTA+Ca^{2+}	—	48	+12
EDTA+Mg^{2+}	—	50	+16

EDTA concentration = 5×10^{-4} M, the concentration of individual ions = 5×10^{-4} M. For other conditions see Table 1.

individual metals given in the absence of auxine or EDTA on the intensity of rooting of tomato plants. The concentrations proving most favorable for rooting were used in further experiments.

Table 5

Effect of metals on the activity of equimolar EDTA solutions and the investigated ions in the process of rooting of tomato leaves

Medium I 24 h	Medium II 10 days	Number of roots per cutting	Stimulation + inhibition — %
Water	Fe^{3+} 5×10^{-4} M	38	
EDTA+Fe^{3+}	Fe^{3+} 5×10^{-4} M	2	—95
Water	Cu^{2+} 10^{-5} M	55	
EDTA+Cu^{2+}	Cu^{2+} 10^{-5} M	44	—20
Water	Ni^{2+} 10^{-6} M	49	
EDTA+Ni^{2+}	Ni^{2+} 10^{-6} M	47	— 4
Water	Co^{2+} 10^{-6} M	47	
EDTA+Co^{2+}	Co^{2+} 10^{-6} M	50	+11
Water	Mn^{2+} 10^{-4} M	34	
EDTA+Mn^{2+}	Mn^{2+} 10^{-4} M	40	+18
Water	Ca^{2+} 5×10^{-4} M	19	
EDTA+Ca^{2+}	Ca^{2+} 5×10^{-4} M	46	+140
Water	Mg^{2+} 5×10^{-4} M	28	
EDTA+Mg^{2+}	Mg^{2+} 5×10^{-4} M	46	+64

Medium I: 5×10^{-4} M EDTA, concentration of particular ions = 5×10^{-4} M. For other conditions see Table 1.

The data concerning the effect of optimum concentrations of metal ions on the rooting of tomato leaves not subjected to any prior treatment are summarized in Table 3. It follows that Fe^{3+}, Cu^{2+}, Ni^{2+} and Co^{2+}-ions exerted a favourable effect, the effect o of Mn^{2+} and Mg^{2+}-ions being much weaker, whereas Ca^{2+}-ions proved to be quite indifferent.

In general, the effect of the mentioned ions on the rooting of cuttings previously incubated in pure EDTA solution is similar to that of Fe^{3+}-ions demonstrated in earler experiments. Addition of metal ions to medium II increases the number of adventitious roots (Table 3) as compared with the effect obtained with ions given alone. The most pronounced effects, however, were obtained in the presence of Fe^{3+}, Ca^{+2} — and Mg^{2+}-ions, the remaining ions exerting a much weaker influence.

According to our expectations, 24-hour incubation of the investigated plants in an EDTA solution supplemented with an equimolar concentration of Fe^{+3}-ions inhibits the effect of EDTA as well as the very process of rooting. A similar inhibitory effect may be observed when Cu + EDTA is given in equimolar concentrations (Table 4), although the rate of inhibition is distinctly slower. Equimolar concentrations of EDTA with Ni—, Co— and Mn— ions are indifferent as regards the effect exerted by pure EDTA, wheareas EDTA together with equimolar concentrations of Ca or Mg ions slightly increases the number of adventitious roots, (by about 12—16% as compared to the effect of the pure substance).

This inhibitory effect of equimolar concentrations of the investigated metals with EDTA can be reduced (Except that of Fe^{3+}) if after a 24-hour incubation in equimolar solution the petioles are transferred to medium II supplemented with a suitable ions (Ca and Mg in particular) given in concentration known to be optimal for rooting (Table 5).

DISCUSSION

The formation of adventitious roots in cuttings (petioles or shoots) may be induced by various agents. EDTA is one of these agents whose effect is similar to that of IAA and whose ability to stimulate the rooting process has been noted on willow twigs (Buczek 1965). The same experiments performed on other plants have confirmed the stimulating effect of EDTA on the induction of adventitious roots. Although in presence of EDTA the intensity of rooting was markedly lower than in presence of IAA, nevertheless the effect of EDTA was very distinct.

The inductive effect of EDTA was not manifested at all if the cuttings had been incubated in solutions of cations in presence of equimolar amounts of EDTA. From the analysis of the data given in Table 4, it follows that the higher the ability of the metal to form a stable complex with EDTA the lower is the effect of the solution on rooting. The results obtained allow to arrange the investigated cations in a series, according to their inhibitory rooting effect exerted in solutions equimolar in respect to EDTA, namely: $Fe^{3+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+}$. This series is analogous to that given by de Kock (1956) for the toxicity in *Synapis*. The order of the ions in this series, is moreover, consistent with the theoretical trend, acording to which stable complexes with EDTA are formed. The above facts suggest that the induction of adventitious roots due to EDTA depends on its chelating properties.

