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The effect of kinetin on the chlorophyll pigments content in leaves of *Zea mays* L. seedlings and accumulation of some metal ions

In this work the relationship between the accumulation of some metals, the content of chlorophyll *a* and *b* in leaves of *Zea mays* L. plants, growth of seedlings and concentration of kinetin in medium has been studied. The experiments were carried out with 7-day old maize plants grown on the Hoagland's medium. The seedlings were exposed to the solution containing kinetin in different concentration ($10^{-9} \div 10^{-5}$ mol · dm^{-3}). The accumulation of metals in maize leaves was measured by emission spectroscopy using the spectrometer with excitation by argon inductively coupled plasma technique (ICP-OES). The amount of chlorophyll pigment was determined by the spectrophotometer UV-Vis. The study shows that kinetin changes the uptake and accumulation of metal ions in leaves of maize plants, changes the growth of seedlings, has influence on the seeds germinations and productivity of fresh and dry mass, and decreases the chlorophyll pigments content in leaves of *Zea mays* L.

Keywords: **kinetin, metals accumulation, chlorophyll *a* and *b*, *Zea mays* L**

Introduction

Hormones of plants are organic substances in small amounts which regulate intracellular processes and change physiological and biochemical reactions in plants. The regulatory function of phytohormones in plants has been established by various workers [1-9]. Plant hormones have been studied for many years because they have been heavily implicated in the control of plant growth and development. They may regulate a wide variety of physiological responses in plants, are central in the regulation of elongation growth and are important in transduction of signals, respiration, water uptake, transport of ions in plant cells, phloem unloading, activation of proteinase inhibitor genes and gas exchange. Phytohormones have been considered to fall into six classes: auxins, cytokinins, gibberellins, abscisic acid, ethylene and brassinosteroids.

Cytokinins are a class of phytohormones that play an important role at all phases of plant development from seed germination to senescence [10-12]. They act at the cellular level by inducing expression of some genes, promotion mitosis and chloroplast development but also on the organ level by releasing buds from apical

dominance or by inhibiting shoot and root growth [13, 14]. The naturally occurring cytokinins are N6-substituted adenine derivatives that contain an isoprenoid or an aromatic derivative side chain [15, 16]. N6-benzyladenine and its derivatives, representing aromatic cytokinins, have been detected in a number of plant species, as minor components of the total cytokinins [17, 18]. Some authors presented in their works recent progress in identification of genes encoding cytokinin biosynthetic (isopentenyltransferases) and degradation of enzymes (cytokinin oxidase/ dehydrogenases), as well as cytokinin receptors, phosphotransmitters, and response regulator proteins [19]. On the other side, cytokinins crosstalk and interaction with other hormones, especially with auxin and ABA (abscisic acid) is well known [11, 20, 21]. In combination with auxin they control cell division, promote juvenility or slow ageing and induce the formation of luteral buds. The data suggested that modification of level of cytokinins affects not only IAA (indole-3-acetic acid) but also ABA levels. On the other hand, there are very limited data in the literature about mechanism of cytokinins transport [20].

Kinetin, the most known cytokinin, has furfuryl ring at the N6-position of adenine and was identified in both animal cellular DNA and plant tissue extracts [15]. Kinetin has important functions in living organisms, in particular in animals and plants [13, 22]. Kinetin is known to be essential to plants and is a necessary hormone for these organisms. Although its role for animals is well known, in the case of plants, it needs further investigation. Kinetin in low concentrations influences plants in a positive way but higher concentrations are toxic.

It is commonly accepted that micro- and macroelements play very significant role in plant cells [23-26]. Micro- and macroelements, similarly to other metals are accumulated in the natural environment at high concentrations and they are taken up by plants [23, 25, 27]. On the other hand, one of the most important factors determining the uptake, accumulation and distribution of the nutrient elements in plant tissues is the interaction between the ions of elements and the effect of the physiologically important substances [28-35]. The interactions between some ions of mineral nutrients in the soil or medium affect the ions uptake, distribution and accumulation and sometimes induce increased or decreased their content in the plants. This interactions are very important for higher plants and well known. On the other side the correlation and interaction between micro- and macroelements and phytohormones is sometimes observed [27, 36, 37].

