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Comparative investigations on the rooting of twigs of *Salix viminalis* due to IAA and EDTA.

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INTRODUCTION

It has been found recently (Bennet-Clark 1956, Heath and Clark 1956, 1960), that ethylenediaminetetraacetic acid (EDTA) as well as some other chelating agents affect the extension of oat and wheat coleoptiles and of the roots of wheat in a way similar to that of 3-indolylacetic acid (IAA). Bennet-Clark and Heath and Clark have proposed an hypothesis upon there observations and assumed that IAA regulates growth due to its chelating properties. Nevertheless, the effects of EDTA and of other chelating agents on growth are not always confirmed in other growth-tests. Fawcett et al., (1956) in the course of their investigations on the influence of EDTA on the growth of coleoptile in wheat have found that the effect of this substance on the extension of coleoptile in wheat was insignificant compared with IAA. It appeared moreover, that EDTA was completely inactive in the pea curvature and tomato leaf epinasty tests. Thimann and Takahashi (1958) have reported that EDTA is not active in the oat mesocotyl growth test. The investigations of Burström and Tullin (1957) and Burström (1961) have shown that EDTA does not inhibit the elongation of root cells in wheat but that it distinctly inhibits the multiplication of those cells in the dark, being practically inactive in the light.

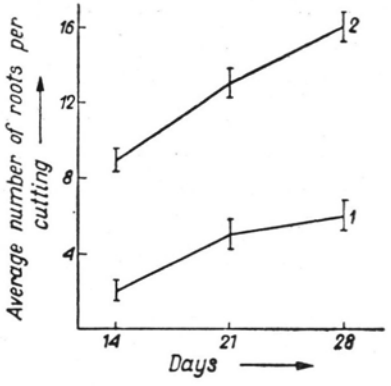
The papers mentioned above indicate great differences in the interpretation of the effect of EDTA on the regulation of growth reactions, therefore further comparative investigations on the role of IAA and EDTA in the regulation of growth-reactions typical for auxins seemed to be necessary. For this reason we have carried out experiments on the effects of IAA and EDTA on the formation of roots in the twigs of *Salix viminalis* during the period of winter dormancy and after the breaking of this dormancy.

METHODS

The experiments have been conducted on the twigs of *Salix viminalis* cut off from the shrubs in the beginning December. The winter dormancy has been broken by dipping the twigs in a 38°C waterbath for six hours. The twigs were afterwards put into solutions of either IAA (10^{-6} M. concentration) or of EDTA (10^{-6} or 10^{-4} M. concentrations; EDTA being added as the di-sodium salt). The twigs were afterwards transfered into big beakers filled with water or a solution of KNO_3 (250 mg/l). Also twigs in a state of unbroken dormancy were put directly into the investigation solutions and after 24 hrs. transfered into the beakers with water or KNO_3. The experiments were carried out at a constant relative air humidity 40—50% and a temperature of 20—22°C. The intensity of the artificial light supplied was equal to 4000 Lx. After 2, 3 and 4 weeks dating from the beginning of the experiments we counted the number of roots formed at the base of separate twigs. The results are given as the average number of roots per twig, calculated from 10 replications for each combination. Each experiment has been repeated at least twice.

RESULTS

Although *Salix viminalis* belongs to easily rooting plants, in the period of winter dormancy its ability of form lateral roots distinctly declines. However by giving the twigs a suitable treatment one can stimulate



Graph 1. The effect of a thermal bath on the formation of lateral roots in *Salix viminalis*.

