

The changes in anatomical structures in *Pisum sativum* L. seedlings after treatment with morphactin IT 3456 and benzyladenine*

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Morphactins, a powerful new group of plant growth regulators, are fluorene derivatives (Merck 1965; Pieniążek and Saniewski 1967).

The present paper is the continuation of our work on the effects of morphactin IT 3456 (IT), containing 80 % methyl-2-chloro-9-hydroxy-fluorene-(9)-carboxylate, and benzyladenine (BA) on the growth and the anatomical structures of dwarf pea cultivar „Cukrowy Hłowiecki” (Saniewski et al. 1968).

• MATERIAL AND METHODS

Pea seeds were soaked for 24 hours in the following solutions of the growth regulators:

1. Morphactin (IT) — 30 mg/l
2. Benzyladenine (BA) — 25 mg/l and 50 mg/l
3. IT plus BA — 30 mg/l plus 25 mg/l and 30 mg/l plus 50 mg/l
4. Control — water.

The seeds were then planted (May 12, 1968) in pots containing Keramzyt (baked clay gravel). The pots were watered with tap water and the plants cultivated for four weeks outdoors, and then fixed in 75 % ethanol for anatomical studies. Paraffin blocks were prepared in the usual way and sectioned on the microtome (5, 10 μ m). Lignin and cellulose were detected after staining with acid fuchsine and malachite green. The presence of lignin in the cell walls was investigated additionally with phloroglucinol in HCl and after staining with iodine in zinc chloride. The degree of lignification was estimated visually on sections of the same thickness using the standardized color reagents. The transections of roots and stems were magnified $\times 32$ and $\times 140$. The transections of stems were made in the middle of the lowest internode, and of the roots 0.5 cm below the hypocotyl.

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RESULTS AND DISCUSSION

The plants obtained from seeds soaked in the solution of morphactin had the roots and shoots greatly reduced in comparison with control plants (Fig. 1). On the main roots along the length of 5–8 cm below the hypocotyl, abundant and very short lateral roots were produced. They were growing at different angles to the longitudinal axis of the main root (Fig. 2).

Similar results were obtained in our previous work, when the seedlings of peas and *Dolichos lablab* were grown all the time in the solution of morphactin. The meristematic tissues were then stimulated along the entire length of the protoxylem bundles but no lateral roots were formed, and only strand like ridges of the stele were produced (Saniewski, Smoliński, Pieniążek 1968).

Benzyladenine (25 and 50 mg/l) reduced the growth of shoots and roots more than morphactin (Fig. 3). It stimulated, however, the growth of cotyledonary axillary buds, and sometimes buds at higher nodes grew out. The lateral shoots were half or the same length as the main shoot, and their diameter equal or even greater than that of the latter. When BA was used at the concentration of 50 mg/l no lateral roots were formed.

Identical effects of BA on the growth of pea seedlings were obtained by Sprent (1968a). Application of BA to dry pea seed delayed the production of amylase and the concomittant breakdown of starch. The utilization of nitrogenous reserve food material was also delayed. Shoot growth was correlated with breakdown of the reserve food (Sprent 1968b).

The seeds treated with BA together with IT produced the plants with the most reduced shoots and roots (Fig. 4). The lateral shoots were also stimulated into growth like in the case of benzyladenine, they were, however, shorter and thicker, especially near their base. The growth of roots was even more reduced than when BA was applied singly.

The roots of the control plants were circular on the cross-sections (Fig. 5a, b). The primary cortex was composed of parenchyma cells arranged rather loosely. The most intensive cambial activity took place between metaphloem and metaxylem, and consequently the secondary xylem components appeared first in this area. As a result of the secondary growth the cambium becomes circular. The conductive elements of the primary and secondary xylem were polygonal on transverse sections, and their walls were highly lignified. The phloem fibers had thick walls, small lumina, and only middle lamellae were lignified.

After morphactin treatment the roots on the transverse sections were almost triangular (Fig. 6a, b). The surface of the section was markedly larger as compared with controls. The primary cortex was split by the emerging numerous lateral roots. As in our previous experiment (Saniewski et al., 1968) the cambium was stimulated mainly above the protoxylem poles which led to the formation of a triangular shaped stele. The secondary xylem was composed of short, thin-walled and poorly lignified vessels and tracheids. The secondary cells between the phloem and the rays of the primary xylem were undifferentiated. The fibers above the phloem had thick, cellulose

walls. In rare cases lignin was found in the corner thickenings of 3—4 cells. The secondary growth on the phloem side consisted of a wide layer of parenchyma dividing in many planes.