The aim of this work was to examine the effect of kinetin on the uptake and accumulation of some metals (K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}) in maize leaves and content of chlorophyll pigments in leaves of the seedlings of *Zea mays* L. Also, the effect of kinetin on the growth reaction of plants seedlings, fresh and dry mass, the content of water and seeds germination was investigated in this study.

1. Materials and methods

1.1. Plant material

The experiments were carried out with seven-day old maize plants (*Zea mays* L. *var* K33xF2) grown on the Hoagland's medium: 0.95 g · dm^{-3} $Ca(NO_3)_2 \cdot H_2O$, 0.61 g · dm^{-3} KNO_3, 0.49 g · dm^{-3} $MgSO_4 \cdot 7H_2O$, 0.005 g · dm^{-3} citric acid Fe(III) salt, 0.60 mg· dm^{-3} H_3BO_3, 0.40 mg · dm^{-3} $MnCl_2 \cdot 4H_2O$, 0.05 mg · dm^{-3} $ZnSO_4 \times 4H_2O$, 0.05 mg · dm^{-3} $CuSO_4 \cdot 5H_2O$, 0.02 mg · dm^{-3} H_2MoO_4 [38]. Maize seeds were cultivated for four days in the darkness at 27°C on moist filter paper. Then, the individual seedlings were transferred into aerated solution containing the macro- and microelements, and later cultivated in a green house for 12 hours in the light and 12 hours in the darkness (12 h photoperiod) at 25°C and an irradiance of ca. 450 μmol · m^{-2} · s^{-1} (white light). The control plants were grown on the Hoagland's medium. The other were exposed to the Hoagland's medium containing kinetin (concentration of $10^{-9} \div 10^{-5}$ mol · dm^{-5}) about 72 hours before the chemical analysis; pH of the medium was 6.5. Seeds of maize were germinated on wet filter paper in darkened chamber thermostat (25°C) in Hoagland's nutrient solution with and without of kinetin. The interrelations between the growth of plants and concentration of kinetin in medium and maize seeds germination was studied.

1.2. Preparation of samples

The application of the ICP-OES technique in the analysis of materials originating from plants requires first mineralization of the sample which corresponds to the decomposition of the organic matrix. Depending on the type of the plant material and the analytical method applied several mineralization techniques can be adopted.

The plant material was dried at the temperature of 70°C to a constant mass. The dry mass of the 0.5 g sample was treated with 65% HNO_3 acid (Merck) and heated in a pressureless mineralizator. Eventually, the 30% H_2O_2 was added until the bright yellow colour was acquired, which proved that the samples were fully mineralized. After mineralization the samples were diluted with redistilled water to a volume of 25 cm^3. The dissolved material was introduced through a teflon connector on a peristaltic pump into a nebulizer. After pulverization with a carrier gas the sample was delivered to the ICP burner and analyzed.

1.3. Reagents and apparatus

The following reagents were used in the experiment: redistilled water, concentrated nitric acid and hydrogen peroxide solution. All chemicals were of analytical quality. The concentrations of investigated metal ions in the leaves of maize was measured by emission spectroscopy using a sequential spectrometer ICP-OES made by Spectro Analytical Instruments (Germany). This technique enables elementary analysis. The spectrometer was used with the following parameters: fre-

quency - 27.12 MHz, power - 1.1 kW, coolant gas - 14.0 $dm^3 \cdot min^{-1}$, auxilary gas - 0.5 $dm^3 \cdot min^{-1}$, nebulizer gas - 1.0 $dm^3 \cdot min^{-1}$, nebulizer - concentric Meinhard, nebulizer pressure - 2.4 bar, sample rate - 1.0 $cm^3 \cdot min^{-1}$, observation height 11 mm, holographic grating - 2400 grooves$\cdot mm^{-1}$, analytical lines were: Zn - 213.856 nm, Mn - 257.610 nm, Fe - 259.940 nm, Mg - 279.553 nm, Cu - 324.754 nm, Ca - 317.933 nm, Na - 589.592 nm, K - 766.490 nm, integration time - 3 s. Standard solution of the investigated element of 1 mg $\cdot$ cm^{-3} (Merck) was used as a reference. Standard samples contained conc. HNO_3 similarly as the samples analyzed after mineralization. In the preparation of the calibration curves five standards were used. The two-point recalibration was applied in the case of the spectrometer.

