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Dynamics of auxins, gibberellin-like substances and growth inhibitors in the rooting process of black poplar cuttings (*Populus nigra* L.)*

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It is generally known that auxins play an essential role in the process of root formation and growth (Audus 1959). There exist also many data pointing to the essential role of growth inhibitors in these processes (Hess 1964; Richards 1964; Fadl and Hartmann 1967; Gesto and al. 1967; Vieitez and al. 1967; Hyun and Hong 1968).

According to some authors, the process of rooting is just depending on the auxin/inhibitor ratio (Turetskaya and al. 1970).

Much less is known about the role of gibberellins in the rooting process. It is known, however, that the roots are the source of gibberellins and there are also suggestions that these growth regulators may be synthesised in roots (for review — Michniewicz and al. 1970). Most authors acknowlege that gibberellins inhibit root initiation though there are some data showing that these substances may stimulate the rooting process (for review — Michniewicz and Kriesel 1970).

It is little known up to now about the changes in growth regulators during the process of rooting of cuttings (Hemberg 1951; Saito and Ogasawara 1960; Kefeli and Turetskaya 1965; Wareing and Smith 1965; Turetskaya and al. 1966 — a, b). These few papers which have been published so far are devoted only to auxins and growth inhibitors isolated together with auxins (Kefeli and Turetskaya 1965; Turetskaya and al. 1966 — a, b).

The purpose of this paper is to investigate the changes in the content of auxins, gibberellins and growth inhibitors (either isolated together with auxins or with gibberellins) during the process of rooting of black poplar cuttings.

METHODS

The cuttings were taken from 10 years old poplar trees growing in naturally occuring stands — Mała Kępa near Ostromecko. Material was gathered in autumn, October 15th, 1968 after the leaves falling. As cuttings 25 cm long one and three year old shoots were used. The bases of the cuttings were immersed in the Hoagland solution up to 1/3 their height. The medium of cuttings was aerated everyday.

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The cuttings were maintained at 25°C under 16 hour photoperiods.

For the analyses the bark of the cuttings containing roots of different stages was taken. The material was gathered 1) immediately after the taking the cuttings, 2) after the emergence of the roots, (after 25 days of cultivation), 3) in the period of root developing (60th day of cultivation), 4) in the period of full roots development (81 days).

Auxins and gibberellins were extracted from the same sample of the frozen plant material with 80% methanol for 48 hrs. Flash evaporation at 30°C removed the methanol, leaving an aqueous residue.

In the case of gibberellins, this residue was adjusted to pH 2 with concentrated H_3PO_4 and fractionated with the method of Murakami (1959). The final ethyl acetate fraction contained acid substances. The acid fraction was evaporated to dryness and partitioned by using ascending chromatography on Whatman's 1 paper. As a solvent system n-butanol, acetic acid, water (19:1:6 v/v) were used. The eluates from chromatograms were bioassayed with the first leaf of oat test (Michniewicz 1961).

The activity of gibberellin-like substances was also checked using the modified lettuce hypocotyl test (cult. Böttner) after Frankland and Wareing (1960), and the test of dwarf pea (var. Cud Ameryki, according to McComb and Carr 1958).

For auxin determination, the residue was acidified up to pH 3,5 with HCl and then shaken with excessive amount of ethyl ether. The joint fractions of ethyl ether were condensed and the residue was diluted in methanol. For the quantitative determination of the auxins the descending chromatography on Whatman's 3 paper (solvent system:isopropanol, ammonia, water 10:1:1 v/v) and the *Avena* straight growth test were used.

An equivalent of 3 g of fresh weight of plant material was always spotted on each chromatogram.

Growth-inhibiting substances were extracted together with gibberellins or auxins. The growth inhibition was expressed in activity units. As a unit 10 per cent of the growth inhibition of first oat leaf (in the case of inhibitors extracted together with gibberellins), or the *Avena* straight growth (in the case of inhibitors extracted together with auxins) in relation to control were adopted.

The position of growth regulators on chromatograms was examined in UV light and compared with the position of IAA and GA_3. The significance of differences was established by estimating LSD at $P = 0.01$.

All experiments were repeated three times.

RESULTS AND DISCUSSION

The auxin-like substances were localized in three zones of chromatograms:near start point, in the zone corresponding to the IAA position and at the front of chromatograms. This is similar to data obtained by Kamieńska (unpublished data) in leaves and inflorescences of these poplars. In our experiments the most active substance was localized at R_f 0,3—0,5 i.e. in the zone corresponding to IAA position.