The diameter of roots after BA treatment did not differ from controls. The diameter of the conductive elements, the thickness and the degree of wall lignification in the primary and secondary walls were like in control plants. The secondary growth took place mainly at the side and above protoxylem bundles (Fig. 7). The phloem fibers had lignified middle lamellae but their secondary wall was unlignified.

The mixture of BA and morphactin produced the same type of secondary growth as in the case of BA applied singly. However, the components of the primary xylem were poorly lignified, and the secondary growth contained very few well differentiated conductive elements (Fig. 8). They were scattered among parenchyma cells, their lumina were small and the walls poorly lignified. Thus in the secondary growth the differentiation and the lignification were altered in the same way as it was the case of plants treated only with morphactin.

The stems of control plants on transverse sections were square, and the primary cortex was composed of loosely arranged parenchyma. Four schizogenous intercellular spaces were evident (Hayward, 1948), and between them two sclerenchymatic bundles. The remaining two collateral vascular bundles were also present. The xylem walls were lignified, and in the mechanical tissue lignin was detected in the middle lamella (Fig. 9a, b).

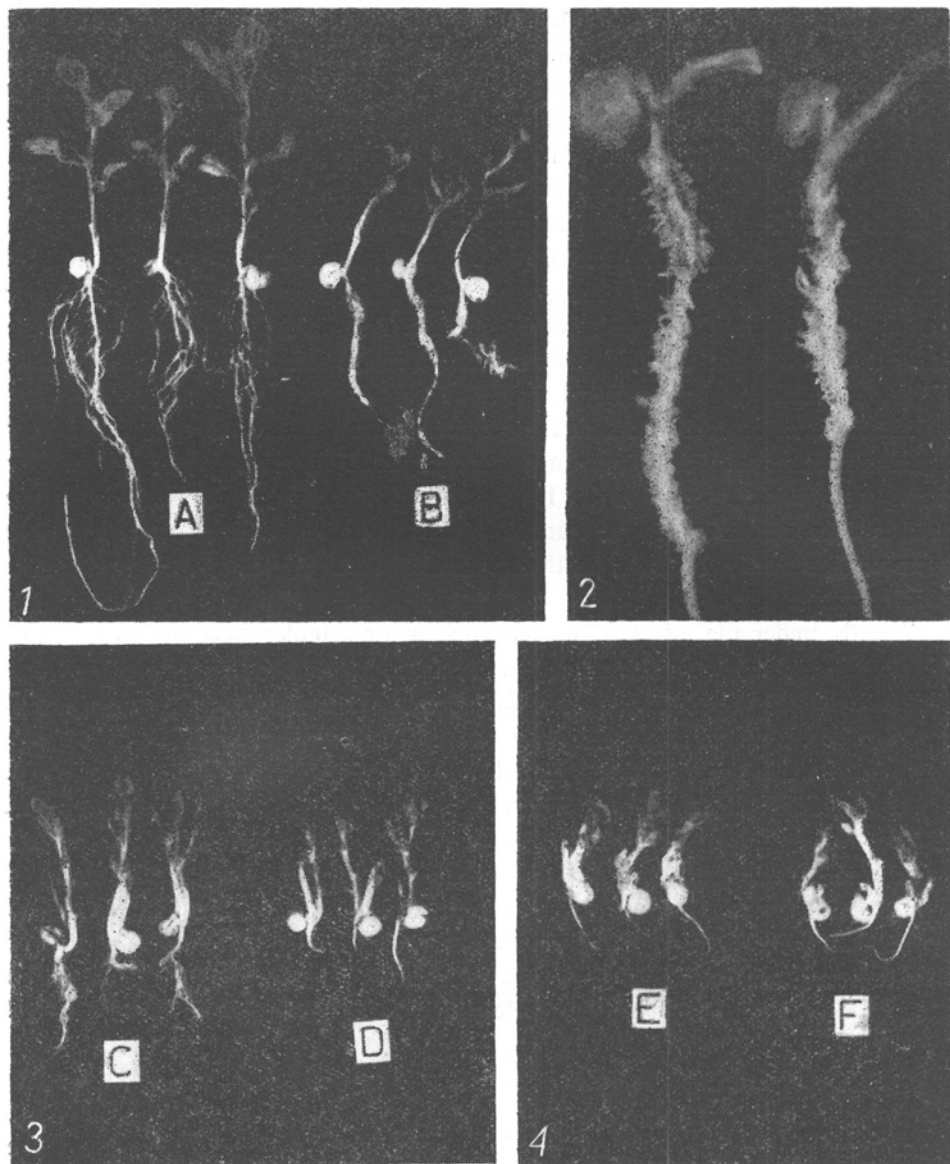
Directly under the pericycle six sclerenchymatic bundles were present, two of them connected with polar bundles (Hayward, 1948). The cells of the mechanical tissue were thick-walled and only middle lamella was lignified. The vessels and tracheids of the secondary xylem were well differentiated, polygonal and of large diameter on transverse sections. The walls were thick and highly lignified (Fig. 9a).

