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Dynamics of gibberellin-like substances in the development of buds, newly formed shoots and adventitious roots of willow cuttings (*Salix viminalis* L.)

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Abstract

It was stated that adventitious roots as well as shoots formed from the buds of willow cuttings contained two GA-like substances. One of them was different in roots and in shoots. The amount of Ga-like substance in roots was much higher than in shoots. The level of these substances increased very intensively in roots while in shoots rather slightly and only in the earlier stages of their growth. The results of later experiments and of others presented here shown that adventitious roots of willow cuttings are the sites of gibberellin biosynthesis. Possible explanation of existing of different gibberellins in roots and in shoots is also discussed.

INTRODUCTION

The results of earlier experiments indicate that gibberellins appear in poplar cuttings after the emergence of adventitious roots, and that the level of these growth substances rises during the root growth (Michniewicz and Kriesel 1970). It was also pointed out in the experiments with willow cuttings that gibberellins present in shoots formed from the buds partly originate in the adventitious roots formed on the cuttings (Michniewicz et al. 1970).

The aim of these investigations is to compare the dynamics of endogenous gibberellins during the development and growth of adventitious roots of willow cuttings to the dynamics of these growth substances during the development of buds and newly formed shoots.

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MATERIALS AND METHODS

As the plant material one year old shoots of *Salix viminalis* growing in natural stands at the Vistula river near Toruń were used. They were gathered in the period of bud dormancy in March 1970.

25 cm cuttings were cut out below the flower buds. The bases of the cuttings were immersed in tap water up to 1/3 of their height.

The cuttings were cultivated on 16 hrs long day at 25°C and light intensity about 2000 lux. The cultures were aerated every day.

For the analyses the bark of the cuttings with roots as well as the buds and new shoots formed out of them were taken.

This plant material was collected seven times in different stages of development of cuttings (Tab. 1 and Fig. 1).

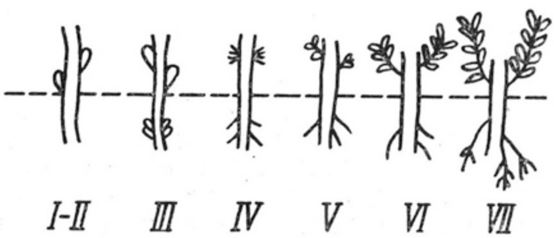

Fig. 1. The stages of development of shoots and adventitious roots of willow cuttings

Table 1

The stages of development of the cuttings

Stage	Data	Roots	Shoots
I	11th March	not emerged	buds in dormant state
II	14th March	not emerged	buds after 3 days of cultivation of cuttings
III	17th March	the period of root emergence	swollen buds
IV	21th March	length of 1 cm	beginning of axial extension
V	26th March	length of 1,5—2 cm	length of 2 cm
VI	2nd April	length of 7—8 cm	length of 4—5 cm
VII	10th April	length of 10 cm	length of 15 cm

The anatomical cross and longitudinal freehand sections of the basal part of cuttings being in the I and II stage of development were examined. It was stated that in freshly cut cuttings resting root primordia have already existed. Three days after the cultivation in tap water the meristematic activity of cells of root primordia was observed.

Gibberellins* were extracted from the frozen plant material with 80% methonal for 48 hrs. Flash evaporation at 30°C removed the methanol, leaving an equous residue. This residue was adjusted to pH 2 with concentrate H_3PO_4 and fractionated according to the method of Murakami (1959). The final ethyl acetate fraction contained acid substances. It was evaporated and partitionated using thin-layer chromatography (Silica gel G solvent system: benzene-acetic acid 10:3 v/v.). An equivalent of 3 g of fresh weight of plant material was spotted on each chromatogram. The eluates from chromatograms were bioassyed using the modified lettuce hypocotyl test (cv. Böttner) after Frankland and Wareing (1960). The acitvity of gibberellins was checked using the test of dwarf pea (cv. Cud Ameryki) according to McComb and Carr (1958).
The measurements of activity of eluates were repeated 3 times.

