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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGUS *GLOMUS INTRARADICES* ON ACCUMULATION OF RADIOCAESIUM BY PLANT SPECIES

The role of arbuscular mycorrhizal fungus *Glomus intraradices* in ^{134}Cs isotope uptake by different plant species is studied. The impact of radiocaesium on mycorrhizal development and functioning of plant photosynthetic apparatus is considered. The possibility of mycorrhizal symbiosis application in phytoremediation of radioactively contaminated areas is analyzed. It is found that colonization of plants with arbuscular mycorrhizal fungus resulted in significant decrease of radiocaesium concentration in their aboveground parts, while it did not have considerable impact on the radionuclide uptake by plant root system.

Keywords: radiocaesium, radioactive contamination of environment, arbuscular mycorrhiza, arbuscular mycorrhizal fungi, plant photosynthetic apparatus, mycorrhizal colonization, phytoremediation.

Introduction

The radiocaesium isotopes have been introduced into the environment via various routes for last several decades. Altogether, roughly 1 EBq (10^{18} Bq) of long-lived ^{137}Cs was released to the Earth's biosphere in the XX – XXIth centuries that resulted in contamination of vast areas all over the world. About 90 % of radiocaesium was originated from atmospheric nuclear testing, approximately 4 % was released by fuel reprocessing and nuclear fuel facilities and roughly 6 % – by Chornobyl and Fukusima accidents. Nowadays the ^{137}Cs absorption by plants and its accumulation, therefore, represents the main source of human exposure to this radionuclide. The principal route of radiocaesium entry into biological food chain in terrestrial ecosystems is the soil-to-plant pathway. This radionuclide is expected to remain in the rooting zone of plants for decades and respectively to be involved in biological migration chains. However, the mechanisms by which radiocaesium is taken up by plant roots are not completely understood.

Recently the alternative strategies, orientated towards the use of plants and micro-organisms, separately or in combination, have been proposed for removing or immobilizing radiocaesium in the soil [1]. Among these micro-organisms, mycorrhizal fungi received a particular attention. An estimated 90 % of terrestrial plants exist in a symbiotic association with soil fungi forming mycorrhizal associations [2]. Among them, the obligate arbuscular mycorrhizal (AM) fungal symbionts are supposed to have a principal role [3]. These fungi are important participants in the Cs cycle in the upper layers of soils. They have strong impact on mobility of radiocaesium in the soil and result to unavailability of this radionuclide to the other components in ecosystems [4]. At the same time, it was demonstrated [5] that AM fungi can transform and immobilize radionuclides and correspondingly limit their toxicity and bioavailability to plants and spreading into the soils. Accordingly, plants growing in contaminated soil could obtain benefit from their AM fungal symbiotic partners.

Nevertheless, the role of arbuscular mycorrhizal fungi on the acquisition of radiocaesium by plants remains poorly understood and controversial. The lack of clear results on the capacity of AM fungi to accumulate or transport Cs could be principally attributed to different and inadequate experimental systems used in previous studies. Furthermore, the various AM fungi and plants studied could also explain the controversial conclusions obtained, since AM fungi and plants have probably different capacity to accumulate and transport radiocaesium. Consequently, the objectives of this work were to identify the capacity of AM fungi to take up and transfer caesium isotopes to their hosts as well as to estimate the influence of arbuscular mycorrhiza on radiocaesium uptake by plants and impact of radiocaesium on development of AM fungal symbioses.

Materials and methods

Four plant species (*Plantago lanceolata, Medicago truncatula, Lolium perenne* and *Helianthus annuus*) capable to form efficient association with a broad range of AM fungi were selected for our study. The plants were cultivated in the presence or absence of AM fungus *Glomus intraradices* (strain BIO, obtained from BIORIZE, Dijon, France). The ^{134}Cs isotope (obtained from "POLATOM" Radioisotope Centre, Otwock-Świerk, Poland) was added to sterilized substrata in pots in form of CsCl water solution. ^{134}Cs activity concentration was adjusted to 100 000 Bq per pot (77 000 Bq·kg^{-1}). The plants were grown in transparent Sun bags (Sigma^{TM} Aldrich, Poznan, Poland) in a growth chamber at 20 °C, with a photoperiod of 12 h light and 12 h darkness, at photosynthetic photon flux density 30 ± 6 µmol·(s·m^2)$^{-1}$ and harvested each three months.