1.4. Chlorophyll analysis

Chlorophyll pigment was extracted from leaves discs with a cork borer from fully expanded, exposed leaves. The pigment was extracted with aqueous acetone (80% v/v) and determined by the spectrophotometer UV-Vis according to Vernon's models - which are modification of Arnon's models:

- chlorophyll *a* [mg$\cdot dm^{-3}$] = 11.63 A_{665} – 2.39 A_{649}
- chlorophyll *b* [mg$\cdot dm^{-3}$] = 20.11 A_{649} – 5.18 A_{665}
- chlorophyll *a*+*b* [mg$\cdot dm^{-3}$] = 6.45 A_{665} + 17.72 A_{649},

where: A_{665} - maximum of chlorophyll *a* absorption, A_{665} - maximum of chlorophyll *b* absorption.

Content of chlorophyll pigment in the leaves of *Zea mays* L. was determined in mg $\cdot$ g^{-1} dry mass of leaves. The values in the figures represent the averages obtained from 10 measurements. The average error was 4÷6%.

2. Results and discussion

The results presented in this paper indicate the relationships between the concentrations of kinetin in plants medium, uptake and accumulation of some micro- and macroelements, and the chlorophyll *a* and *b* content in the leaves of maize seedlings. The effect of kinetin on the growth reaction of plant seedlings, and seeds germination was stated, too. Also, the correlation between the concentration of kinetin in the external medium and productivity of plants fresh mass, content of dry mass and the water in the seedlings leaves of *Zea mays* L. were conformed. The results our work were summarized in Figures 1-5.

The dependence between content of investigated ions in plant leaves and kinetin concentration in the external medium is shown in Figure 1. We have noticed the decrease of potassium ions in leaves of plants cultivated on medium, where concentrations of phytohormone were in the range $10^{-9} \div 10^{-5}$ mol $\cdot$ dm^{-3}. On the other hand, we have also noticed the strong increase of sodium ions content in plants cultivated at the concentration of phytohormon 10^{-6} mol $\cdot$ dm^{-3}, and for the remaining

plants, similarity to potassium, the observed fluctuations of the Na^+ ions content were at the level of the experimental errors. In our work the distribution of Ca^{2+} to the leaves of investigated plant organs was analyzed, too. The increase of accumulated Ca^{2+} has been observed only in case of the presence of kinetin (10^{-9} mol · dm^{-3}) compared to control. However, we have observed the strong decrease of its accumulation for experiments with kinetin in concentration 10^{-8}÷ ÷10^{-5} mol · dm^{-3}.

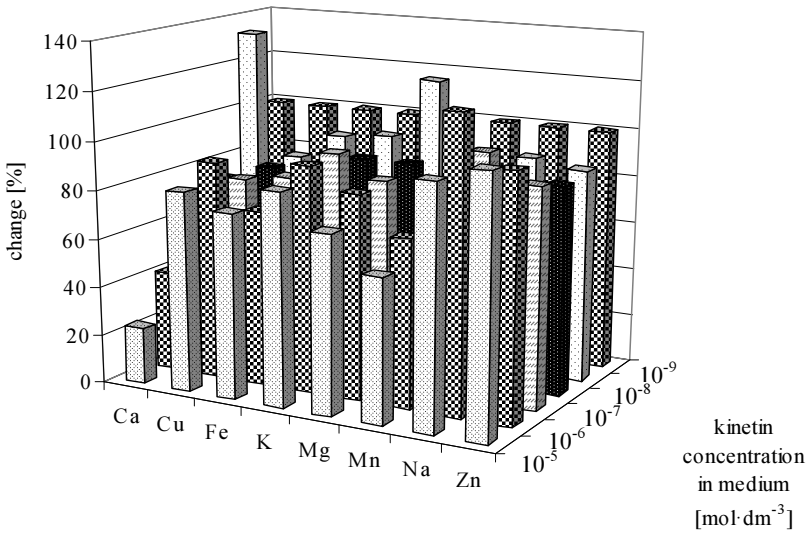

Fig. 1. Change of metal contents (%) in the leaves of 7-day seedlings maize (average from 10 measurements)