From the experiments described above it follows that incubation of cuttings in pure solution of versenate resulted beyond doubt in the formation of some endogenic EDTA complexes. This process is sufficient to induce intensive rooting, but this treatment has also some side effects, namely the progressive chlorosis of leaves. This can be avoided if, after incubation in pure EDTA, the leaves are placed in medium II supplemented with Fe^{+3} - or Cu^{+2}-ions. It is probable that these cations release a certain agent from the EDTA complex, or that the addition of iron compensate its deficiency in the plant.

Effect similar to that exerted by pure EDTA was obtained when the solution of EDTA was supplemented with equimolar quantities of Ni, Co, Mn, Ca and Mg ions. It seems, however, that the effect of Ni, Co and Mn ions is reduced to the favourable action of those metals on the very processes of rooting. This hypothesis results from the fact that the above mentioned ions and their equimolar solutions with EDTA (Table 5) do not cause significant differences in rooting. Pure calcium appeared to be indifferent, whereas magnesium slightly increased the number of adventitious roots (Table 3). However, Ca or Mg ions added to medium II, after a 24-hour incubation in pure solution of EDTA, or in solutions of Ca + EDTA or Mg + EDTA exerted a positive effect on rooting.

The above facts allow to infer that chelation of Ca^{2+} and Mg^{2+}-ions (like of Fe^{3+}-ions) by EDTA takes chiefly place when this factor is inducing rooting. It seems, however that Fe^{3+}-ions are not so essential for the very process of rooting as for other metabolic processes occurring in the rooted leaves. This hypothesis results from the fact that Fe^{3+} added to medium II exerted a positive effect if, and only, if, the leaves were previously incubated in pure EDTA solution. On the other hand, addition of iron to medium II after a 24-hour incubation in an equimolar solutions of Fe^{3+} + EDTA distinctly inhibited the rooting, while the leaves or shoots showed symptoms typical of excessive doses of iron. The above described relations can be explained in the following way: the stimulation of rooting due to EDTA is possible if this agent introduced from outside is able to form chelating compounds within the plant. This treatment, however, is accompanied by a progressing chlorosis. The plants recover after subsequent addition of iron, which, in turn, appears favourable for the process of rooting. If, however, the leaves are incubated in equimolar solutions of EDTA + Fe^{3+} or Cu^{2+}, ions known to form very stable complexes with EDTA (Hill-Cottingham 1961), then the probability that EDTA will form endogenic chelates is almost null. Consequenty, no increase in the formation of adventitious roots is observed.

The induction of new organ — in our case of roots primordia — depends first of all on the nuclear metabolism of DNA (Fellenberg 1965). Since EDTA induces formation of adventitious roots, we may suppose that this chelate affects the metabolism of nucleoproteides in some obscure way. This is a hypothesis, although probable, bearing in mind that EDTA is able to remove divalent ions (probably Ca- and Mg-ions) which link some number of nucleotides with protein (Mazia 1953; Ts'o 1958; Hanson 1960). The removal of divalent ions (Ca and Mg) from the plant cells due to chelation results in degradation of the microsomal fraction of RNA within plant tissue (T s'o 1958). This degradation is strongly arrested by Ca and Mg ions.

It follows from our experiments that the addition of Ca or Mg ions to medium II, after 24-hour incubation in pure EDTA solution considerably increases the rooting of cuttings. It is also possible that EDTA as a chelating agent, is able to affect the trend of the metabolism of polynucleoproteides by controlling the calcium and magnesium ratio.

SUMMARY

The effect of EDTA on the rooting of tomato and bean leaves was investigated, in relation to the chelating properties of this agent and its ability to stimulate willow twigs.

From the comparison of the effects of IAA and EDTA it follows, that both agenst stimulate the process of rooting, though the effect of IAA is considerably higher. EDTA appeared to be most effective when after 24-hour incubation of cuttings in pure EDTA solution the leaves were placed in medium II supplemented with equimolar quantities of Fe^{3+}, Ca^{2+} or Mg^{2+}-ions. The effect of Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+}-ions is relatively weak. Equimolar solutions of these cations with EDTA appeared to be either inactive or inhibitory (Fe or Cu).