For closer identifications of the purified fractions UV and IR spectra on Spectrophotometer Unicam and Spectrophotometer UR-10, respectively, were performed.

RESULTS AND DISCUSSION

Gibberellins of shoots as well as roots of willow cuttings were localized in two zones of chromatograms. Shoot gibberellins were localized at R_f 0.2—0.3 and R_f 0.6—0.8 (Fig. 2) but gibberellins of roots were detectable in zones corresponding to R_f 0.2—0.3 and R_f 0.8—0.9 (Fig. 3).

Gibberellins localized near the start point were more active than gibberellins localized near the front of chromatograms. These growth substances appeared earlier in shoots (stage II), than in the basal parts of cuttings (stage III), but gibberellins localized near the front of chromatograms appeared in shoots and roots at the same time (stage III).

The dormant buds did not contain any gibberellines, they appeared in swollen buds. These results are also in accordance with the data of earlier experiments with willow (Michniewicz et al. 1970), poplar (Kamieńska 1966), pine and larch (Kopewicz et al. 1967).

The amount of gibberelin localized at R_f 0.2—0.3 increased during the development of newly formed shoots, however the level of substance localized at R_f 0.6—0.8 which appeared in the III stage of development

* This term stands for gibberellin-like substance since their chemical structure is not yet determined.

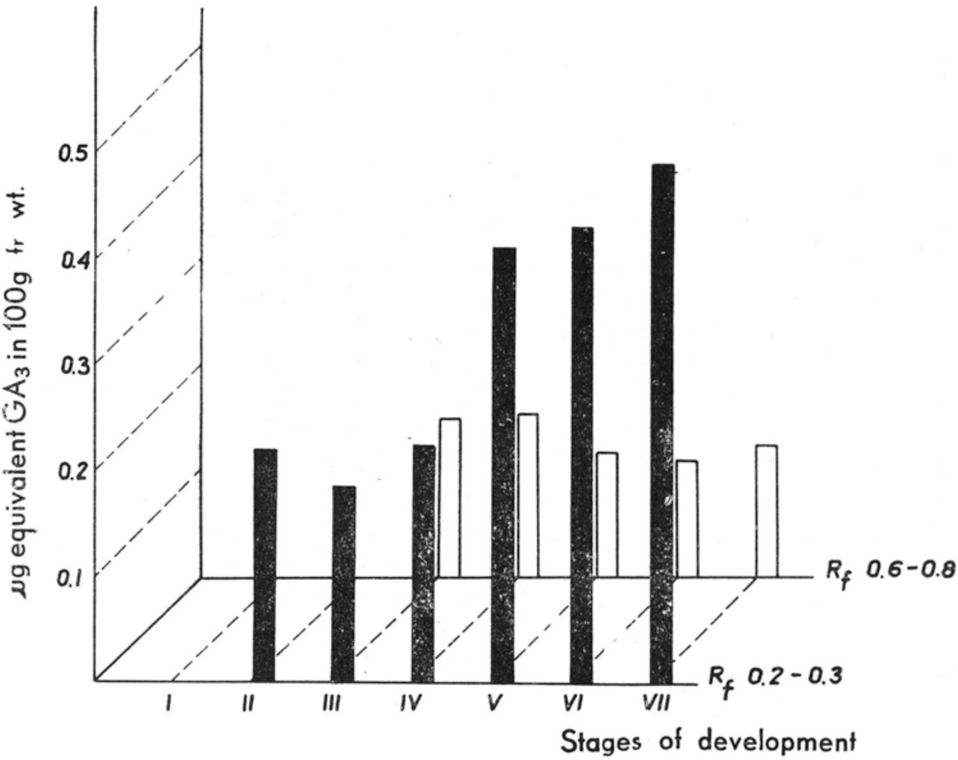

Fig. 2 Chromatographic analysis of GA-like substances isolated from buds and newly formed shoots of willow cuttings in different stages of their development

did not change at this time. As we see from the data presented in Fig. 4 the total amount of gibberellins slightly increased during the shoot development but only in its earlier stages.