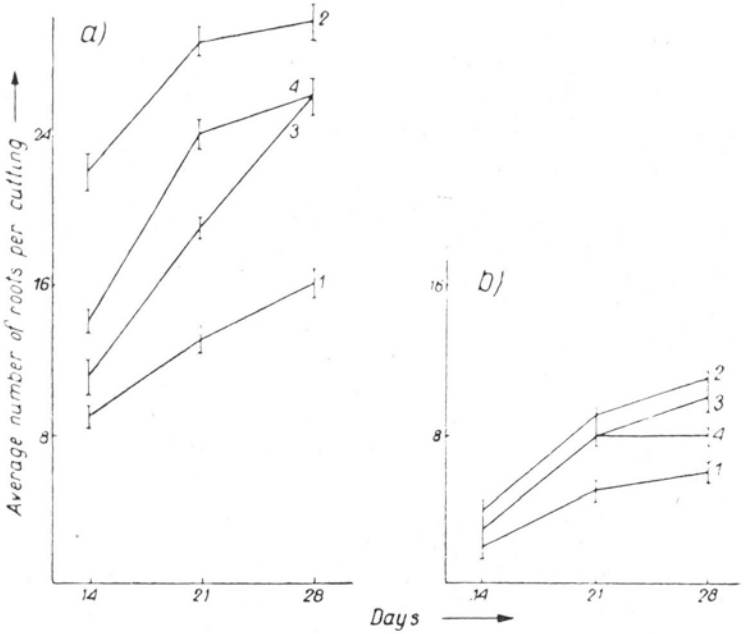
1 — During the period of winter dormancy, 2 — After the breaking of winter dormancy.

the cambium of those plants to a more intensive activity. This eventually leads to the formation of lateral roots at the base of the cut off twigs. Graph 1 shows the result of such treatments; at the end of experiment the twigs dipped in thermal bath for 6 hours (38°C) have produced more lateral roots than the twigs not dipped in warm water.

This experiment was a starting point to farther investigations. The

next experiment has been conducted in two variants: a) the twigs were dipped in the thermal bath (winter dormancy broken) and b) the twigs were not dipped in the thermal bath (winter dormancy not broken).

The effects of IAA and of EDTA on the formation of lateral roots in the twigs of *Salix viminalis* after breaking winter dormancy are presented in graph 2a. Although the effect of IAA appeared to be much

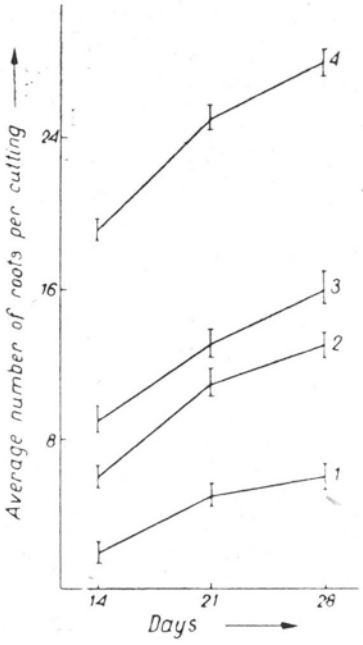


Graph 2a and 2b. Effects of IAA and EDTA on the formation of lateral roots in *Salix viminalis*.

a — After the breaking of winter dormancy, b — During the period of winter dormancy, 1 — Control., 2 — IAA 10^{-6} M., 3 — EDTA 10^{-6} M., 4 — EDTA 10^{-4} M.