The activity concentration of ^{134}Cs in roots and shoots of plants was determined using a gamma-spectrometer with semiconductor p-type coaxial high purity HP-Ge detector with a relative efficiency of 15 % and resolution of 2.5 keV at 1.33 MeV, shielded by 10 cm of lead with inner lining with 2 mm Cd and 18 mm Cu.

For the estimation of mycorrhizal colonization, the roots of plants were carefully washed with tap water, softened in 10% potassium hydroxide for 24 hours, washed in water again, acidified in 5% lactic acid in water for 12 – 24 h and stained with 0.01% aniline blue in lactic acid (to visualize AMF) for 24 h at room temperature. The root fragments were mounted and squashed on the slide covered with lactoglicerole. The parameters of AM colonization were assessed according to the method developed in [6] that assumes six levels of mycorrhizal colonization (from 0 to 5). The relative mycorrhizal root length (M%), intensity of colonization within individual mycorrhizal roots (m%), relative arbuscular richness (A%) and arbuscule richness in root fragments where the arbuscules were present (a%) were evaluated using Nikon Eclipse 800 light microscope equipped with Nomarski contrast and fluorescence.

The photosynthetic activity of plants was evaluated using a Plant Efficiency Analyzer fluorimeter (Hansatech Instruments, UK) estimating Chlorophyll *a* fluorescence transients of intact plant leaves. The Chl *a* fluorescence transients (OJIP transients) were induced by a red light pulse (peak at 650 nm) of 600 W·m^{-2} intensity provided by an array of three light-emitting diodes. The transients were recorded for 1 s with 12 bit resolution, starting 10 µs after the onset of illumination. Each transient was analyzed according to the OJIP-test based on the theory of energy fluxes in biomembranes [7]. The selected original data were processed by means of their utilization for the calculation of biophysical parameters by the JIP-test equation, and the number of biophysical parameters were calculated. Among them, the most important parameters are the performance indexes PI_{abs} (evaluated on the base of light absorption) and PI_{total} (total performance index). PI_{abs} and PI_{total} comply all basic biophysical parameters and represent the photosynthetic system vitality.

Results and discussion

^{134}Cs uptake by plants

P. lanceolata inoculated with *G. intraradices* contained 66846 ± 11029 Bq·kg^{-1} of ^{134}Cs in their shoots, that is considerably lower in comparison with the radionuclide activity concentration in nonmycorrhizal plant shoots (87500 ± 12333 Bq·kg^{-1}). At the same time, ^{134}Cs activity concentration in roots of mycorrhizal and nonmycorrhizal *P. lanceolata* was not differed significantly, although the slightly higher radiocaesium content (18 ± 10 %) was found in roots of nonmycorrhizal plants (Fig. 1A). Due to the higher biomass of mycorrhizal *P. lanceolata*, the ^{134}Cs activity in roots and shoots of single mycorrhizal and nonmycorrhizal plant (Bq·plant^{-1}, dry weight) and correspondingly the total radiocaesium activity in a single plant (i.e. shoots plus roots) were not differed substantially ($p < 0.05$). The root/shoot ratios of ^{134}Cs activity concentration in *P. lanceolata* colonized with *G. intraradices* were slightly (about 11 %) higher as compared to those of nonmycorrhizal ones. As it is known [8], the higher root/shoot ratios of caesium content in plants indicates the reduced root to shoot translocation of this element, thus the tendency of the mycorrhiza to reduce radionuclide translocation from *P. lanceolata* roots to shoots was revealed.