The above facts suggest that: 1. EDTA is capable of stimulating rooting in plants. 2. It is probable that this stimulation is due to certain agent, essential for formation of root primordia, which is chelated by EDTA. 3. It would seem that Ca^{2+} and Mg^{2+} ions are this agent, while Fe^{3+} plays an important part in other processes accompanying rooting.

Department of Plant Physiology,
University,
Wrocław, Kanonia 6/8.

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REFERENCES

Bennet-Clark T. A., 1956, The chemistry and mode of action of plant growth substances, Butterworth Scientific Publications, London.

Brown J. C., L. O. Tiffin, R. S. Holmes, 1960, Competition between chelating agents and roots as factor affecting absorption of iron and other ions by plant species, Plant Physiol. 35:878—886.

Buczek J., 1965, Comparative investigations on the rooting of twigs of *Salix viminalis* due to IAA and EDTA, Acta Soc. Bot. Pol., 34:389—397.

Buczek J., 1966, Ukorzenianie liści fasoli i pomidorów pod wpływem kwasu β-indolilooctowego i mineralnych związków azotu, Hodowla Roślin, Aklimatyzacja i Nasiennictwo 10:275—281.

Burström H., 1963, Growth regulation by metals and chelates; In Advances in Bot. Res. 1:73—100.

De Kock P. C., 1956, Heavy metal toxicity and iron chlorosis, Ann. Bot. 22:133—141.

Fellenberg G., 1963, Über Organbildung an in vitro kultivierten Knollengewebe von Solanum tuberosum, Z. Bot. 51:113—141.

Fellenberg G., 1965, Hemmung der Wurzellbildung an etiolirten Erbsenepikotylen durch Bromuracil und Histon, Planta 64:287—290.

Hanson J. B., 1960, Impairment of respiration, ion accumulation, and ion retention in root tissue treated with ribonuclease and ethylenediamine tetaraacetic acid, Plant Physiol. 35:372—379.

Heath O. V. S. J. E. Clark, 1956, Chelating agents as plant growth substances Nature 177—1118—1121.

Heath O. V. S., J. E. Clark, 1960, Chelation in auxin action, J. Exp. Bot. 11:167—187.

Hill-Cottingham D .G., C. P. Lloyd-Jones, 1961, Absorption and breakdown of iron-ethylenediamine tetraacetic acid by tomato plants, Nature 189:312.

Martell A. E., M. Calvin, 1952, Chemistry of the metal chelate compounds, Prentice Hall, New York.

Mazia D., 1953, Cell division, Sci. Amer. 189:53.

Ts'O P. O. P., 1958, Structure of microsomal nucleoprotein particles from pea seedlings, [In: Microsomal Particles and Protein Synthesis. P. 156—168. Symposium Biophys. Soc., Washington] Acad. Sci., Washington, D. C.

Weinstein L. H., M. Meiss, R. L. Uhler, E. R. Purvis, 1956, Growth-promoting effects of ethylenediamine tetra-acetic acid, Nature. 178:1188.

Rola Na_2-EDTA i kationów metali w indukowaniu korzeni przybyszowych

Streszczenie

W oparciu o chelatowe właściwości EDTA oraz jego zdolność do stymulowania ukorzeniania gałązek wikliny (Buczek 1965) przebadano wpływ tego czynnika na ukorzenianie liści i pędów pomidorów odmiany 'Best of all' i liści fasoli odmiany 'Saxa'.

Porównując działanie IAA oraz EDTA stwierdzono, że obydwa czynniki stymulują proces ukorzeniania aczkolwiek wpływ IAA był znacznie silniejszy. Najlepsze efekty z EDTA uzyskano, gdy do roztworu II, po 24-godzinnej inkubacji w samym roztworze EDTA dodano równoważnych ilości jonów Fe^{3+}, Ca^{2+}, względnie Mg^{2+}. Efekt kationów: Cu^{2+}, Ni^{2+}, Co^{2+} lub Mn^{2+} był niewielki w porównaniu z wpływem wyżej wymienionych trzech kationów. Ekwimolarne roztwory badanych kationów z EDTA okazały się nieczynne albo działały hamująco (z żelazem i miedzią).

Powyższe fakty sugerują, że: 1° EDTA jest zdolny do stymulowania ukorzeniania roślin, 2° stymulacja ta polega prawdopodobnie na chelatowaniu przez EDTA jakiegoś czynnika istotnego dla powstawania zawiązków korzeni przybyszowych, 3° czynnikiem tym prawdopodobnie są kationy Ca^{2+} i Mg^{2+}, natomiast żelazo odgrywa zapewne ważną rolę w innych procesach towarzyszących ukorzenianiu.