Jolanta MOLAS[1] and Maria CHRZĄSTEK[1]

COMPARISON OF POLYAMINE CONTENT IN LEAVES OF BARLEY PLANTS GROWN IN NICKEL STRESS CONDITIONS, CAUSED BY INORGANIC AND CHELATIC NICKEL

PORÓWNANIE ZAWARTOŚCI POLIAMIN W LIŚCIACH JĘCZMIENIA UPRAWIANEGO W WARUNKACH STRESU POWODOWANEGO PRZEZ NIKIEL W FORMIE NIEORGANICZNEJ I CHELATOWEJ

Abstract: In a pot experiment the effect of inorganic and chelatic nickel on the metabolism of polyamines in leaves of spring barley plants cv. Poldek was examined. Nickel was applied to acid soil in the form of $NiSO_4 \cdot 7H_2O$ and Ni-EDTA (M:L 1:1) in the doses of 50 and 75 mg Ni $\cdot$ kg^{-1} of soil. After a 10-day exposition of plants to the effect of inorganic and chelatic nickel, the content of putrescine in leaves increased, whereas the content of spermine and spermidine decreased. In stress conditions caused by inorganic nickel the increase of putrescine content and the decrease of spermine and spermidine content in barley leaves were much bigger than in stress conditions caused by chelatic nickel.

Keywords: nickel stress, spermidine, spermine, spring barley, putrescine

In stress conditions caused by different factors metabolism of polyamines in plants changes and the intensity and direction of these changes depend on the genotype of the plant as well as on the type, concentration and duration of the effect of the stress factor [1-3]. Results of studies conducted so far have shown that metabolism of polyamines changes also in response to nickel stress [4-6]. Specificity of these changes lies in the fact that accumulation of putrescine in plant tissues increases, whereas the content of spermine and spermidine decreases. In spite of the fact that putrescine takes part in inactivating free radicals generated in plant cells in stress conditions [5], putrescine is believed to be a hormone which speeds up the ageing of plants [7, 8]. On the other hand, spermine and spermidine delay the process of plant ageing and their main function in stress conditions consists in protecting cell membranes from damage [9-12].

The research on the metabolism of polyamines in plants in response to nickel stress conditions conducted so far have concentrated on the stress caused by inorganic form of this metal. However, nickel, as a *d*-electrone metal, easily forms chelate compounds with low-molecular-weight organic compounds in the environment [13-16]. It is in the form of chelates that this metal appears in the environment as a pollutant, ie as a component of sewage and industrial waste and of waste sludge [14-16]. Consequently, it is accessible to plants in large amounts in the form of complex ions. The main purpose of this study was to find out the direction of changes of metabolism of polyamines in the leaves of spring barley cv. Poldek exposed to nickel stress conditions caused by inorganic and cheltic forms of this metal.

[1] Department of Plant Biology, Faculty of Agricultural Sciences in Zamosc, University of Life Sciences in Lublin, ul. Szczebrzeska 102, 22-400 Zamość, phone 84 677 27 32, email: jolanta.molas@up.lublin.pl

Materials and methods

Spring barley plants cv. Poldek were grown in pot cultures on average-quality soil, whose granulometric composition was that of silt. The soil had the following properties: float particles - 28%, organic C - 0.8 mg · kg^{-1}, sorption capacity - 11.8 cmol(+)kg^{-1}, pH 5.2, total Ni content - 11.8 mg · kg^{-1}, content of Ni soluble forms in 1 mol · dm^{-3} HCl - 2.4 mg · kg^{-1}. Basic fertilization was applied in the experiment; fertilization amounts per pot were the following: 1 g of N in the form of NH_4NO_3, 0.5 g of P - $Ca(H_2PO_4)_2$, 0.8 of K - KCl and 0.3 mg of Mg in the form of $MgSO_4$. Plant vegetation was conducted in growth chamber at 20ºC and at relative humidity of 75%, for a long (16-hour) photoperiod with light intensity of 180 µmol · m^{-2} · s^{-1}. Nickel was added to the soil at the stage of plant germination in the form of $NiSO_4 \cdot 7H_2O$ and Ni-EDTA (M:L 1:1) in the doses of 50 and 75 mg Ni · kg^{-1}.