The colonization of *M. truncatula* with *G. intraradices* also caused a significant reduction of radiocaesium uptake in plant shoots. Thus, ^{134}Cs activity concentration in aboveground part of mycorrhizal *M. truncatula* was 86888 ± 20022 Bq·kg^{-1}, whereas shoots of nonmycorrhizal plants contained 132100 ± 15505 Bq·kg^{-1} of this radionuclide. At the same time, the mycorrhiza resulted in considerable (19 ± 6 %) increase of radiocaesium activity concentration in *M. truncatula* roots in comparison with that in nonmycorrhizal plants (see Fig. 2B). The distribution of ^{134}Cs activity between aboveground and underground parts of mycorrhizal and nonmycorrhizal *M. truncatula* was differed. Thus, the radionuclide activity concentration in roots of mycorrhizal *M. truncatula* was 46 ± 15 % lower than in their shoots. The opposite tendency was observed in case of nonmycorrhizal plants, where ^{134}Cs activity concentration in shoots was 24 ± 9 % higher than in roots (see Fig. 2B). No statistically significant differences were found between dry masses of mycorrhizal and nonmycorrhizal plants, although both roots and shoots of AM inoculated *M. truncatula* grown on ^{134}Cs spiked substrata had slightly higher weight (about 14 and 12 % correspondingly) as compared to those of nonmycorrhizal plants. The colonization of plants grown on radioactively contaminated substrata also led to moderate (about 10 %) increase of their shoot length. The evaluated ^{134}Cs activity in shoots of single mycorrhizal alfalfa was 9.3 ± 0.6 Bq, whereas shoots of nonmycorrhizal *M. truncatula* contained significantly higher amount of radiocaesium (12.3 ± 0.6 Bq). On the contrary, the radionuclide activity in roots of mycorrhizal alfalfa was substantially higher (3.1 ± 0.1 Bq) as compared to that of nonmycorrhizal plants (2.3 ± 0.2 Bq). Consequently, mycorrhizal *M. truncatula* had significantly lower total activity of radiocaesium (12.4 ± 0.7 Bq) when compared to that of nonmycorrhizal plants (14.6 ± 0.8 Bq). The radionuclide translocation from underground to aboveground parts of plants was more intensive in case of nonmycorrhizal alfalfa. Their root/shoot ratio of ^{134}Cs activity concentration was 0.81 ± 0.28 being considerably lower in comparison with that of mycorrhizal *M. truncatula* (1.45 ± 0.41).

The harvested plants of *L. perenne* mycorrhizal with *G. intraradices* had more than two fold lower ^{134}Cs activity concentration both in their roots and shoots as compared to those of nonmycorrhizal plants. The ^{134}Cs distribution within *L. perenne* demonstrated that the radionuclide activity concentration in aboveground parts of both mycorrhizal and nonmycorrhizal ryegrass was about three times lower when compared to that of plant underground parts (see Fig. 1C). Dry weights of mycorrhizal and nonmycorrhizal *L. perenne* and their shoot length were not differed considerably, however the biomass of plants colonized with *G. intraradices* and grown on substrata spiked with ^{134}Cs was slightly (less than 10 %) higher when compared to that of nonmycorrhizal species. Hence, the colonization with *G. intraradices* resulted in considerable decrease of ^{134}Cs activity (Bq per plant) in shoots (76 ± 23 %) and roots (53 ± 16 %) of single mycorrhizal ryegrass as compared to that of nonmycorrhizal plants.

As opposed to plant species considered above, the colonization of *H. annuus* with *G. intraradices* resulted in significant increase of ^{134}Cs uptake by plants. Thus, the radiocaesium activity concentrations both in underground and aboveground parts of mycorrhizal sunflowers were nearly 10 fold greater when compared to those of nonmycorrhizal plants (see Fig. 1D). At the same time, roots of both mycorrhizal and nonmycorrhizal sunflowers had about 50 % higher ^{134}Cs activity concentrations when compared to plant shoots.