After morphactin treatment the diameter of the stem was not affected, however, the nodes were thicker than that of the control plants. The schizogenous intercellular spaces were larger, and the cells of the mechanical tissue smaller than in the control plants. The lignin was found only in the cell corners, the radial and tangential lamellae were free of lignin. The components of xylem and phloem had smaller diameter than that found in the stems of control plants. The secondary growth contained very few well differentiated xylem cells, and their walls were poorly lignified (Fig. 10).

After benzyladenine treatment the diameter of the stem on the transverse section was larger than that of controls. This increase was due to the wide layer of the primary cortex, and it was caused by the growth in size of parenchyma cells. The intercellular spaces were smaller, and the components of wood and mechanical tissue did not differ in the lignin content from the controls.

In the central stele the width of the secondary xylem and phloem was somewhat larger than in the controls. The central stele was oval, and the xylem formed a closed ring around the primary xylem. The vessels and tracheids were of regular, polygonal shape with highly lignified walls. A larger number of xylem components with bigger lumina was found than in the control stems (Fig. 11).

Plate I



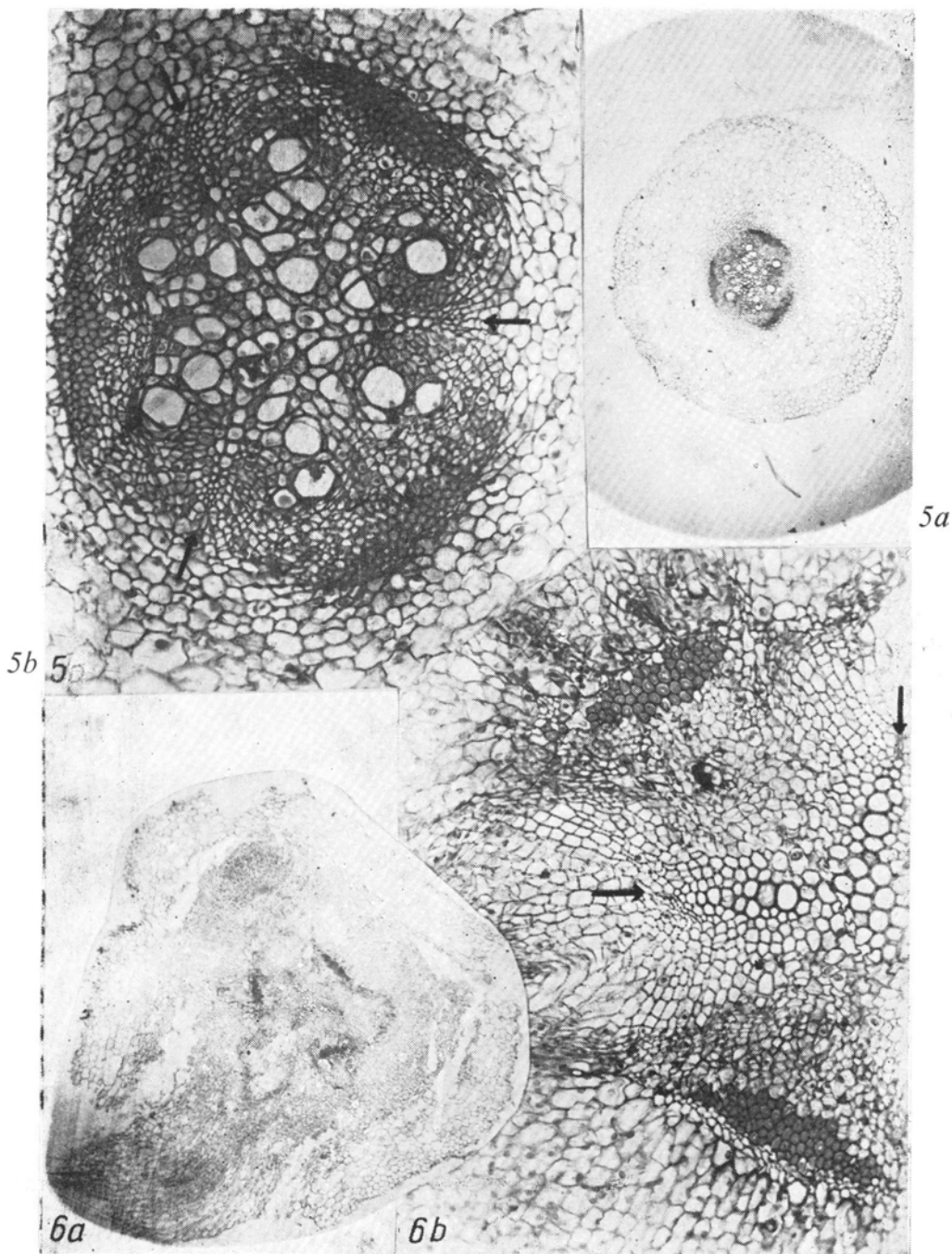
The appearance of seedlings grown from seeds soaked in morphactin IT 3456 (IT) and benzyladenine (BA)