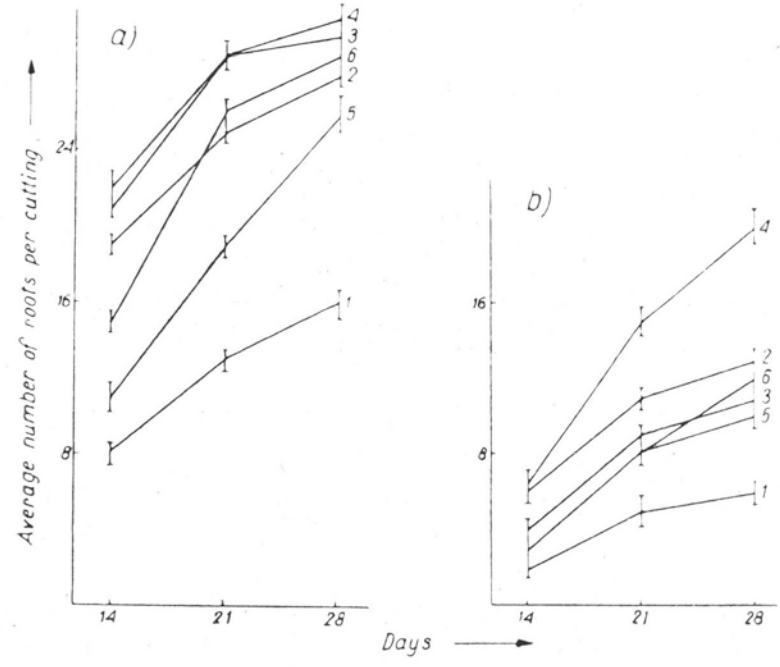
stronger than that of the two combination of EDTA, EDTA being a typical chelator has distinctly increased the number of roots produced in comparison with the control. In the twigs whose winter dormancy has not been broken the effects of EDTA and IAA were similar (graph 2b); slight differences are within the limits of error.

We know from the experiment of Luckwill (1956) that some mineral salts have a considerable effect on the formation of lateral roots in the cut off twigs. In our experiments we have investigated the effect of certain mineral salts [KCl, $CaCl_2$, $Ca(NO_3)_2$, KNO_3, $(NH_4)_2SO_4$] on the rooting of *Salix viminalis* twigs. Positive effects have been obtained only in experiments with the solution of KNO_3 (250 mg/l). The results



Graph 3. The effect of KNO_3 on the formation of lateral roots in *Salix viminalis*.

1 — Control and 2 — KNO_3 during the period of winter dormancy, 3 — Control and 4 — KNO_3 after the breaking of winter dormancy.



Graph 4a and 4b. Effects of IAA and EDTA supplemented with KNO_3 on the formation of lateral roots in *Salix viminalis*.

a — After the breaking of winter dormancy, *b* — During the period of winter dormancy. *1* — Control., *2* — KNO_3., *3* — IAA 10^{-6} M., *4* — IAA 10^{-6} M + KNO_3., *5* — EDTA 10^{-6} M., *6* — EDTA 10^{-6} M + KNO_3.

are presented in graph 3. The solution of KNO_3 in both variants of the experiment (winter dormancy broken and not broken) has increased more than two times the number of roots produced in comparison with the control.

In a further experiment we have investigated the effects of IAA and EDTA solutions supplemented with KNO_3 on the rooting process.

The results presented in graphs 4a and 4b may be summarized in the following way:

A. The winter dormancy broken (graph 4a).

1. The number of roots in the combination IAA + KNO_3 was increased in comparison with the solution of KNO_3 only, whereas such an increase of the number of roots has not been observed in the combination EDTA + KNO_3 when compared with the solution of KNO_3 only.

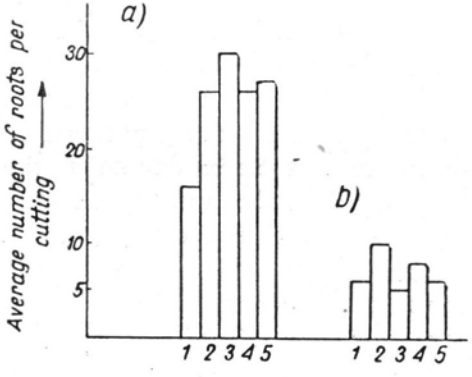
2. In an experiment with IAA the combination IAA + KNO_3 did not cause any differences in number of the roots, while in an analogical experiments with EDTA and EDTA + KNO_3 differences have been found.

3. The number of roots produced by twigs treated with KNO_3 is considerably greater than in twigs treated with EDTA, being, however, much smaller than in twigs treated with the solution of IAA only.

