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Original scientific paper

REACTION OF WINTER OILSEED RAPE VARIETIES TO ELEVATED CONCENTRATIONS OF LEAD

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Remediation methods allow the removal of metals from contaminated soil, and phytoremediation a technology for cleaning contaminated soil and waste material by plants, is becoming increasingly used. *Brassica napus* L., as one of the main oilcrops and high-biomass producing species, is becoming more and more interesting for the use in phytoextraction as it is proved to be tolerant to higher concentrations of heavy metals. The aim of this study was to examine the specific responses

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of three commercial winter rapeseed varieties, Banaćanka, Slavica and Kata, to the increased concentrations of lead *in vitro*. Significant reduction in root length of plants treated with lead was observed only in the variety Slavica, indicating susceptibility of this variety to the increased concentrations of this heavy metal. As in variety Kata a significant reduction in the length of the above-ground part due to the treatment with lead was detected, it could be concluded that the variety Banaćanka is the most tolerant to the applied concentrations of lead since there were no significant changes in the growth and biomass accumulation in all treatments except one, and could be recommended for further use in phytoremediation studies.

Key words: Brassica napus L., lead, tolerance, phytoremediation

INTRODUCTION

Heavy metals are a group of elements that have a specific weight greater than 5. The largest number of these metals is located in soluble minerals in rocks and soils made by decomposition of these minerals contain more heavy metals (KASTORI *et al.,* 1997).. In addition, these elements often get into soil by anthropogenic influence by industrial pollution or application of some fertilizers and pesticides (KASTORI *et al.,* 1997).

Different plant species and genotypes react differently to the presence of increased concentrations of heavy metals. Generally, heavy metal toxicity is expressed when their concentration in the tissues is above average values (KASTORI *et al.,* 1997). They can then affect the virtually all the physiological and biochemical processes of plants.

Remediation methods allow the removal of metals from contaminated soil, and phytoremediation a technology for cleaning contaminated soil and waste material by plants, is becoming increasingly used. The use of plants for extraction of metals from contaminated soil is introduced and developed in 1980 (FULEKAR *et al.*, 2009).

The natural heavy metal hyper-accumulating plant species are well represented by the members of *Brassicaceae* (GRATAO *et al.*, 2005). *Brassica napu*s L., as one of the main oilcrops and high-biomass producing species (MARJANOVIĆ-JEROMELA *et al.*, 2008, MARINKOVIĆ *et al*. 2003), is becoming more and more interesting for the use in phytoextraction as it is proved to be tolerant to higher concentrations of heavy metals (PRASAD and FREITAS, 2003).

The aim of this study was to examine the specific responses of commercial winter rapeseed varieties to increased concentrations of lead *in vitro*.

MATERIALS AND METHODS

Three commercial winter rapeseed varieties, Banaćanka, Slavica and Kata, of Institute of Field and Vegetable Crops, Novi Sad, Serbia, were used in the experiment. Surface sterilized seeds of the tested varieties were placed in plastic pots containing 100 ml MS medium (MURASHIGE and SKOOG, 1962) supplemented with different concentrations of lead - without Pb^{+2} (control); with 5 µMl^{-1} Pb^{+2} (variant 1), with 10 µMl^{-1} Pb^{+2} (variant 2), with 400 µM^{-1} Pb^{+2} (variant 3) and with 600 µMl^{-1} Pb^{+2} (variant 4). Each variant was set in three repetitions. After 14 days of culture fresh (FM) and dry mass (DM) of root and above-ground part, as well as shoot and root length of each plant were measured. Obtained data were analyzed by ANOVA for significance level p=0.05.

RESULTS

In Banaćanka, lead treatment did not have significant influence on FM of above-ground part, although it induced the decrease in average values of this parameter (Table 1). The same stands for all other traits except fresh mass of root, where a significant decrease compared to the control, was observed in treatment with 10 µM Pb.