The results concerning the dynamics of gibberellins in the developing adventitious roots were different. These substances appeared in the III stage of root development after the root emergence and their amount markedly increased simultaneously with the growth of roots. Contrary to the shoots, the amount of growth substances localized nearer the front of the chromatogram increased very intensively during root growth. So, the activity of root gibberellins was much higher than the activity of gibberellins isolated from shoots (Fig. 4).

These data are also in accordance with the results of experiments with poplar and with conclusion indicating that gibberellin play an essential role in the root growth (Michniewicz and Kriesel, 1970). On the other hand the results of the experiments presented here, indicating that the level of gibberellin in roots rises up more intensively than in shoots, support the supposition presented in our earlier paper (Michniewicz et al. 1970) that the adventitious roots of wilow cuttings are

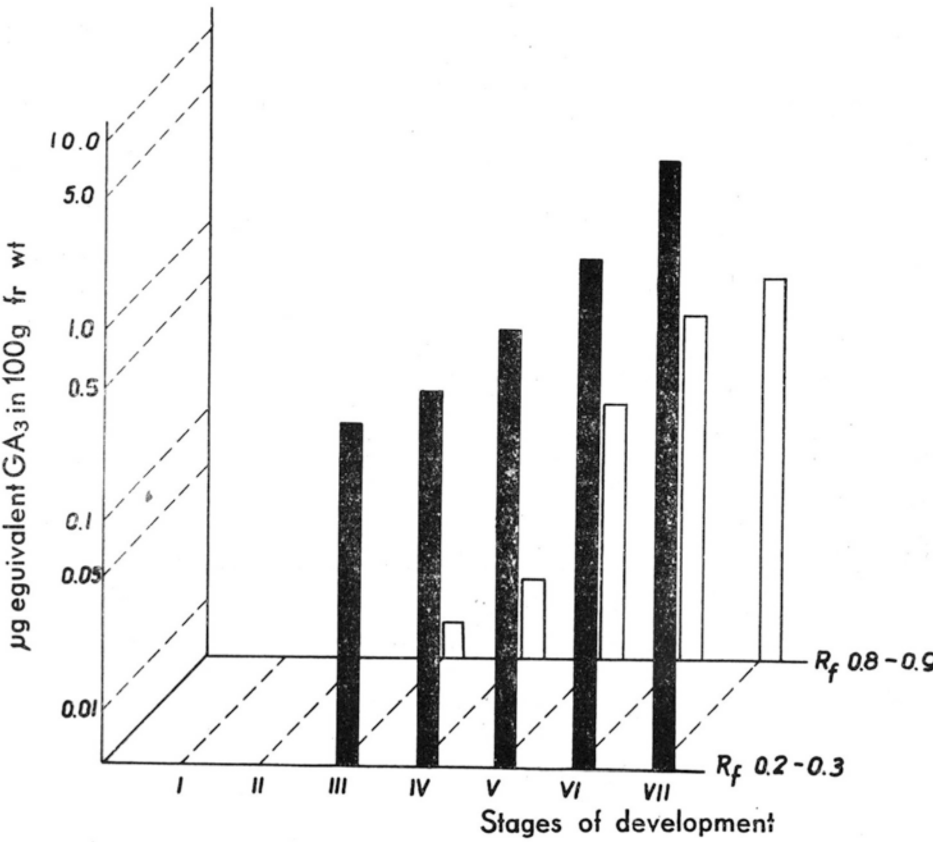

Fig. 3 Chromatographic analysis of GA-like substances isolated from the bark containing adventitious roots of willow cuttings in different stages of their development

the site of gibberellin biosynthesis. It was supported also by the results of analyses of apical and basal parts of adventitious roots of willow cuttings. It was stated that apical segments characterized by high activity of biochemical processes contained more gibberellins than the basal segments (Fig. 5).