The distribution of magnesium to the leaves tissues of *Zea mays* L. plants (plants growing on the control medium) is lower than in the leaves of plants cultivated on medium with kinetin in concentration 10^{-9} mol · dm^{-3}. However, we have observed the decrease of its content for experiments with kinetin in concentration 10^{-8}÷10^{-5} mol · dm^{-3}. The increase of accumulated Mg^{2+} has been observed only in case of the presence of kinetin (10^{-9} mol · dm^{-3}) compared to control. On the other side, the kind of distribution of magnesium ions to leaves of maize for investigated concentrations of kinetin is similar to the distribution of calcium ions. The Fe^{2+} accumulation was established in the leaves of plants in comparison to control plants. The content of iron in the leaves, compared to control, in all cases, was decreased. On the other hand, the distribution of iron ions from the roots *via* mesocotyles, to the leaves expresses the reversed tendency compared to Zn^{2+} content (Fig. 1). Content of Mn^{2+} which we are presented in this study, is relatively to control low in case of the presence of kinetin in external medium. In the work the distinct decrease of magnesium ions for in all range of kinetin concentrations in medium was observed. The accumulation of copper ions decreases slightly in plant leaves in the presence of kinetin. Our results indicate that accumulation of zinc ions in the leaves

tissues of plants, which are grown in a medium containing kinetin, are decreased compared to control plants.

The date of our framework shows that kinetin, the essential component of plant cells, affects the uptake and accumulation of nutrient elements in plant tissues and induce concentration changes of someone of them in plants.

The nutrient elements investigated in our study have significant role in the higher plant metabolism [23-25].

Potassium (K), sodium (Na) and calcium (Ca) are the trace elements, which have some important functions in living organisms. These elements are known to be essential and necessary for plants. Plants and other living organisms utilize the following three elements: potassium (K), sodium (Na) and calcium (Ca) for regulation of cell membrane potential and turgor. The uptake of K^+, Na^+ and Ca^{2+} occurs through ion channels in the plasma membrane of the cells. The concentrations of potassium, sodium and calcium induced changes of membrane permeability that facilitates solute diffusion into plants tissues. On the other hand, the build up of cell structures and the capacity for cell elongation and cell division are dependent on the accessibility of calcium, the influence of Ca^{2+} on carbohydrate metabolism is known, too.

Magnesium (Mg) is one of the key nutrients for plant. Mg^{2+} influences the organization of protoplasmatic structures in cells, growth of plants, on plant pigments synthesis, activates many enzymatic systems of photosynthesis and respiration and acts on phosphorus management in plants and Mg is constituent of chlorophyll molecules. Magnesium deficiency induce chlorosis and necrosis of the leaves and easy reutilization.

Iron (Fe) also plays an important role in cell function and is an essential metal for some metabolic processes in plants. Iron enters many plant enzymes which play the dominant role in oxidoredox reactions of photosynthesis and respiration. It also takes part in chlorophyll pigment synthesis, synthesis of some proteins and in the accumulation of nitrogen in plants. Iron participates in content of many enzymes: cytochromes, ferredoxine, SOD (superoxide dismutase), catalases, peroxidases and nitratereductases. The deficiency of Fe^{2+} in plants causes significant changes in the plant metabolism and induces chlorosis. One of the most important factors determining uptake of this element is the form and concentration of Fe in the soil. Iron in low concentrations influences in a negative way on plants induces toxicity of manganese but the higher concentrations of iron are toxic and induces deficiency Mn^{2+}. At high concentrations Fe is toxic to plants.