Fig. 1. A — control, B — IT (30 mg/l)

Fig. 2. The detail of the roots from IT treatment (fig. 1B), magn. $\times 3$

Fig. 3. C — BA (25 mg/l), D — BA (50 mg/l)

Fig. 4. E — IT (30 mg/l) plus BA (25 mg/l); F — IT (30 mg/l) plus BA (50 mg/l)

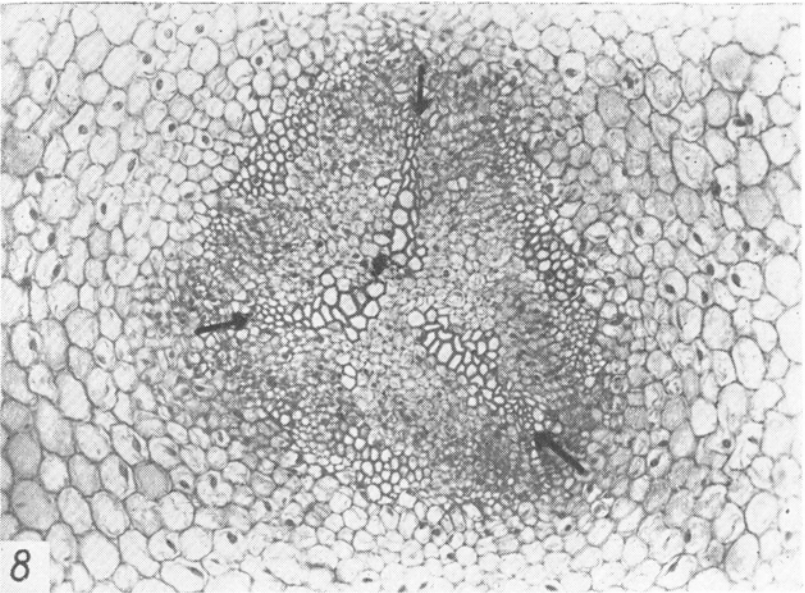
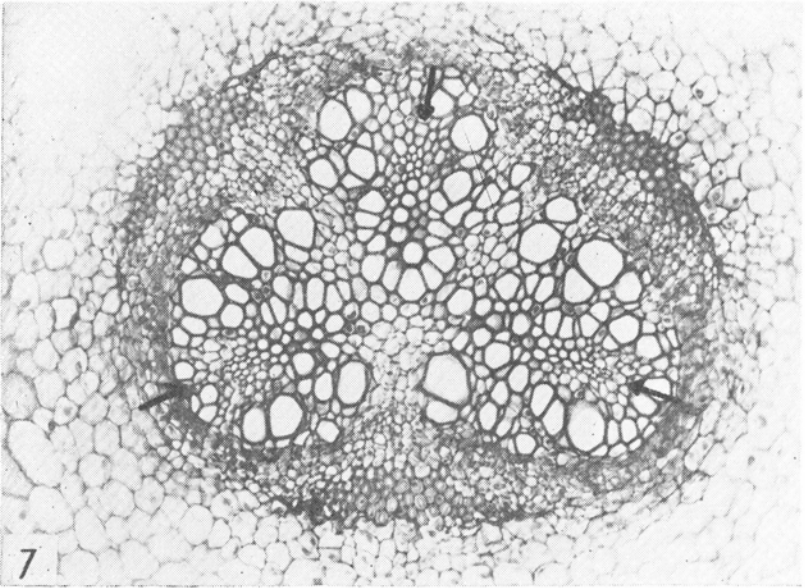