However, the statment that gibberellins appear in soots earlier than in roots indicate that these growth substances can be produced also in shoots, probably from the gibberellin precursors present in dormant buds. These consulsions are also in accordance with the results of earlier experiments with willow cuttings (M i c h n i e w i c z et al. 1970). The question of the origin of gibberllin precursors present in dormant buds remains still unsolved.

It is interesting to compare the quality of gibberellins isolted from shoots and roots of willow cuttings. As can be seen from the data pre-

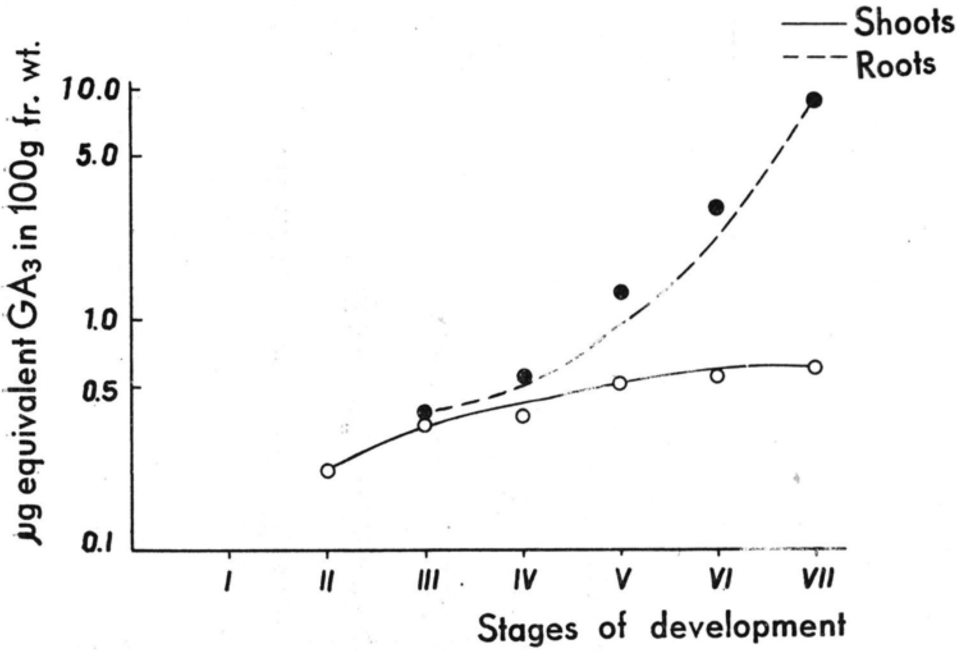

Fig. 4 Total activity of GA-like substances in shoots* and adventitious roots** of willow cuttings

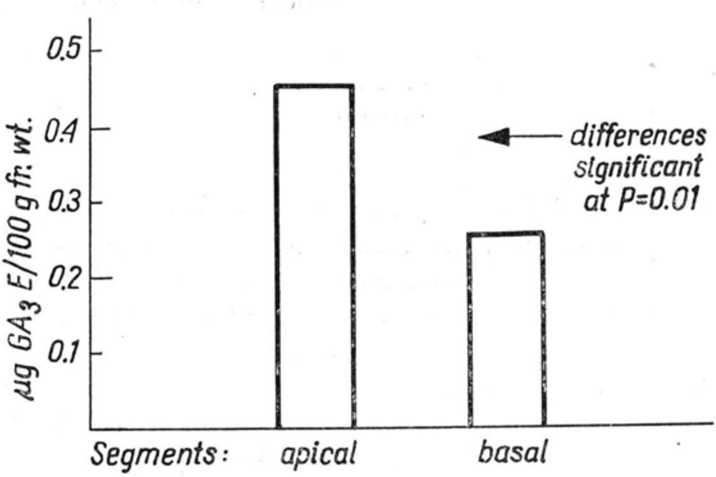

Fig. 5 The level of gibberellin-like substances in the apical and basal segments of adventitious roots of willow cutings (for analyses the segments including 1/3 length of roots were taken)