Manganese (Mn), in turn, is regarded as activator of many different enzymatic reactions and takes part in photosyntesis. Mn^{2+} activates decarboxylases, dehydrogenases, is constituent of complex PSII-protein, SOD and phosphatase. Deficiency of Mn induces inhibition of growth, chlorosis and necrosis, falling the leaves, the low reutilization.

Copper (Cu) is an essential micronutrient for plant metabolism, enters the oxidoredox enzymes, acts as a component of several enzymes, involved in carbohy-

drate, nitrogen (N) and cell wall metabolism. Copper takes part in protein synthesis and in the synthesis of some amino acids. Cu^{2+} is important to seed production, disease resistance and the water relations in the plant. Visible symptoms of Cu toxicity are small chlorotic leaves and early leaf fall, the growth is stunted and initiation of roots and development of root laterals are poor. Cu^{2+} ions inhibit photosynthesis and respiration and copper also decreased the chlorophyll content in the leaf cells. Copper plays an important role in cell function and is essential in structural stability of chromosomes and energy transfer. Deficiency of Cu induces chlorosis of leaves.

Zinc (Zn) is an essential trace element for every living organism. About two hundred enzymes and transcription factors require Zn^{2+} as a functional component there for, zinc affects major metabolic processes, as well as regulation of the cell cycle and cell division. This metal plays an important role in the protein synthesis, as in the carbohydrate, takes part in metabolism regulation of sacharides, nucleic acid and lipid metabolism. One of the first symptom of zinc deficiency is an inhibition of cell growth and proliferation. The toxic concentrations of Zn negatively affect photosynthetic electron transport and photophosphorylation and effect on the photosynthetic enzymes. Zinc affects on the biosynthesis of chlorophyll. One of the primary mechanisms of Zn toxicity may be an increased permeability of root membranes, which will cause nutrients to leak out from the roots.

It is commonly accepted that micro- and macroelements play significant role in plant cells [22, 23, 25, 27]. On the other hand, in the interactions between some ions of mineral nutrients in the soil or medium affect the ions uptake, distribution and accumulation and sometimes induce increased or decreased their content in the plants [22, 28, 29, 31-35]. The interactions between some ions of elements in plant cells are very important and well known. On the other side, the correlation and interaction between macro- and microelements and fitohormons is sometimes observed [27, 36, 37, 39]. In the present article there have been determined too: germination of maize seeds, the fresh and dry weight of plants, content of the water and the amount of chlorophyll pigments in the seedlings (Figs. 2-5).

The results of chlorophyll *a*+*b*, *a* and *b* content indicated generally a decrease with increasing concentration of kinetin in the culture medium in comparison to control samples (Fig. 2). On the other hand, we have observed the slightly increase of chlorophyll pigments *b* and *a*+*b* content *per* dry mass of maize seedlings treated by kinetin in concentrations $10^{-8} \div 10^{-5}$ mol·dm^{-3} (in comparison to control samples). We found a greater sensitivity (response) to kinetin on the chlorophyll *a* than *b*. Generally, in the medium containing different concentrations of kinetin we have observed the increase of *b* and *a*+*b* chlorophyll content and decrease of the *a* photosynthetic pigment and *a*/*b* range. Photosynthetic pigments (especially chlorophylls *a* and *b* and carotenoids) are important factors of photosynthetic processes and are very important for plants growth and development [7, 40-43].