The presence of ^{134}Cs did not have appreciable impact on *H. annuus* growth parameters, and the most distinct was the mycorrhiza influence. Thus, the mycorrhizal *H. annuus* grown on radioactive and clean substrata produced correspondingly 12 and 11 % longer shoots as compared to those of nonmycorrhizal plants. The shoots dry weight of mycorrhizal *H. annuus* grown both on radioactively contaminated and non-polluted soil exceeded substantially (about 70 and 80 % respectively) shoots dry weight of nonmycorrhizal ones. The degrees of ^{134}Cs translocation from roots to shoots of mycorrhizal and nonmycorrhizal *H. annuus* were not differed considerably due to similar root/shoot ratios of the radionuclide activity concentration (1.54 ± 0.10 and 1.47 ± 0.21 correspondingly).

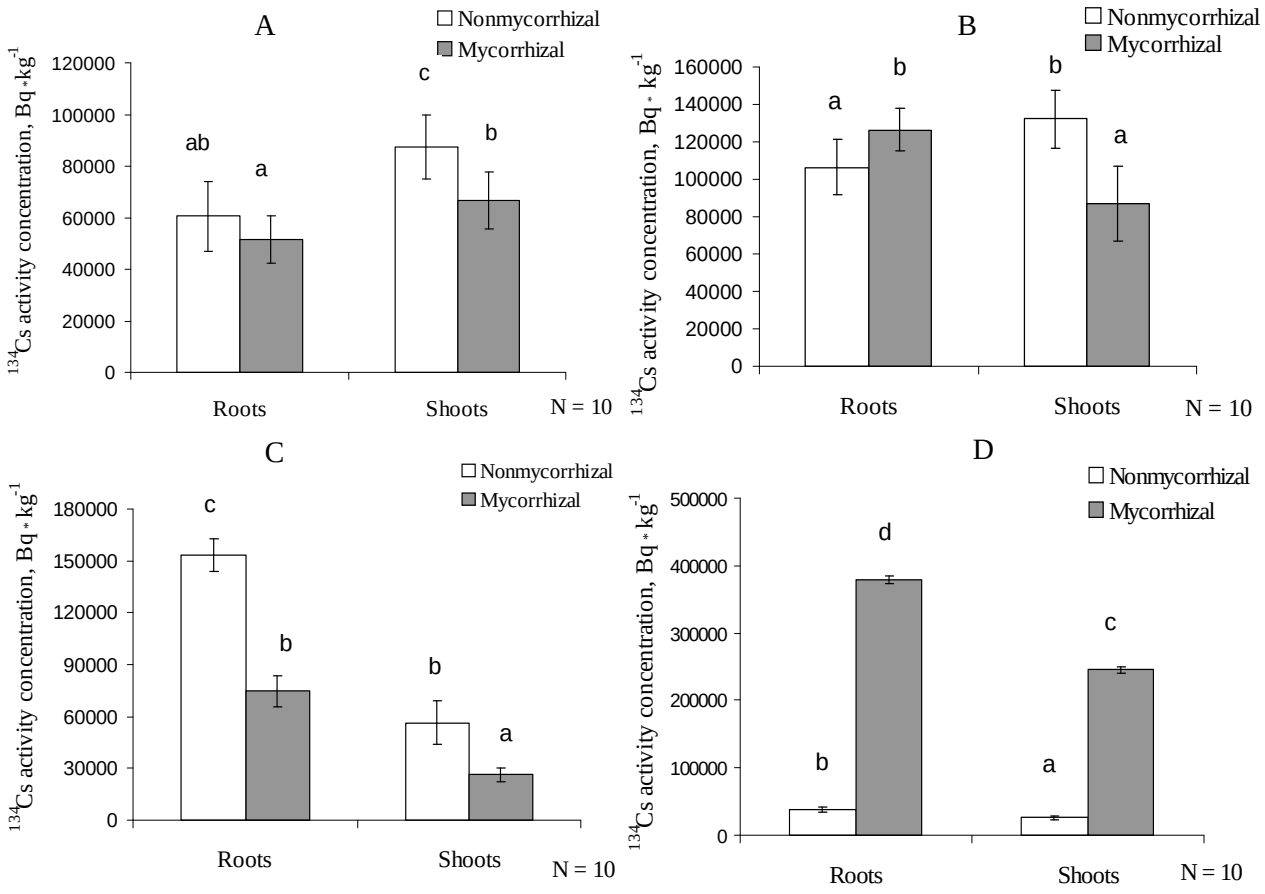

Fig. 1. ^{134}Cs activity concentration (Bq·kg^{-1}) in roots and shoots of *Plantago lanceolata* (A), *Medicago truncatula* (B), *Lolium perenne* (C) and *Helianthus annuus* (D) mycorrhizal or not with *Glomus intraradices* and grown on substrata spiked with ^{134}Cs (77 000 Bq·kg^{-1}). The results are presented as mean ± standard deviation. The different letters above bars mean statistically significant differences ($p < 0.05$).

Functioning of plant photosynthetic apparatus

The spiking of soil with radiocaesium did not have an appreciable impact on functioning of photosynthetic apparatus of studied plant species. The most of photosynthesis biophysical parameters both in mycorrhizal and nonmycorrhizal plants cultivated on substrata