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* the buds and nevly formed shoots
** the bark containg adventitious roots

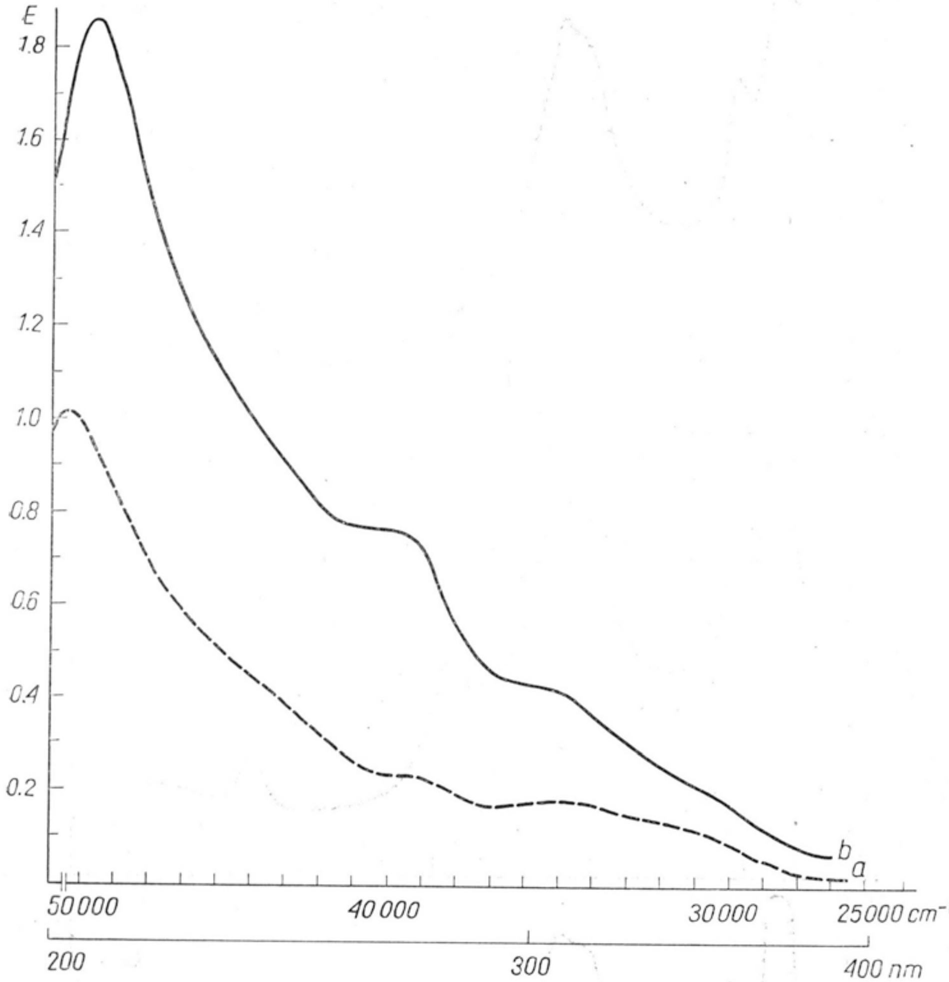

Fig. 6 UV spectra of gibberellin-like substances isolated from shoots and localized at R_f 0.6—0.8 (*a*) and from roots, localized at R_f 0.8—0.9 (*b*)

sented in Fig. 2 and Fig. 3 one of the gibberellins isolated from shoots and from roots localizaed near the start point, has the same chromatographic properties. However, chromatographic properties of the other gibberellins localized nearer the front of chromatograms were different in roots and in shoots.