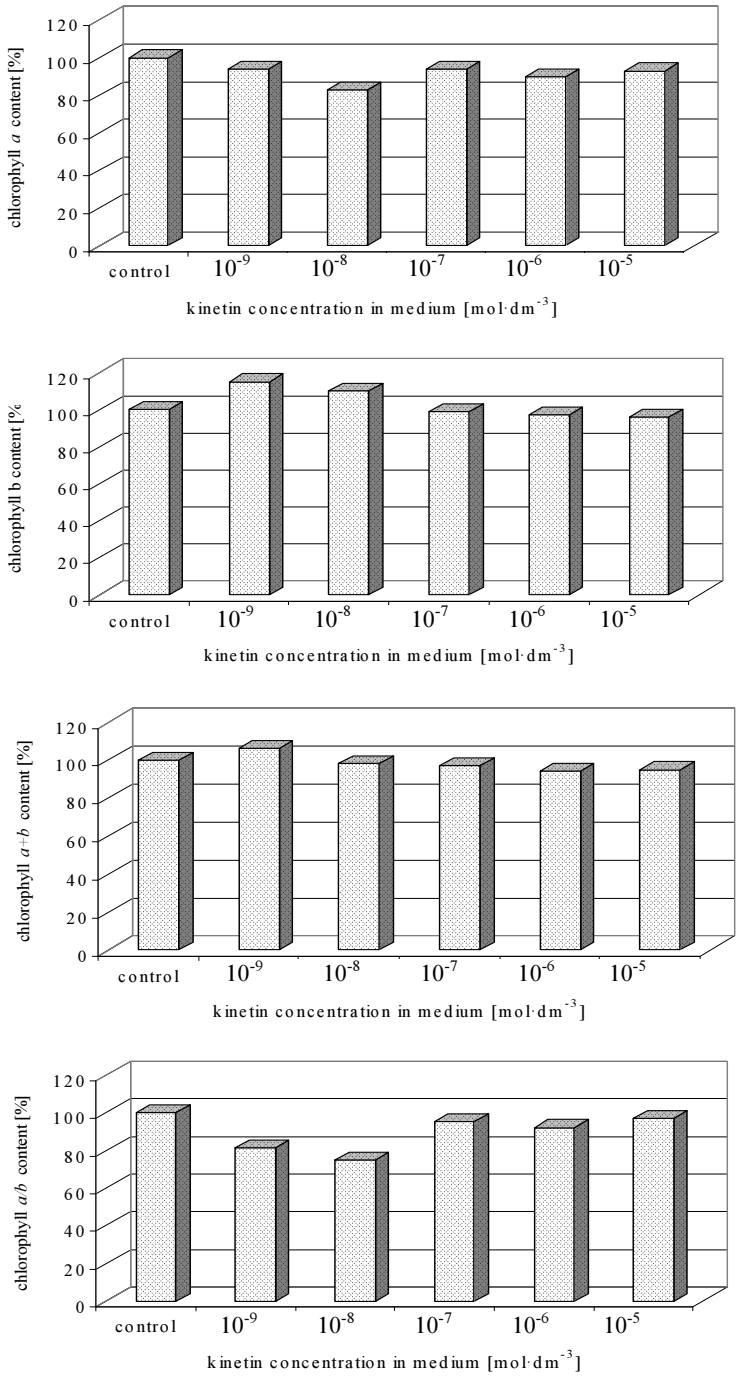

Fig. 2. Percentage of chlorophyll *a*, *b*, *a+b* content and *a/b* ratio *per* dry mass of maize seedling leaves treated by kinetin in different concentrations during growing (average from 10 measurements)

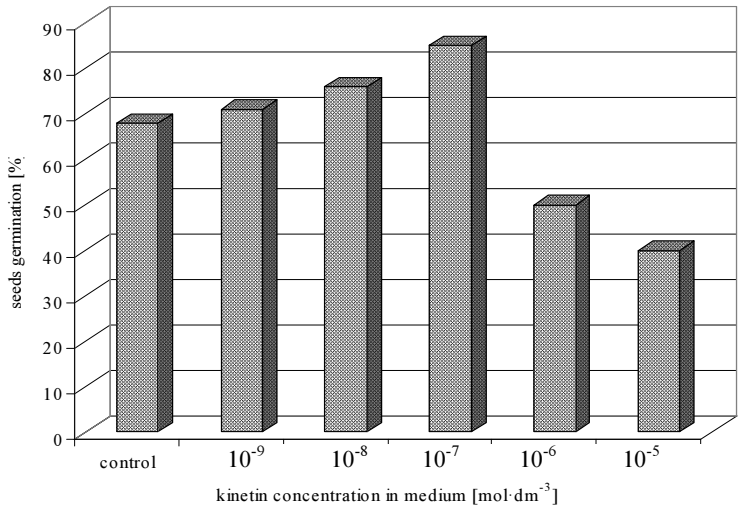

Fig. 3. *Zea mays* L. seeds germination in control medium and in the medium (%) where seeds were treated by kinetin (averages for 10 experiments)