B. The winter dormancy not broken (graph 4b).

1. The effects of the combinations of IAA, EDTA and EDTA + KNO_3 on the number of roots produced were similar.

2. The solution of KNO_3 gave better results than the remaining combinations, except for the combination IAA + KNO_3, where the number of the produced roots was almost two times as great as in the remaining combinations.



Graph 5a and 5b. Effect of EDTA on the formation of lateral roots in Salix viminalis after 28 days dating from the beginning of experiment.

a — After the breaking of winter dormancy, *b* — during the period of winter dormancy. 1 — Control., 2 — EDTA 10^{-6} M and 4 — EDTA 10^{-4} M soaked for 24 hours., 3 — EDTA 10^{-6} M and 5 — EDTA 10^{-4} M constantly present in the solution.

The next experiment has been carried out with the solution of EDTA in such a way that one part of the twigs was dipped into the investigated solutions for 24 hours (as in the former experiments), while the other part was left in the solutions as for the whole experiment. Results are

presented in graphs 5a and 5b. From these experiments it appears that neither the 24 hours period nor the constant treatment with EDTA have caused any inhibitory or toxic effect on the formation of roots.

DISCUSSION

The experiments discussed above show that EDTA applied in the period of winter dormancy has affected the formation of roots in cut off twigs of *Salix viminalis* to the same degree as IAA. After the breaking of winter dormancy, the effect of EDTA was slightly smaller than that of IAA, exceeding, however, the results obtained for control twigs.

It is believed (Bünning 1953) that IAA is an agent stimulating the cells of cambium to a more intense activity, whereas the rooting ability of the cut off twigs depends probably on their predisposition, irrespective of the presence of auxins. The role of stimulators is not always performed by auxins; we know a number of substances that cause the formation of roots in plants. It is possible that EDTA is one of substances able to stimulate the cambium cells to activity, but so far the very mechanism by means of which these substances are stimulating the activity of cambial cells is not completely known. It is possible that some substances which possess chelating properties play in the process of rooting a role similar to auxins or to other substances that stimulate the cells of cambium.

We know from the investigations of Heath and Clark (1956, 1960) that the effect of EDTA on the extension in plants is similar to that of IAA. The above mentioned authors have carried out their investigations on the coleoptiles of Wheat and on the roots of wheat. In both cases the effects of EDTA and IAA have been identical. Heath and Clark interpret their investigations accepting the hypothesis of Bennet-Clark which suggests that the mechanism of auxin activity is due to their chelating properties. Our experiments performed on the rooting test of *Salix viminalis*, though essentially different from the tests ob Heath and Clark's confirm to a certain extent their hypothesis, which suggests that the effects of IAA and EDTA are similar.

Fawcett et al., (1956) carried out their investigations on the growth of segments of wheat coleoptiles and came to another conclusion. From their experiments it appeared that the effect of EDTA on the growth of the coleoptile segments was insignificant when compared with IAA. Since the doses of EDTA were rather large (10^{-4} M.) approaching toxic levels in Fawcett's opinion, this chelating agent was not acting as true growth substance. He suggested that EDTA affected a change in the permeability of the cell membrane and in the structure of the cell wall because of its high concentration.

In our experiments, however, the results obtained for EDTA have been similar or identical with those obtained for IAA, and the same was observed at high concentration of EDTA (10^{-4} M.) as well at low one (10^{-6} M.) which corresponded to the optimal concentration of IAA. In no case have toxic effects of EDTA been found.

In the author's opinion EDTA may affect a number of growth reactions characteristic for the activity of the auxins due to its chelating properties. As we know growth is closely related with the general metabolism of a plant. It is possible that chelating agents are in some cases able to exert reactions normal for auxins.