The anatomical changes in the roots induced by morphactin and benzyladenine.

On cross sections the arrows point to the primary xylem bundles

Fig. 5. Control, *a* — magn. $\times 32$, *b* — magn. $\times 140$

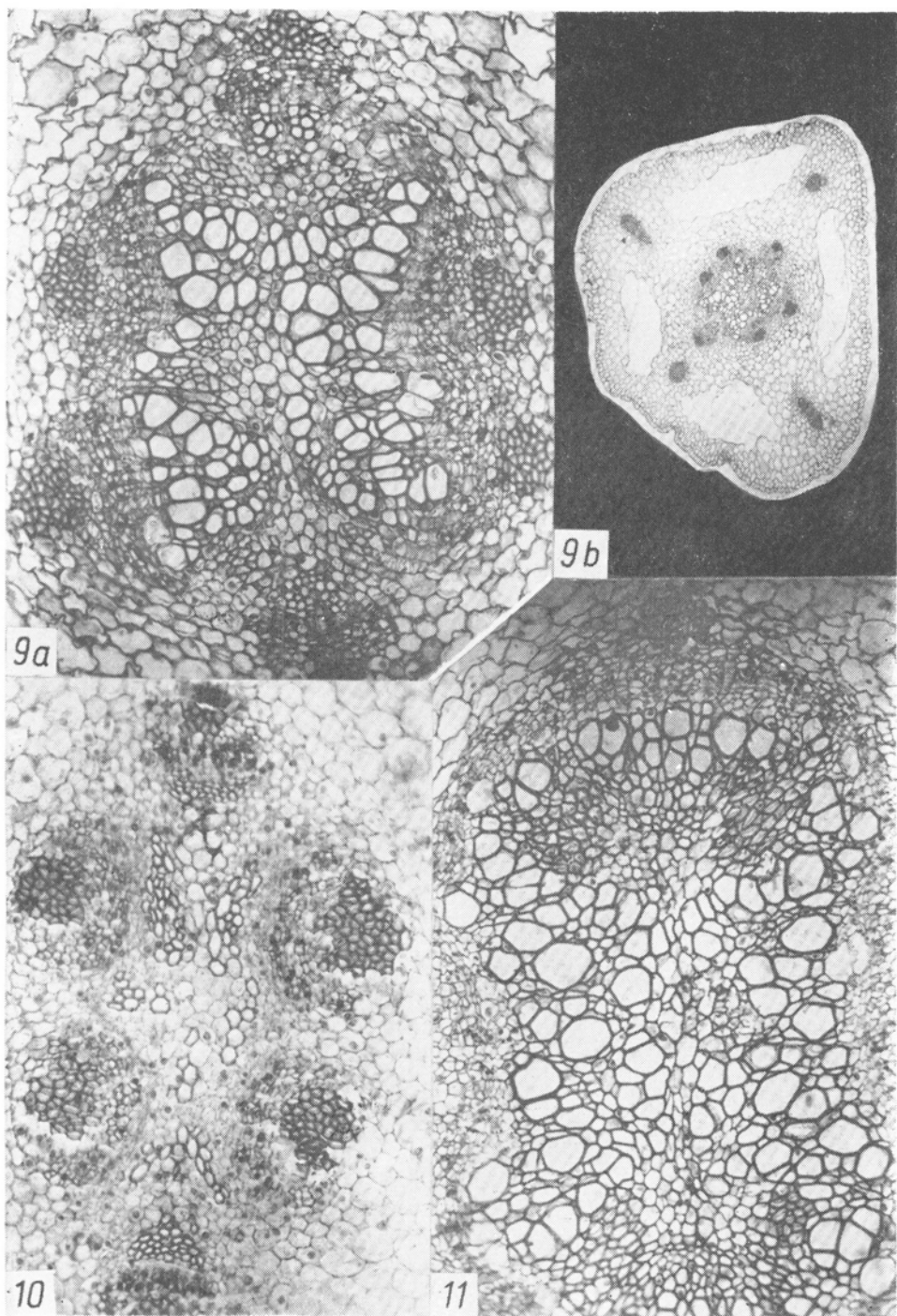
Fig. 6. IT (30 mg/l), *a* — magn. $\times 32$, *b* — magn. $\times 140$