In our work we were studied the influence of different concentrations of kinetin on the vegetation of plants, as well. The seeds germination of *Zea mays* L. (Fig. 3) increased with concentration of kinetin in medium ($10^{-9} \div 10^{-7}$ mol · dm^{-3}) and had his optimum for concentration 10^{-7} mol · dm^{-3}. On the other side, kinetin at the higher concentrations ($10^{-6} \div 10^{-5}$ mol · dm^{-3}) reduced this process. The fresh and dry mass of the plant leaves, as well as content of the water in the leaves tissues, are sensitive for kinetin, too. In comparison with the control plants, kinetin disturbed productivity of fresh and dry mass, and content of the water at all used concentrations in the work (Fig. 4).

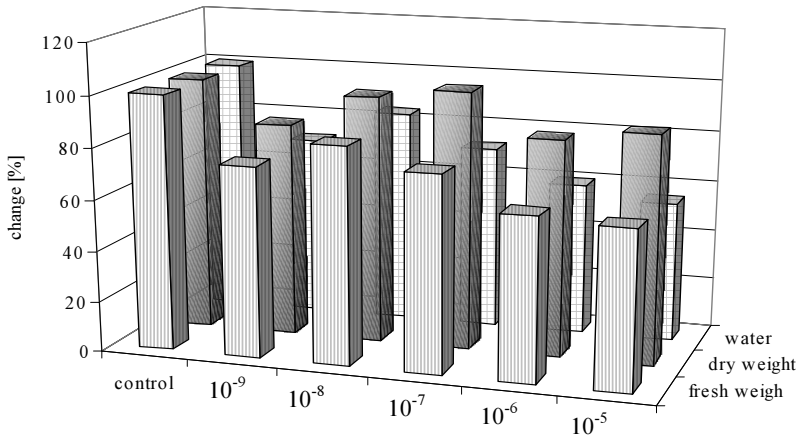

Fig. 4. Productivity of the plant fresh and dry weight and content of the water (%) in the tissue of maize leaves (averages for 10 experiments)

The interrelations between the growth of plants and concentration of kinetin in medium was showed in Figure 5. The results of our work showed, that the addition of kinetin to plant medium affects the growth of maize seedlings. The type of dependence of average length of seedlings for investigated concentrations of kinetin is similar to the dependence of maize seeds germination (Figs. 3 and 5). The lower concentrations of kinetin in plant medium increase the plant growth ($10^{-9} \div 10^{-8}$ mol · dm^{-3}) and higher considerably decrease ($10^{-7} \div 10^{-5}$ mol · dm^{-3}).

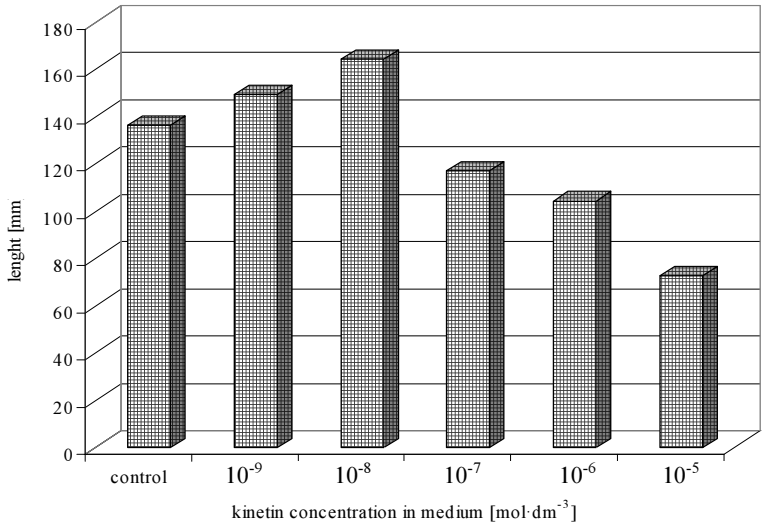

Fig. 5. Length of maize seedlings (mm) treated by kinetin in different concentrations during growing (average from 10 measurements)