Results of our experiments suggest the existence of separate mechanisms for these two substances (graphs 4a and 4b). If we compare the effect of separate combinations on the rooting in *Salix viminalis* twigs during the period of winter dormancy, it apears that pure solutions of IAA and EDTA exert identical effects on the process of rooting. Their activities however are decidelly different after the twigs have been soaked in the solution of KNO_3. Potassium nitrate has intensified the effect of IAA, and was completely indifferent for EDTA. Similar conclusions can be obtained from the experiments conducted after breaking winter dormancy. In these experiments we do not observe any interaction of KNO_3 with EDTA. Although the number of roots in the combination EDTA + KNO_3 was much greater than in a pure solution of EDTA it was equal to the number of roots produced in the plants soaked in a solution of KNO_3. Thus an augmentation in the number of roots can be solely ascribed to the action of KNO_3 and not to the interaction of EDTA with KNO_3. In this variant of the experiment KNO_3 has not caused any intensification of the IAA effect, while in former experiment this intensification has been observed. We must remember, however, that in the experiment carried out after breaking the winter dormancy, the type of metabolism in twigs was completely different, uncomparable with the metabolism observed in plants whose dormancy has not been broken. The breaking of dormancy releases a very intense metabolism mobilizing the food stored within the twigs. As it has been shown by the research of Overbeek et al., (1946), the ratio of dissolved nitrogen and carbohydrates present in twigs was of special importance in the process of root-formation. It is possible that KNO_3 appeared to be such an effective agent in our experiments, because it supplied the twigs with assimilable nitrogen. It seems, however, highly probable that KNO_3 being an agent intensifying the effect of auxins (graph 4b.), does not play any important role while in the presence of EDTA. The formation of lateral roots at the base of the cut off twigs begins from an intense multiplication of endodermal and pericycle cells. IAA and — as it follows from our experiments — EDTA

are able to induce this process. However, in the light of the investigations of Burström (1961) conducted on the roots of wheat, EDTA strongly inhibits the multiplication of the cells in those roots. According to Burström's opinion this inhibition is due to chelation of iron in roots by EDTA. On the other hand, in our experiments we have found that EDTA affects distinctly the formation of lateral roots by increasing their number in comparison with the control. In no experiment have we found an inhibitory effect of EDTA regardless of whether this chelating agent was active for 24 hours or whether was constantly present in the solution for the whole experiment (graph 5a and 5b).

CONCLUSIONS

1. EDTA affects the formation of lateral roots in cut off twigs of *Salix viminalis* during winter dormancy in the same way as IAA.

2. After breaking winter dormancy the effect of EDTA is somewhat smaller than that of IAA, exceeding however, distinctly the results obtained for the control twigs.

3. The solution of KNO_3 intensifies the effect of IAA on twigs of *Salix viminalis* during winter dormancy showing no interaction in combination with EDTA, which seems to confirm our assumption that these two substances produce the same effect by means of different mechanisms.

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Porównawcze badania nad ukorzenianiem się gałązek Salix viminalis pod wpływem IAA i EDTA

Streszczenie

Badano wpływ IAA w stężeniu 10^{-6} M i EDTA w stężeniu 10^{-4} M i 10^{-6} M oraz KNO_3 (250 mg/l) na tworzenie się korzeni bocznych u *Salix viminalis*. Doświadczenia przeprowadzono na gałązkach wikliny (ścinanych z krzewu w grudniu), w dwu wariantach: a) Przerwany okres zimowego spoczynku (przez zanurzenie gałązek do wody o temperaturze 38°C na okres 6 godzin) i b) nie przerwany okres zimowego spoczynku. Stwierdzono, że EDTA w okresie zimowego spoczynku wpływa w tym samym stopniu na powstawanie korzeni jak IAA, natomiast po przerwaniu zimowego spoczynku efekt EDTA jest nieco słabszy w porównaniu z wpływem IAA, ale wyraźnie przewyższa kontrolę. Roztwór KNO_3 wzmaga działanie IAA u gałązek wikliny pozostających w zimowym spoczynku, natomiast jest bez wpływu na działanie EDTA w tym samym okresie. Powyższe wyniki przemawiają za oddzielnym mechanizmem działania tych dwu substancji na proces tworzenia się korzeni bocznych u gałązek *Salix viminalis*.