The cuttings taken for the analyses immediately after cutting were characterized by relatively high level of auxins. The level of these growth substances reached its maximum in the period immediately after roots emergence. In the later period, during the roots growth, the quantity of these substances decreased markedly (Fig. 1).

These data are in accordance with the results of Wareing's and Smith's experiments (1965) showing that in the poplar cuttings taken in the period of dormancy, the amount of acid auxins (similar in properties to IAA) increased during first six days of cultivation, but after this period the level of these substances decreased so, that after 21 days it was already very low.

The results of our investigations are also in agreement with data obtained by some membres of the scientific staff of Plant Physiology Institute Academy of Science in USSR, Moscow. In the experiments on the rooting of bean (Turetskaya and al. 1966 — b) and grape (Turetskaya and al. 1966 — a) these scientists pointed out that the greatest activity of auxin type substances was found in the cuttings being in the period of root formation. Afterwards, in the period of roots growth the amount of auxin decreased.

Different data to our results were obtained by Soviet scientists in similar ex-

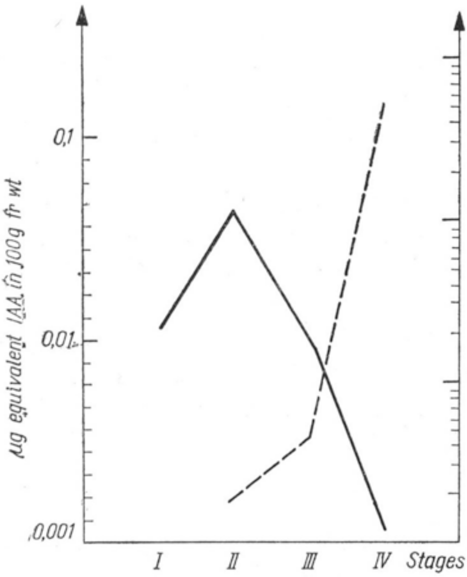

Fig. 1

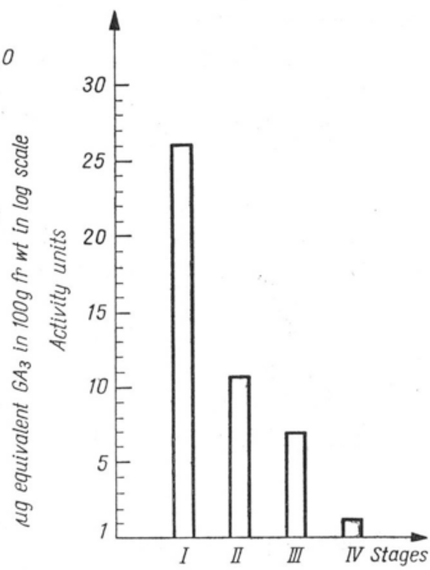

Fig. 2

Fig. 1 The content of auxin- (continued line) and gibberellin-like substances (broken line) in the part of bark of the poplar cuttings containing roots in different stages of their development.

Fig. 2 The content of growth inhibitors in the part of bark of the poplar cuttings containing roots in different stages of their development.

periments with willow (Kefeli and Turetskaya 1965; Turetskaya and al. 1966 — b).

The level of auxins during the rooting of cuttings was investigated also by Hemberg (1951) in experiments with beans and by Saito and Ogasawara (1960) in experiments with willow. These authors applied however other methods of investigations so, theirs results can not be compared with data obtained in our experiments.

The gibberellin-like substances were localized in three zones of chromatograms: R_f 0,3—0,4, 0,5—0,6 and 0,8—1,0. The greatest amounts of these substances were found at the front of chromatograms. The cuttings being in the period of roots growth were characterized by the highest activity of gibberellin-like substances (Fig. 1).

In the cuttings taken for the analyses immediately after cutting no GA-like substances were found. These substances appeard only immediately after roots emergence and their amount increased simultaneously with growth of roots.

The cuttings received immediately after cutting were characterized by the great amount of inhibitors isolated together with gibberellins. As it is shown in our previous papers such inhibitors reduce the growth induced by auxin and gibberellin and are not active in microbiological test (Michniewicz and Kopcewicz 1966; Michniewicz 1970; Michniewicz and Kopcewicz 1970). During the period of rooting the amount of these substances rapidly decreased (Fig. 2).