Table 1. Influence of lead on the morphological parameters of rapeseed variety Banaćanka

Variant	FMS	FMR	DMS	DMR	SL	RL
Control	0.401^{a}	0.045^{a}	0.026^{a}	0.0031^{a}	4.52^{a}	8.98^{a}
5 µM Pb	0.355^{a}	0.044^{ab}	0.023^{a}	0.0029^{a}	3.78^{a}	8.48^{a}
10 µM Pb	0.361^{a}	0.034^{b}	0.026^{a}	0.0027^{a}	4.48^{a}	7.21^{a}
400 µM Pb	0.346^{a}	0.036^{ab}	0.023^{a}	0.0028^{a}	3.43^{a}	8.61^{a}
600 µM Pb	0.38^{a}	0.042^{ab}	0.024^{a}	0.0027^{a}	4.22^{a}	8.7^{a}
LSD ($p \leq 0.05$)	0.055	0.009	0.004	0.0006	1.133	2.604

FMS - fresh mass of above-ground part; FMR - fresh mass of root; DMS - dry mass of above-ground part; DMR - dry mass of root; SL - shoot length; RL - root length

Table 2. Influence of lead on the morphological parameters of rapeseed variety Slavica

Variant	FMS	FMR	DMS	DMR	SL	RL
Control	0.474^{a}	0.046^{a}	0.03^{a}	0.0036^{a}	4.48^{a}	8.63^{a}
5 µM Pb	0.511^{a}	0.052^{a}	0.032^{a}	0.0032^{a}	4.5^{a}	7.3^{ab}
10 µM Pb	0.392^{a}	0.037^{a}	0.026^{a}	0.0038^{a}	3.42^{a}	5.22^{c}
400 µM Pb	0.432^{a}	0.044^{a}	0.028^{a}	0.003^{a}	4.63^{a}	6.53^{bc}
600 µM Pb	0.495^{a}	0.047^{a}	0.03^{a}	0.0034^{a}	4.26^{a}	7.3^{ab}
LSD ($p \leq 0.05$)	0.167	0.015	0.012	0.0012	1.536	1.92

FMS - fresh mass of above-ground part; FMR - fresh mass of root; DMS - dry mass of above-ground part; DMR - dry mass of root; SL - shoot length; RL - root length

The analysis of mean fresh and dry masses in Slavica showed that Pb treatment did not have significant influence on biomass production in this variety (Table 2). The increase in the Pb concentration also did not cause any significant change in length of above-ground part. Root length, however, shows much greater variability between different treatments, which shows the dependence of this parameter, the influence of lead. Statistically significant differences were observed between control and treatment with 10 and 400 µM Pb for root length. Lead treatment caused significant decrease of root length in this variety, with the lowest value observed in treatment with 10 µM.

In variety Kata lead treatment did not have significant effect on dry and fresh mass as well as root length. Treatment with 10, 400 and 600 µM Pb lead to significant decrease in shoot length, with the highest decrease determined at lead concentration of 600 µM.

Table 3. Influence of lead on the morphological parameters of rapeseed variety Kata

Variant	FMS	FMR	DMS	DMR	SL	RL
Control	0.403 [a]	0.045 [a]	0.027 [a]	0.0032 [a]	3.41 [a]	7.67 [a]
5 µM Pb	0.386 [a]	0.04 [a]	0.025 [a]	0.0031 [a]	3.26 [a]	8.35 [a]
10 µM Pb	0.368 [a]	0.043 [a]	0.025 [a]	0.0029 [a]	2.83 [b]	7.32 [a]
400 µM Pb	0.404 [a]	0.041 [a]	0.027 [a]	0.0031 [a]	2.42 [c]	7.99 [a]
600 µM Pb	0.37 [a]	0.047 [a]	0.024 [a]	0.0029 [a]	2 [d]	8.13 [a]
LSD ($p \leq 0.05$)	0.069	0.016	0.005	0.001	0.3584	2.213

DISCUSSION

Numerous studies confirm that many heavy metals accumulate mainly in the underground part of plants (NICHOLLS and MAL, 2003). In earlier studies, it was found that lead reduces root growth by limiting cell division (KIBRIA, 2009), and cell elongation (KOPITTKE *et al.*, 2007). The reduction of root growth is also attributed to the possible replacement of calcium in the cell wall by lead (KIBRIA, 2009). In our work lead treatment affected the root growth in Slavica, with the significant decrease appearing at concentration of 10 and 400 µM.