with ^{134}Cs were not varied considerably as compared to those of control ones (nonmycorrhizal, without radiocaesium). The exception was observed only in case of *M. truncatula* grown on radioactively contaminated substrata that demonstrated the distinct negative response of plant photosynthetic apparatus to the radiocaesium impact. Thus, alfalfas mycorrhizal with *G. intraradices* and cultivated on substrata spiked with ^{134}Cs had considerably lower efficiency of trapped exciton movement into electron transport chain ($\psi_{Eo} = ET_0/TR_0$) and maximum yield of electron transport ($\varphi_{Eo} = ET_0/ABS$**)** when compared to those of control plants from clean substrata. Also, the total and absorption vitality indexes (PI_{abs} and PI_{total}) of these alfalfas were respectively 33 ± 13 and 42 ± 19 % lower than those of control plants (see Fig. 2B).

In turn, the fungal colonization of plant species grown on radioactive substrata had considerable positive impact on functioning of *H. annuus* photosynthetic apparatus (see Fig. 2D). In this case, the AM inoculation of plants cultivated on radioactive soil improved considerably vitality indexes of sunflowers. Thus, PI_{abs} of mycorrhizal *H. annuus* was correspondingly 41 ± 11 and 36 ± 13 % higher than those of nonmycorrhizal plants grown on soil with ^{134}Cs and control plants. In turn, PI_{total} of mycorrhizal sunflowers exceeded considerably those of nonmycorrhizal plants cultivated on radioactive substrata as well as control plants (40 ± 10 and 45 ± 12 % respectively, see Fig. 2D).