The anatomical changes in the roots induced by morphactin and benzyladenine.
On cross sections the arrows point to the primary xylem bundles

Fig. 7. BA (50 mg/l), magn. $\times 140$,

Fig. 8. IT (30 mg/l) plus BA (50 mg/l), magn. $\times 140$

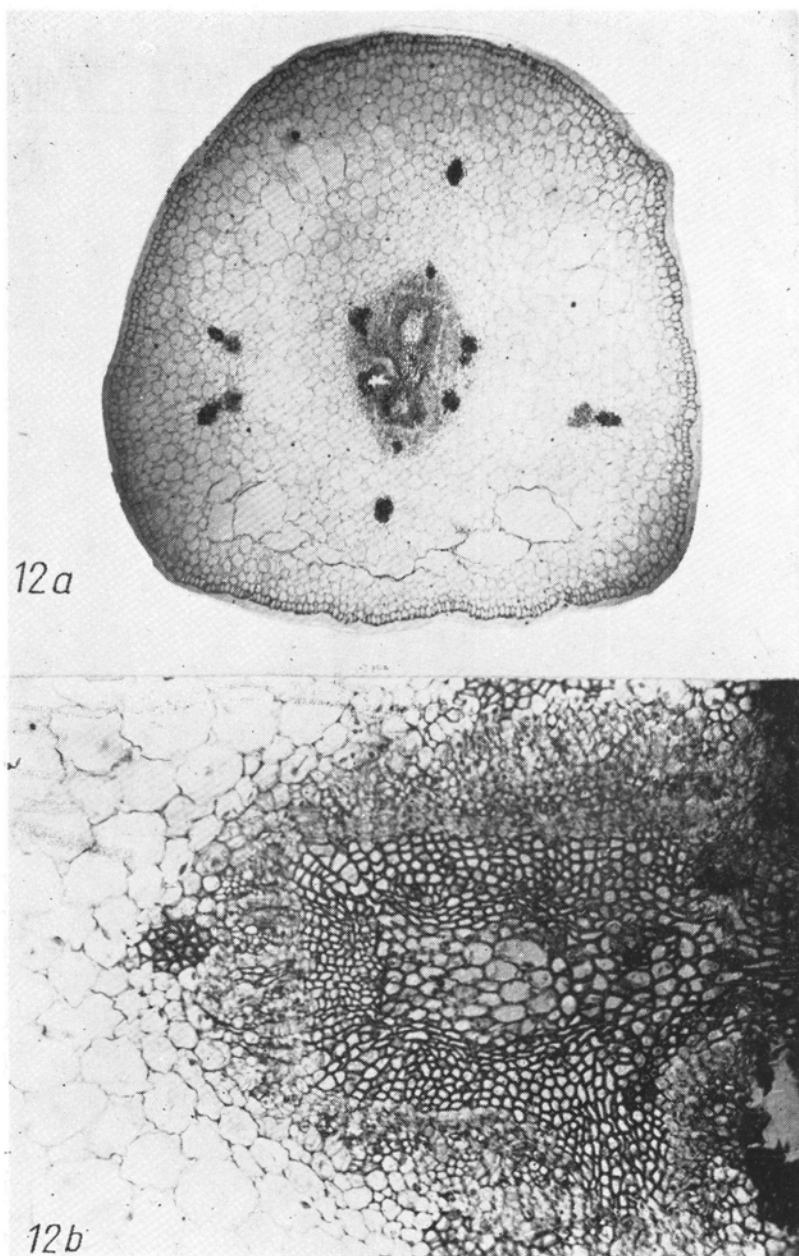


The anatomical changes induced in the shoots by morphactin and benzyladenine. Cross sections.