Plant metabolism may be affected by kinetin in different ways and particularly processes of biosynthesis of chlorophylls in higher plants are susceptible to kinetin. Under reduced chlorophyll synthesis the photosynthesis is reduced, as shown in Figure 2. The results of chlorophyll content indicated a decrease with increasing concentration of kinetin in the culture medium. On the other hand, photosynthesis is one of the most important studied physiological processes in plant sciences. Plant metabolism is affected by photosynthesis and particularly photosynthetic processes in higher plants decided about plant growth and development. Kinetin penetrates into chloroplast seems to be a very important factor protecting photochemical activity. The photosynthetic activity of chloroplasts is related to the presence of many factors. One of them are chlorophyll pigments.

Conclusions

The results presented in the work indicate the relationship between the uptake and accumulation of some macro- and microelements in leaves of maize plants and the presence of kinetin in the external medium for growing plants, similary to

growth, productivity of fresh and dry weight of plants and water content in the leaves. The study shows that the content of chlorophyll *a* and *b* in maize leaves depends on the concentration of kinetin in external medium and generally amount of these photosynthetic pigments.

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Wpływ kinetyny na zawartość barwników chlorofilowych i kumulację wybranych metali w liściach *Zea mays* L.

Cytokininy, w tym kinetyna, są fitohormonami, których głównym zadaniem jest kontrola wzrostu, rozwoju komórek i ich dyferencjacji oraz biogenezy chloroplastów. Biorą one udział także w formowaniu się organów i ich regeneracji oraz w procesach deetiolacji i wielu innych. Wśród nich znajduje się również szlak syntezy chlorofilu podczas zielenienia etiolowanych roślin. Cytokininy należą do związków stymulujących ten proces, co odbywa się kilkoma drogami, między innymi mają swój wpływ na aktywność enzymów biorących udział w syntezie zielonego barwnika. Celem niniejszej pracy było zbadanie oddziaływania naturalnego hormonu roślinnego kinetyny na syntezę barwników chlorofilowych (chlorofilu *a* i *b*) w liściach siewek kukurydzy *(Zea mays* L.) oraz na zawartość wybranych makro- i mikroelementów.

Doświadczenia przeprowadzono na 7-dniowych siewkach kukurydzy hodowanych w ciemni i na świetle w temperaturze 27°C, na pożywce Hoaglanda; pH pożywki wynosiło 6,5. Natężenie światła w trakcie hodowli roślin było około 450 µmol · s^{-1}. Do badań użyto kinetyny w stężeniach 10^{-9}÷10^{-5} mol · dm^{-3}. Barwniki chlorofilowe oznaczano metodą spektrofotometryczną, natomiast stężenie badanych metali w tkance liści mierzono, posługując się techniką optycznej spektrometrii emisyjnej ze wzbudzeniem w plazmie sprzężonej indukcyjnie (ICP-OES).

Uzyskane w pracy wyniki wskazują na obniżenie zawartości chlorofilu *a* (6÷17%) oraz stosunku chlorofilu *a*/*b* (5÷25%) w zakresie badanych stężeń kinetyny. Z kolei zawartość chlorofilu *b* dla stężeń kinetyny w pożywce w zakresie 10^{-7}÷10^{-5} mol·dm^{-3} była zbliżona do wyników uzyskanych dla roślin kontrolnych i wyższa dla stężeń 10^{-9}÷10^{-8} mol · dm^{-3} (10÷15%). Podobne wartości uzyskano dla sumarycznej zawartości chlorofilu *a*+*b*. Z kolei kinetyna zmniejsza kumulację niektórych metali w tkance liściowej siewek kukurydzy. Uzyskane wyniki wskazują również na oddziaływanie kinetyny na proces kiełkowania nasion, produkcję masy roślinnej, zawartość wody oraz wzrost siewek.

Słowa kluczowe: kinetyna, akumulacja metali, *Zea mays* L., chlorofil *a* i *b*