The proof that these are different gibberellins was given by UV and IR spectra (Fig. 6 and 7). Presence of different gibberellins in shoots and roots were also stated in *Tulipa gesneriana* by gas-liquid chromatography (A u n g et al. 1971).

So, it is possible that shoots and roots may produce different gibberellins. It is also possible that gibberellin biosynthesized in roots is con-

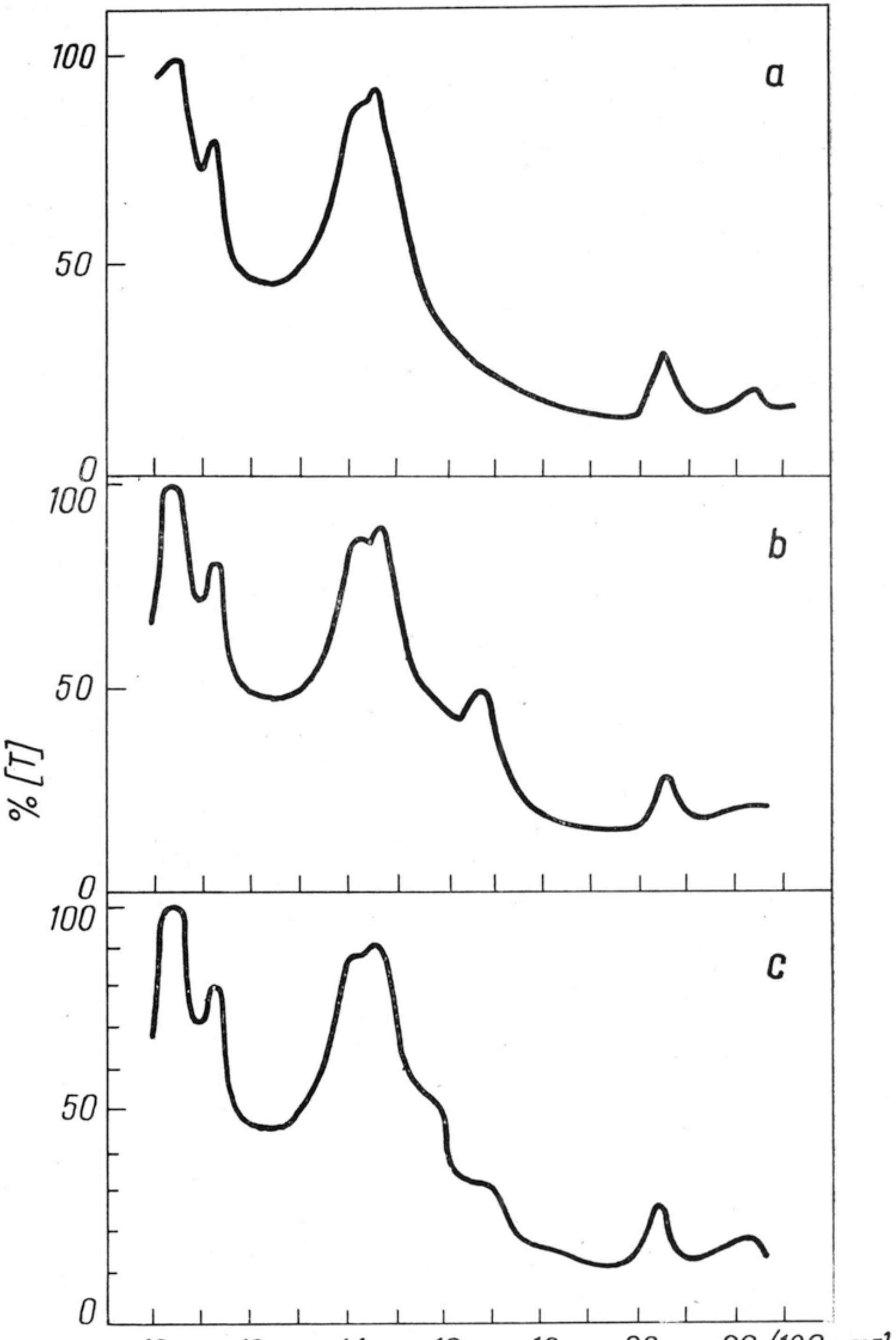

Fig. 7 IR spectra of gibberellin-like substances isolated from shoots and localized at R_f 0.6—0.8 (*b*) and from roots, localized at R_f 0.8—0.9 (*c*). (Spectrum of pure methanol — *a*)

verted in shoots or *vice versa*. Solving of this question would be of great importance for the explaining of the problem of growth correlations between shoots and roots.

CONCLUSIONS

Adventitious roots as well as shoots formed from the buds of willow cuttings, collected in March, contained two gibberellins. One of them was different in roots and in shoots.

Gibberellins appeard in swollen buds and slightly increased their amount in the earlier stages of development of newly formed shoots.

In the bark of basal parts of cuttings, gibberellin appeared after the emergence of adventitious roots. The level of this growth substances rises very intensively during root growth.

The amount of gibberellins in roots was much higher than in shoots.

The results of these experiments support the earlier conclusion that the adventitious roots of willow cuttings are the sites of gibberellins synthesis. They also indicate to the important role of gibberellins in the processes of root growth.

These data indicate that gibberellins can be produced also in shoots, probably from the gibberellin precursors present in dormant buds.

The possibility is here suggested that the shoots and roots may produce different kinds of gibberellins or that gibberellins biosynthesized in one organ may be converted in another organ.