Samples of leaves were harvested after 10 days of plant growth in the conditions of excess of nickel for the analysis of polyamine content. The content of polyamines (PAs) was determined by means of the spectrofluorometric method described by Kaur-Sawhney et al [17]. Extraction of PAs was conducted in 5% $HClO_4$. Dansylation of PAs was conducted by means of dansyl chloride (5 mg/cm^3 acetone) and 200 mm^3 of saturated solution of Na_2CO_3. The dansylated PAs were extracted in 0.5 cm^3 of toluene and after a careful separation the organic fraction was collected on plates covered with silica gel G, in system chloroform: triethylamine (25:2 v/v). Derivatives of the particular polyamines were located by comparing them with appropriate standards in UV light. After TLC, the dansylopolyamine bands were scraped, the residue is eluted in 2 cm^3 of ethylacetate; then the acetate was evaporated and dry samples were dissolved in 5 cm^3 of methanol. Quantitative determination of dansyl derivatives of PAs was made by means of the spectrofluorometric method, at an excitation band λ = 336 nm, and an emission band λ = 515 nm.

Statistical analysis: Variance analysis (ANOVA) of obtained data was carried out, followed by a test checking the significant difference (LSD) with probability of 0.05.

Results and discussion

Nickel used in fertilization of spring barley (*Hordeum vulgare* L.) cv. Poldek influenced on metabolism of polyamines in leaf tissues (Fig. 1). In general, after a 10-day exposure of spring barley to the effect of inorganic and chelatic nickel, the content of polyamines in leaves decreased in the case of both chemical forms; however, the decrease was small (Fig. 1). When the soil was contaminated with inorganic nickel added in the amount of 50 and 75 mg · kg^{-1}, the content of all the three polyamines (Putrescine+Spermine+Spermidine) was smaller by about 4.1% and 4.6% as compared with the control. When the soil was contaminated with Ni-EDTA added in the amount of 50 and 75 mg · kg^{-1}, the content of these polyamines was lower than in the control by 0.5% and 4.1%, respectively. The direction of changes in metabolism of polyamines in barley leaves was specific to this metal and did not depend on its chemical form; the content of putrescine increased and the content of spermine and spermidine decreased (Fig. 1). However, the intensity of metabolism of polyamines depended on the doses and chemical form of nickel applied to the soil; it should be noted that it depended on its chemical form to a greater extent (Fig. 1). After 10 day's growth on soil contaminated with nickel sulphate added in the amount of 50 and 75 mg · kg^{-1}, the content of putrescine in barley leaves increased by 64.4 and 80.6%, respectively, as compared with the content of this polyamine in control barley leaves (Fig. 1). After the same time of growth of plants in the presence of Ni-EDTA chelate which was applied to the soil in the amount of 50 mg Ni · kg^{-1}, the content of putrescine in the leaves increased only by 11.5% (Fig. 1). A significant increase in the content of this polyamine (by 32.2% as compared with the control) was observed when the soil was contaminated with a larger amount of nickel chelate, ie 75 mg Ni · kg^{-1} of the soil; however, also in this case the increase in the content of putrescine was smaller than when the soil was contaminated with inorganic nickel (Fig. 1).

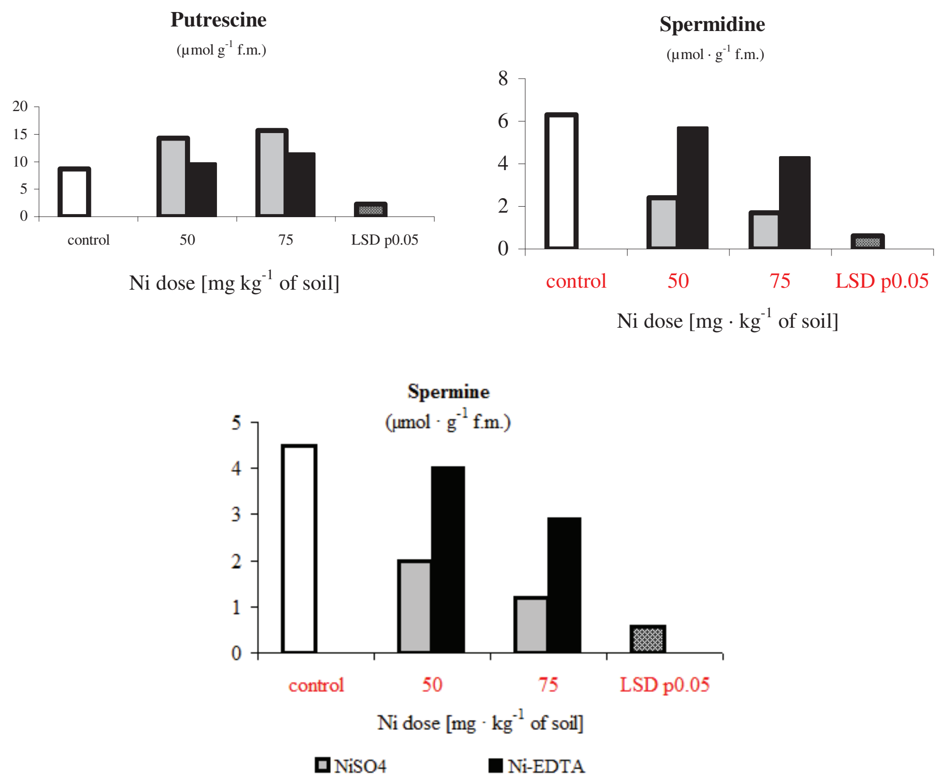

Fig. 1. The comparison of polyamines content in leaves of barley plants grown in the presence of inorganic and chelatic nickel excess, LSD p0.05 - the least significant difference with a probability of 0.05

