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PHYTOTOXIC EFFECT OF SOME METAL IONS ON SELECTED RAPESEED CULTIVARS REGISTERED IN SLOVAKIA

WPŁYW FITOTOKSYCZNOŚCI JONÓW NIEKTÓRYCH METALI NA WYBRANE ODMIANY UPRAWNE RZEPAKU ZAREJESTROWANE NA SŁOWACJI

Abstract: The aim of this study was to investigate the phytotoxic effects of seven metal ions (Cd(II), Cr(VI), Cu(II), Hg(II), Ni(II), Pb(II) and Zn(II)) on length of roots of five rapeseed (*Brassica napus* L. *subsp. napus*) cultivars registered in Slovakia (Atlantic, Baldur, Californium, Oponent and Verona). The phytotoxic effect of metals was evaluated using IC_{50} values. The studied metal ions inhibited germination and root growth of rapeseed seedlings. In general, the toxicity of metal ions decreased in the following order Cu > Cr >Hg > Cd > Pb > Ni > Zn. Atlantic, Baldur and Californium were more sensitive to Cd than to Ni, for Oponent and Verona higher toxicity exhibited Ni. From the studied rapeseed cultivars Atlantic and Californium were found to be most sensitive to tested metals. On the other hand, high tolerance to metal treatment was determined for Baldur. Czech cultivar Opponent showed high tolerance to Cd, Cr, Cu and Pb, but it was sensitive to Hg and Ni. The above-mentioned results confirmed differences in the metal tolerance of tested rapeseed cultivars.

Keywords: toxic metals, *Brassica napus* L., phytotoxicity, root growth

Rapeseed (*Brassica napus* L. *subsp. napus*) is the most important oil crop in Slovakia with a wide spectrum of utilization: as an agricultural crop (food industry), worthful nutritive animal fodder (rapeseed press cakes) and technical crop. From rapeseed oil biocomponent FAME for biodiesel is prepared [1, 2]. Some *Brassica* species (*Brassica napus* and *Raphanus sativus)* showed a moderate tolerance against several metals and thus, rapeseed can be used for remediation of soils contaminated by toxic metals [3, 4]. If soils contaminated with heavy metals are phytoremediated by oil crops (such as *Brassica* ssp.), biodiesel production from the resulting plant oil could be a viable option to generate bioenergy [5].

Elongation growth is a complex process including turgor changes, synthesis of cell membrane components and content of growth regulators. Excess of heavy metals inhibits elongation plant growth [6, 7]. Some authors consider that inhibition of the cell cycle is the basis for growth inhibition [8]. Moreover, many toxic metal ions are efficient catalysts in the formation of several reactive oxygen species and other free radicals which adversely affect physiological processes in plants, including plant growth [6, 9].

Uptake of toxic amounts of metal by higher plants or algae can result in inhibition of several enzymes and in increase in activity of others. Metal accumulation in the cellular compartment is a prerequisite for enzyme inhibition *in vivo*. Binding of toxic metals to sulfhydryl groups, involved in the catalytic action of structural integrity of enzymes results in inhibition of enzyme activity. On the other hand, the induction of some enzymes (eg

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SOD, CAT, etc.) is considered to play a significant role in the stress metabolism, induced by metal phytotoxicity [10].

This study is aimed to investigate the effect of seven metals ions (Cd(II), Cr(VI), Cu(II), Hg(II), Ni(II), Pb(II) and Zn(II)) on root growth of five cultivars of *Brassica napus* plants registered in Slovakia (Atlantic, Baldur, Californium, Oponent and Verona) and to determine differences in intraspecific sensitivity of rapeseed to metal induced stress.

Material and methods

For experiments following compounds were used: $Cd(NO_3)_2 \cdot 4H_2O$, $K_2Cr_2O_7$, $CuCl_2 \cdot 2H_2O$, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, $Pb(NO_3)_2$ and $ZnCl_2 \cdot H_2O$. These analytical reagent-grade chemicals purchased from Lachema (Brno, Czech Republic) were employed for the preparation of all solutions.

For experiments seeds of following five rapeseed cultivars registered in Slovakia were used: Atlantic, Baldur, Californium, Oponent and Verona. The seeds were purchased from Slovak Centrum of Agricultural Production, Research Institute of Plant Production in Piestany, Slovakia. Atlantic is a medium-early and high type genotype suitable for potato production region; Baldur is a medium-early and medium-high type of genotype suitable for all production regions; Californium is a medium-early to early and medium-high type of genotype suitable for maize production region; Oponent is a late and high type of genotype suitable for potato production region and Verona is a late and medium-high genotype suitable for maize and potato production regions [2, 11].

The seeds of *Brassica napus* were placed in Petri dishes with a 14 cm diameter and filter paper on the bottom. In each Petri dish 58 seeds were evenly displayed on the surface of filter paper and the amount of solution used was 10 cm^3 per dish. Each concentration was duplicated. After 72 hours exposure at mean air temperature (25ºC) in the dark the length of roots and shoots was measured. The applied concentration range of studied compounds was 10^{-6} to 10^{-3} mol dm^{-3}.