Fig. 9. Control, *a* — magn. $\times 140$, *b* — magn. $\times 32$

Fig. 10. IT (30 mg/l), magn. $\times 140$

Fig. 11. BA (50 mg/l), magn. $\times 140$



The anatomical changes induced in the shoots by morphactin and benzyladenine.

Cross sections

Fig. 12. IT (30 mg/l) plus BA (50 mg/l), *a* — magn. $\times 32$, *b* — magn. $\times 140$

In the treatment with BA and morphactin the diameter of the stems was greatly increased (Fig. 12a). The primary cortex was thicker due not only to the increase in the size of the parenchyma cells but also to their greater number. The lignification of cell walls both in the conductive and mechanical tissues was as after treatment with morphactin. The secondary xylem formed a continuous ring around the primary xylem. The xylem components had smaller lumina, and they were less lignified than in control and BA treated plants (Fig. 12 b).

The data presented herein suggest the interaction between cytokinin and morphactin with endogenous auxins in cambial activity and xylem differentiation. It is known from other experiments (Krelle and Libbert, 1967; Köhler, 1968) that morphactins are not competitive antagonists of gibberellins. Hashimoto (1961) working with the segments of pea stem has found an increase in thickness of epidermis, cortex and pith after treating the stem segments with kinetin and auxin. He concluded that the thickening of the intact stem is regulated by kinetin or kinetin-like substance(s) in the plant in co-operation with endogenous auxin.

It is then likely that morphactins interact with auxins and cytokinin, as it was suggested in our previous paper (Pieniążek and Saniewski 1968).

SUMMARY

Morphactin IT 3456 and benzyladenine were applied to the seeds of pea cv. Cukrowy Hłowiecki singly or in mixture. The principal morphological and anatomical effects were as follows:

Morphactin — it inhibited the elongation of shoots and to a lesser degree that of roots. It stimulated the formation of lateral roots. It affected the rate of differentiation of xylem both in shoots and roots, and the conductive elements had smaller lumina than controls. The walls of xylem components and of mechanical tissue contained less lignin.

Benzyladenine — it stimulated the branching in peas, and it inhibited the elongation of shoots, roots and leaf growth. It increased the xylem increment and the diameter of xylem components. It did not affect the lignification of the cell walls.

The mixture of morphactin and benzyladenine — it inhibited even more the elongation of shoots and roots. It increased the radial growth of stems, and it decreased the lignification of cell walls (like in the case of morphactin applied singly).

The interaction between cytokinin and morphactin with endogenous auxins in cambial activity and xylem differentiation is suggested.

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*Zmiany w budowie anatomicznej siewek grochu (Pisum sativum L.)
pod wpływem morfaktyny IT 3456 i benzyloadeniny*

Streszczenie

Nasiona grochu karłowego odmiany 'Cukrowy Hłowiecki' moczone w roztworach morfaktyny (IT 3456), benzyloadeniny (BA) oraz w mieszaninie tych dwóch substancji.

Morfaktyna hamowała wzrost elongacyjny pędów w większym stopniu niż korzenia głównego. Stymulowała tworzenie się licznych korzeni bocznych. Poza tym morfaktyna powodowała słabe różnicowanie się drewna i zmniejszenie średnicy elementów przewodzących drewna. Zmniejszona była również lignifikacja ścian elementów przewodzących i tkanki mechanicznej.

Benzyloadenina stymulowała tworzenie się pędów bocznych, zahamowała wzrost elongacyjny łodyg i korzeni oraz rozwój liści. Zwiększony był przyrost masy drewna, elementy przewodzące wykazywały większą średnicę niż analogiczne u roślin kontrolnych. Nie stwierdzono zmian w lignifikacji ścian komórkowych.

Mieszanina BA plus IT 3456 zahamowała silniej wzrost elongacyjny łodyg i korzeni niż każda z tych substancji z osobna. Powodowała wzrost średnicy łodygi. Stopień zdrewnienia ścian komórkowych pod wpływem BA plus IT był taki sam jak w przypadku zastosowania samej morfaktyny.

Dyskutuje się możliwość współdziałania cytokinin i morfaktyny z endogennymi auksynami w kontrolowaniu działalności kambium i różnicowaniu ksylemu.