In spite of the fact that putrescine has the ability of inactivating free radicals generated in cells of plants exposed to heavy metal stress [5], it is generally considered to be a hormone which causes plant ageing because it speeds up the apoptosis of cells [8, 18]. Moreover, it has been shown that in stress conditions, parallel to accumulation of putrescine, such unfavourable consequences of stress as depolarisation of membranes, effect of potassium ions, a drop in protein content as well as withering and necrosis of plant organs can be observed [8]. In the conducted experiment the level of putrescine corresponded to morphological symptoms of nickel toxicity, such as leaf chlorosis and necrosis. These symptoms appeared at the top of the youngest leaves when the soil was contaminated with inorganic nickel, ie when the largest increase in putrescine content was observed.

Studies conducted so far have shown that the mechanism of nickel toxicity is connected with initiation of free-radical reactions *in vivo*, including lipid peroxidation of cell membranes [19, 20]. Studies conducted so far have also shown that polyamines, including putrescine, takes part in inactivating free radicals [5, 9]. A larger increase in the content of this polyamine, which was observed in the presence of nickel sulphate, as compared with the increase noted in the presence of Ni-EDTA chelate, suggests that inorganic nickel induced a stronger oxidative stress in the cells of barley leaves than Ni-EDTA chelate. The difference could result from a different content of this metal *in vivo*. Earlier studies showed that spring barley cv. Poldek assimilated more nickel from acid soil contaminated with the sulphate form of this metal than with Ni-EDTA [21].

Studies conducted so far have also shown that there are differences in the level of spermine and spermidine in barley leaves grown in the presence of inorganic and chelatic form of nickel (Fig. 1). The level of these polyamines was markedly lower in the presence of $NiSO_4$ than in the presence of Ni-EDTA. These differences could result from different phytoassimilation of this element from the two chemical forms.

Studies conducted so far have shown that in stress conditions spermine and spermidine protect cytoplasmic membranes [10-12]. According to Roberts et al [10] spermine and spermidine associate with the surface of membranes, stabilizing their permeability. Results (to be published in Ecol. Chem. Eng.) obtained by the authors show that the level of these two polyamines correlates negatively with the degree of damage to membranes, measured by the content of MDA *in vivo* and the outflow of electrolytes from plant tissues.

Conclusion

In stress conditions caused by inorganic ($NiSO_4 \cdot 7H_2O$) and chelate (Ni-EDTA) forms of nickel the metabolism of polyamines in leaves of spring barley cv. Poldek changed. The direction of the changes in the metabolism of these compounds was specific to this metal and did not depend on the chemical form in which it was applied to the soil; the content of putrescine increased, whereas the content of spermine and spermidine decreased. However, intensity of metabolism of polyamines depended on the chemical form in which nickel was applied to the soil. In the presence of excess of inorganic nickel ($NiSO_4 \cdot 7H_2O$) an increase in the content of putrescine and a decrease in the content of spermine and spermidine in barley leaves were much bigger than in the presence of excess of chelatic nickel (Ni-EDTA).

References

[1] Bouchereau A., Aziz A. and Larher F.: *Polyamines and environmental challenges: recent development.* Plant Sci., 1999, **140**, 103-125.

[2] Kubiś J.: *Poliaminy i ich udział w reakcji roślin na warunki stresowe środowiska*. Kosmos, 2006, **55**(2-3), 209-215.

[3] Liu J-H., Kitashiba H., Wang J., Ban Y. and Moriguchi T.: *Polyamines and their ability to provide environmental stress tolerance to plants.* Plant Biotechnol., 2007, **24**, 117-126.

[4] Zhao J., Shi G. and Yuan Q.: *Polyamines content and physiological and biochemical responses to ladder concentration of nickel stress in Hydrocharais dubia (Bl.) Backer leaves*. Biometals, 2008, **21**, 665-674.

[5] Sharma S.S. and Dietz K.J.: *The significance of amino acids and amino acid-derivated molecules in plant responses and adaptation to heavy metal stress*. J. Exp. Bot., 2002, **57**, 711-726.

[6] Chrząstek M., Świst-Kawala A., Furmańczuk A. and Molas J.: *Permeability of cell membranes vs polyamine content in seedling leaves of oat (Avena sativa L.) two cultivars in nickel stress conditions*. Zesz. Probl. Post. Nauk Roln., 2010, 556 (in press).