Lead can produce a number of effects that can result in a decrease in biomass production at certain plants. Preserved production of biomass in the presence of high concentrations of Pb can contribute to reducing lead concentrations in plant tissue (the biological effect of dilution), which improves the survival of plants on sites contaminated with the lead. As in work of MILADINOVIĆ *et al.* (2011) lead did not affect biomass production of tested genotypes after 14 days of culture. The only exception was Banaćanka, where treatment with 10 µM Pb caused decrease in fresh mass of root. The lack of negative effect of Pb on growth and biomass production of plants exposed to it has already been observed by other authors (BEGONIA *et al.*, 2005; BAILON RUIZ, 2006).

Treatment 10, 400 and 600 µM Pb lead to decrease of mean values of the length of the above-ground part in Kata, indicating a reduction in growth due to the toxic effects of lead. The tests on the root meristem cells of *A. sativum* exposed to low concentrations of lead, have revealed the existence of rapid and efficient defence system, however, when the level of lead in cytosol is increased, these cells are severely damaged (KOPITTKE *et al.*, 2007).

Generally, in this experiment the concentration of 10 µM Pb caused the reduction of some of the measured parameters in all genotypes. This is in contrast to the results of MILADINOVIĆ *et al.* (2011) who found that this concentration had either stimulant or no effect on tested rapeseed varieties.

All tested varieties showed some reactions to the lead treatment, but most of them were not statistically significant. That is why, it could be doubted whether the medium composition affected lead solubility and bioavailability. Consequently, in further research some changes in medium composition should be made, as it was found that the decrease of pH and addition of chelates into the medium could stimulate Pb accumulation (MCBRIDE, 1994; MILLER *et al.*, 2008).

CONCLUSIONS

In plants, exposure to or treatment with heavy metals affects roots much more than the above-ground parts and the root growth is the quickest indicator of the effect of heavy metals on plants exposed to them (NICHOLLS and MAL, 2003). In our tests significant reduction in root length of plants treated with lead was observed only in the variety Slavica, indicating susceptibility of this variety to the increased concentrations of this heavy metal. As in variety Kata a significant reduction in the length of the above-ground part due to the treatment with lead was detected, it could be concluded that the variety Banaćanka is the most tolerant to the applied concentrations of lead since there were no significant changes in the growth and biomass accumulation in all treatments except one. This variety has already been found to be resistant to increased concentrations of cadmium and nickel (MILADINOVIĆ *et al.*, 2008; MAKSIMOVIĆ *et al.,* 2010) and could be recommended for further use in phytoremediation studies.

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REAKCIJA SORTI OZME ULJANE REPICE NA POVEĆANE KONCENTRACIJE OLOVA

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Metode remedijacije omogućavaju uklanjanje metala iz kontaminiranog zemljišta, a fitoremedijacija, tehnologija za čišćenje kontaminiranog zemljišta i otpadnih materijala uz pomoć biljaka, se sve više koristi u ove svrhe. *Brassica napus* L., kao jedna od najznačajnijih uljanih biljnih vrsta sa visokom produkcijom biomase, postaje sve više i više zanimljiva za upotrebu u fitoekstrakciji, s obzirom da se pokazala tolerantnom prema višim koncentracijama teških metala. Cilj rada je bio da se ispita reakcija tri komercijalne sorte ozime uljane repice, Banaćanke, Slavice i Kate, na povećane koncentracije olova u *in vitro* uslovima. Značajno smanjenje dužine korena biljaka tretiranih sa olovom je primećeno samo kod sorte Slavica, što ukazuje na osetljivost ove sorte na povećane koncentracije ovog teškog metala. Kako je kod sorte Kata uočeno značajno smanjenje dužine nadzemnog dela prilikom tretmana sa olovom, može se konstatovati da je sorta Banaćanka najtolerantnija na primenjene koncentracije olova, jer nije bilo značajnih promena u rastu i akumulaciji biomase pri svim tretmanima osim jednog, tako da se ova sorta može se preporučiti za dalju upotrebu u fitoremedijaciji.

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