The data concerning the dynamics of gibberellin-like substances and these kinds of growth inhibitors are not reported so far in literature. There are however some data concerning the quantitative changes of growth inhibitors isolated together with auxins. It is generally assumed that the high level of inhibitors characterizes the cuttings analyzed immediately after cutting (Kefeli and Turetskaya 1965; Turetskaya and al. 1966 — a, b).

Contrary to these data we did not discover this kind of growth inhibitors in freshly taken cuttings. Their presence was stated however during the period immediately after the root initiation and in the early stages of roots growth. These results are scarce though and they were not included in this paper.

The results reported in the present paper ilustrate the data obtained in the experiments with one year old cuttings. The results of analyses of three years old cuttings proved to be similar. The content of auxins and gibberellins in one year old cuttings was however higher than in the three years old cuttings (Table 1).

Table 1

The amount of auxins and gibberellin-like substances in cuttings, in the period immediately after roots emergence (in μg/100 g fresh weight).

Age of shoots	Auxins	Gibberellins
one year old	0.0681	0.1933
three years old	0.0285	0.1333

Table 2

Average number of roots on cuttings of different age.

Age of shoots	Number of roots after:				
	10	12	17	21	29 days
one year old	0.2	2.8	5.4	7.8	8.5
three years old	1.0	2.9	4.2	4.9	4.9

These data would be in accordance with the results of our earlier experiments showing that the plants being in the stage of intensive growth are characterized by higher level of auxins and gibberellins than the plants growing less intensively (Kopcewicz and al. 1967, 1970).

From our experiments it can be seen that the amount of gibberellin-like substances in poplar roots is higher than the amount of auxin — type substances. The auxins and gibberellin-like substances were also previously determined in the leaves and inflorescences of this plant by Kamieńska (1966, 1967 and unpublished data).

Table 3

The maximal amounts of auxins and gibberellin-like substances found in different organs of black poplar (in μg/100 g fresh weight).

Plant organs	Auxins	Gibberellins
Leaves	7.867^{+}	1,2–1,3^{++}
Inflorescences	1.699^{+}	0.565^{+++}
Adventitious roots of one year old shoots	0.068	50.289

after Kamieńska: $^{+}$ – unpublished date, $^{++}$ – 1966, $^{+++}$ – 1967.

As it can be seen from the data in Table 3 the roots are richer in gibberellins and poorer in auxins than the above mentioned organs of poplar trees.

CONCLUSIONS

The level of auxins, gibberellin-like substances and growth inhibitors in the bark of the basal parts of poplar cuttings depends on the rooting stage.

The amount of auxins increases at the first, reaching the maximal value after roots emergence and then decreases.

Gibberellin-like substances appear only after roots emergence. Their level rises during roots growth.

Growth inhibitors extracted together with gibberellins occur in great quantities in freshly cut cuttings. During the process of root formation and growth, their level markedly decreases.

The results of experiments point to the essential role of auxins in the process of root development and the impoıtant role of gibberellin in root growth.

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Dynamika auksyn, substancji giberelinopodobnych i inhibitorów wzrostu w procesie ukorzeniania zrzezów topoli czarnej (Populus nigra L.)

Streszczenie

Określano poziom auksyn, substancji giberelinopodobnych oraz dwóch różnych inhibitorów wzrostu (izolowanych łącznie z auksynami lub giberelinami) w różnych etapach ukorzeniania zrzezów topoli czarnej.

Jako zrzezów użyto jedno i trzyletnie pędy pobrane jesienią z dziesięcioletnich drzew. Analizowano korowinę bazalnych części zrzezów na których tworzyły się korzenie.

Wyniki doświadczeń przedstawiają się następująco:

1) Poziom badanych regulatorów wzrostu w korowinie bazalnych części zrzezów topoli zależał od fazy ukorzeniania.

2) Poziom auksyn początkowo wzrastał i osiągnął wartość najwyższą po wytworzeniu zaczątków korzeni, a następnie malał.

3) Substancje giberelinopodobne ujawniły się dopiero po wytworzeniu zaczątków korzeniowych, a ilość ich rosła w miarę wzrostu korzenia.

4) Inhibitory wzrostu izolowane razem z giberelinami występowały w dużych ilościach w sadzonkach bezpośrednio po ścięciu. W procesie tworzenia się korzeni i ich wzrostu ilość tych substancji malała.

Wyniki tych doświadczeń pozwalają wnioskować, że auksyny spełniają istotną rolę w procesie prowadzącym do wytworzenia korzeni przybyszowych, natomiast gibereliny mają istotne znaczenie w procesie wzrostu korzenia.

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