Results and discussion

Previously it was found that rapeseed is a suitable plant species for investigations of metal phytotoxicity [12]. In the present study *root tolerance index* (RTI) was calculated as the mean seminal root length in the metal treatment divided by the mean seminal root length in the control. From the dependence of RTI on the concentration of applied toxic metals IC_{50} values, ie molar concentrations of the studied metals causing a 50 % inhibition of the studied parameter, were determined (Table 1).

In general, the toxicity of metal ions decreased in the following order Cu > Cr >Hg > Cd > Pb > Ni > Zn. Atlantic (A), Baldur (B) and Californium (C) were more sensitive to Cd than to Ni, for Oponent (O) and Verona (V) higher toxicity exhibited Ni. The sensitivity of studied cultivars treated with toxic metals decreased as follows: for Cd: A > C > V > B > O; for Cr: A = C = V > B = O; for Pb: A > V > C > B > O, for Zn: A > C > O > V > B, for Cu: A > C = V > B > O , for Hg: O > C > A = V > B and for Ni: O = A >V > B > C. Thus, it can be concluded that from the studied rapeseed cultivars Atlantic and Californium were found to be most sensitive to tested metals. On the other hand, overall high tolerance to metal treatment was determined for Baldur. Czech cultivar Opponent showed high tolerance to Cd, Cr, Pb and Cu but it was sensitive to Hg and Ni.

Table 1

IC_{50} values related to root growth inhibition of five rapeseed cultivars by tested metals (C.L. - *confidence limits*)

Metal	**IC_{50} ± $C.L._{0.05}$ [mmol dm^{-3}]**				
	Atlantic	**Baldur**	**Californium**	**Oponent**	**Verona**
Cd	**0.554** 0.295÷1.090	**0.659** 0.320÷1.47	**0.571** 0.200÷1.912	**0.684** 0.427÷1.180	**0.615** 0.353÷1.150
Cr	**0.114** 0.056÷0.264	**0.122** 0.048÷0.400	**0.114** 0.060÷0.252	**0.124** 0.060÷0.308	**0.128** 0.054÷0.324
Cu	**0.065** 0.037÷0.125	**0.088** 0.061÷0.145	**0.071** 0.039÷0.146	**0.089** 0.047÷0.208	**0.072** 0.036÷0.192
Hg	**0.392** 0.222÷0.742	**0.539** 0.230÷1.542	**0.375** 0.175÷0.826	**0.369** 0.237÷0.588	**0.396** 0.192÷0.878
Ni	**0.609** 0.443÷1.078	**0.644** 0.379÷1.142	**0.748** 0.488÷1.204	**0.607** 0.428÷0.868	**0.612** 0.359÷1.084
Pb	**0.581** 0.299÷1.261	**0.736** 0.376÷1.575	**0.701** 0.416÷1.240	**0.880** 0.644÷1.241	**0.696** 0.351÷1.480
Zn	**0.803** 0.404÷1.863	**1.168** 0.808÷1.757	**0.834** 0.410÷1.905	**0.850** 0.576÷1.347	**1.130** 0.596÷2.287

Toxic effect of metal ions depends on their physico-chemical properties. Nieboer and Richardson [13] classified metals based on their ionic and covalent bonding tendencies and donor-atom preference of metals. These authors used the dependence of the covalent index *versus* the ionic index as a base for classification of metal and metalloid ions to three classes. Metals of class A are oxygen donor-atom seekers, whereas those of class B are nitrogen and sulphur seekers and metals ranged to borderline metals are characterized by ambivalent affinity for all three donor-atom. According to Nieboer et al [14] from the investigated seven metals Hg^{2+} was classified as a metal of class B, the other ones as borderline (intermediate) metals. On the other hand, Duffus [15] assigned Cd^{2+} and Pb^{2+} to the metals of class B.

Ivanov et al [16] investigated the effect of different metal ions on root growth inhibition of maize plants and found that the inhibitory effectiveness decreased in the following order: $Cu^{2+} > Hg^{2+} > Cd^{2+} > Zn^{2+}$ and the IC_{50} values related to the inhibition of root growth showed correlation with the affinity of metal ions to –SH groups. The rank of inhibitory effectiveness for phytotoxic effects of above metals on maize roots was the same as determined in our experiment for rapeseed plants. It is known that toxic metals act as mitotic inhibitors what is reflected in the reduction of root growth. Jiang et al [17] observed toxic effect of $CuSO_4$ (10^{-4}÷10^{-2} mol dm^{-3}) on the morphology of chromosomes. Doncheva [18] found that copper interrupts the nuclei formation at the crucial- G_1/S transition point of the cell cycle, when it prevents their entry into mitosis. Reduced root growth could be connected with copper effect on proliferation of root meristem cells. Yadav and Srivastava [19] confirmed inhibitory effect of Cd^{2+} ions on mitotic index as well as on active mitotic index for *Hordeum vulgare* and *Setaria italica.* These authors observed different types of mitotic aberration due to Cd^{2+} action. Ni^{2+} ions belong to strong mitotic inhibitors causing modification on membrane permeability, inhibition of ascorbate peroxidase. However, these negative effects could be reduced by synthetic cytokinins [20].

Summarizing the obtained results it can be concluded that root tolerance index (RTI) was found to serve as good biomarker for evaluating the relative toxicity of toxic metals to rapeseed cultivars.

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