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Dynamika substancji giberelinopodobnych w rozwoju pączków i powstałych z nich pędów oraz w korzeniach przybyszowych zrzezów wierzbowych (Salix viminalis L.)

Streszczenie

Określano poziom substancji giberelinopodobnych w pączkach i w powstałych z nich pędach oraz w korzeniach przybyszowych wierzby w siedmiu fazach rozwojowych (Tab. 1., Fig. 1). Zrzezami były jednoroczne pędy pobrane w okresie spoczynku pączków, w marcu 1970. Badania anatomiczne takich zrzezów wykazały w nich obecność zaczątków spoczynkowych korzeni.

W wyniku doświadczeń stwierdzono, że zarówno pędy, jak i korzenie zawierały po dwie substancje giberelinopodobne. Jedna z nich występowała w obu organach, natomiast druga giberelina była inna w korzeniach i inna w pędach.

W pączkach śpiących giberelin nie stwierdzono. Obecność ich ujawniono dopiero w pąkach nabrzmiałych. W pierwszych fazach wzorstu pędu ilość ich nieco wzrastała.

W korowinie bazalnych części zrzezów (warstwy peryferyczne sięgające łyka) obecność związków giberelinopodobnych stwierdzono dopiero po ujawnieniu się zaczątków korzeni przybyszowych. W miarę wzrostu korzeni ilość giberelin wyraźnie wzrastała. Poziom ich w korzeniach był znacznie wyższy aniżeli w pędach.

Wyniki tych doświadczeń potwierdzają wnioski wysunięte w poprzedniej pracy (Michniewicz et al. 1970) mówiące, że korzenie przybyszowe wierzby są miejscem biosyntezy gibereliny, oraz że substancje te spełniają istotną rolę w procesie wzrostu korzeni.

Dane uzyskane w tej pracy wskazują również na możliwość produkowania giberelin w pędach prawdopodobnie z prekursorów znajdujących się w pączkach spoczynkowych.

W pracy rozważa się możliwość, że korzenie i pędy produkują różne gibereliny, lub też gibereliny wytworzone w jednym organie ulegają przekształceniu w drugim z organów.