[7] Takao K., Rickhag M., Hegardt C., Oredsson S. and Persson L.: *Induction of apoptotic cell death by putrescine*. Int. J. Biochem. Cell Biol., 2006, **38**, 621-628.

[8] Tiburcio A.F., Kaur Sawhney R. and Galston A.W.: *Polyamine metabolism*. [In:] Stress response in plants: adaptation and acclimatization mechanism, B.J. Lea (ed.). Wiess-Liss, New York 1990, 283-325.

[9] Drolet G., Dumbroff E.B. and Legge R.L.: *Radical scavenging properties of polyamines*. Phytochemistry, 1986, **25**, 367-371.

[10] Roberts D.R., Dumbroff E.B. and Thomson J.E.: *Exogenous polyamines after membrane fluidity in bean leaves - a basis for potential misinterpretation of their true physiological role*. Planta, 1986, **167**, 395-401.

[11] Bandurska H., Stroiński A. and Kubiś J.: *The effect of jasmonic acid on the accumulation of ABA, proline and spermidine and its influence on membrane injury under water deficit in two barley genotypes*. Acta Physiol. Plant., 2003, **25**, 279-285.

[12] Kubiś J.: *Exogenous spermidine ylaers in different way membrane permeability and lipid peroxidation in water stressed barley leaves*. Acta Physiol. Plant., 2006, **28**, 27-33.

[13] Lancaster J.R., Jr.: The bioinorganic chemistry of nickel. VCH Publishers, Inc., New York, 1988.

[14] Kabata-Pendias A. and Pendias H.: Biogeochemia pierwiastków śladowych. Wyd. Nauk. PWN, Warszawa 1999.

[15] Adriano D.C.: Trace element in terrestrial environment. 2nd edn., Springer, New York 2001.

[16] Nieminen T.M., Ukonmaanaho L., Rausch N. and Shotyk W.: *Biogeochemistry of nickel and its release into the environment.* Metal. Ions Life Sci., 2007, **2**, 1-30.

[17] Kaur-Sawhney R., Shekhawat N.S. and Galston A.W.: *Polyamine levels as related to growth, differentiation and senescence in protoplast-derived cultures of Vigna aconitifolia and Avena sativa*. Plant Growth Regul., 1985, **3**, 329-337.

[18] Panicot M., Masgrau C., Borrell A., Cordeiro A., Tiburcio A.F. and Atabella T.: *Effects of putrescine accumulation in tobacco transgenic plants with different expression level of oat arginine decarboxylase*. Physiol. Plant., 2002, **114**, 281-287.

[19] Gonnelli C., Galardi F. and Gabbrielli R.: *Nickel and copper tolerance and toxicity in three Tuscan population of Silene paradoxa*. Physiol. Plant., 2001, **113**, 507-514.

[20] Baccouch S., Chaoui A. and El Ferjani E.: *Nickel toxicity induces oxidative damage in Zea mays roots.* J. Plant Nutr., 2001, **24**, 1085-1097.

[21] Molas J. and Baran S.: *Relationship between the chemical forms of nickel applied to the soil and its uptake and toxicity to barley plants (Hordeum vulgare L.)*. Geoderma, 2004, **122**, 247-255.

PORÓWNANIE ZAWARTOŚCI POLIAMIN W LIŚCIACH JĘCZMIENIA UPRAWIANEGO W WARUNKACH STRESU POWODOWANEGO PRZEZ NIKIEL W FORMIE NIEORGANICZNEJ I CHELATOWEJ

Katedra Biologii Roślin, Wydział Nauk Rolniczych w Zamościu, Uniwersytet Przyrodniczy w Lublinie

Abstrakt: W doświadczeniu wazonowym zbadano wpływ niklu na metabolizm poliamin w liściach jęczmienia jarego odmiany Poldek. Nikiel aplikowano do gleby kwaśnej w formie $NiSO_4 \cdot 7H_2O$ oraz Ni-EDTA (M:L 1:1), w dawkach 50 i 75 mg Ni $\cdot$ kg^{-1} gleby. Po 10-dniowej ekspozycji roślin na działanie obu form chemicznych niklu zwiększyła się zawartość putrescyny, a obniżyła zawartość sperminy i spermidyny w liściach jęczmienia. W warunkach stresu powodowanego przez siarczan niklu zarówno poziom wzrostu zawartości putrescyny, jak i obniżenia sperminy i spermidyny w liściach był znacznie większy niż w warunkach stresu powodowanego przez chelat Ni-EDTA.

Słowa kluczowe: jęczmień jary, spermidyna, spermina, stres niklowy, putrescyna