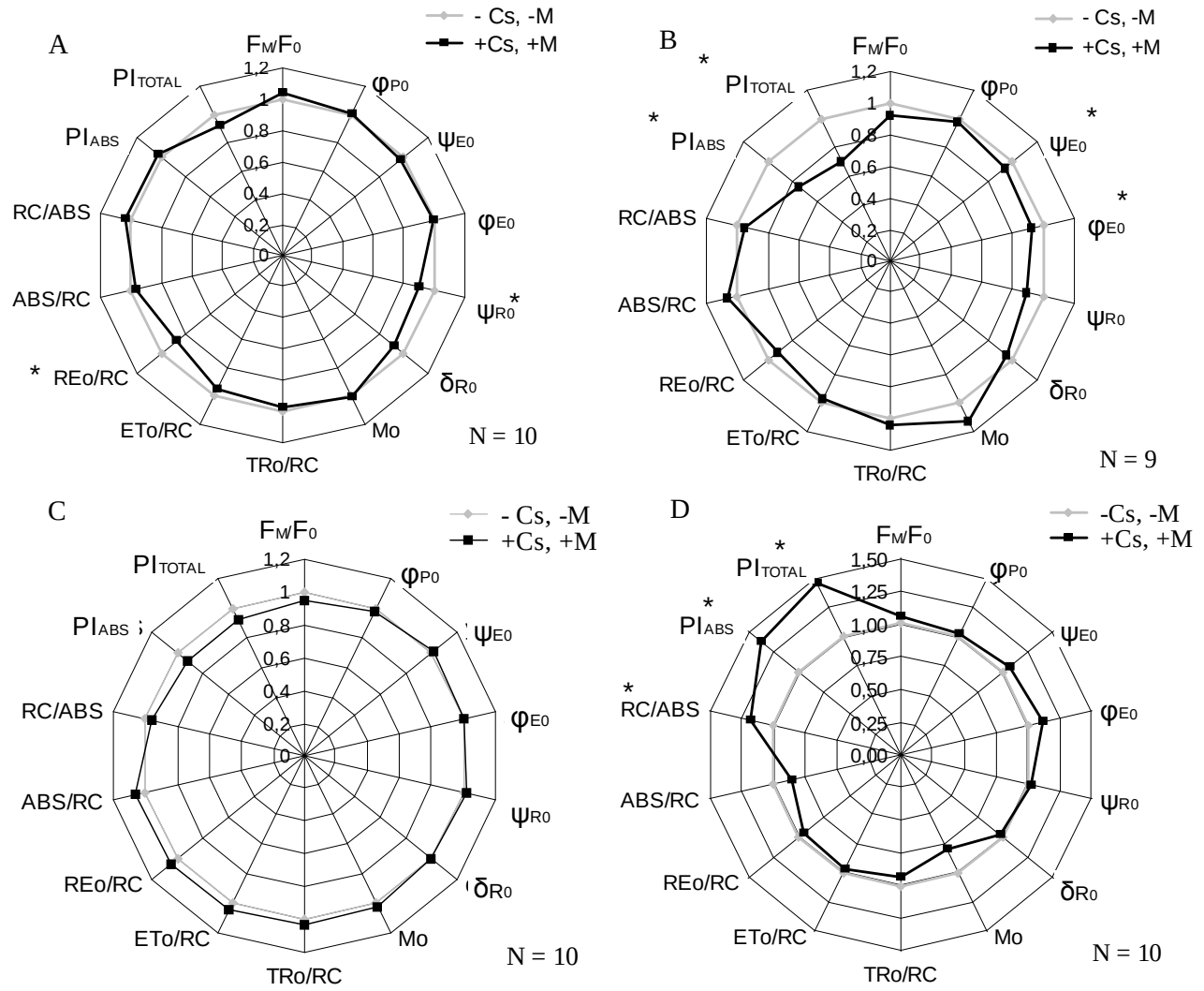

Fig. 2. Biophysical parameters of photosynthesis of *Plantago lanceolata* (A), *Medicago truncatula* (B), *Lolium perenne* (C) and *Helianthus annuus* (D): nonmycorrhizal (control) plants grown on clean soil (-Cs, -M) and plants mycorrhizal with *Glomus intraradices* and cultivated on substrata spiked with ^{134}Cs (+Cs, +M). Values on plots are presented in relative units and normalised on those of the control plants. * - means statistically significant difference ($p < 0.05$).

Arbuscular mycorrhizal colonization of plants

Both treated with radiocaesium and control plant species were characterized with high mycorrhizal frequency (F%) that exceeded 90 %. The AM colonization was uniform in all studied root fragments, and the intraradical structures of the AM fungus were morphologically typical for Arum-type mycorrhizae. The intraradical hyphea of *G. intraradices* within the roots propagated between cortical cells at the long distances and formed lateral branches, which penetrated cells and produced arbuscules inside them (Fig. 3). The presence of numerous intercellular vesicles was characteristic for nearly 80 % of studied root fragments. The spores of *G. intraradices* that have thicker walls in comparison with vesicles were found only in several root fragments. A very low number of intracellular hyphal swellings were observed in plant roots.

The most of AM colonization parameters of plants cultivated on substrata spiked with ^{134}Cs and non-polluted soil were not differed significantly (Fig. 4 A, C, D). Although, in case of *M. truncatula* the presence of radiocaesium resulted in considerable (about 30 %) decrease of mycorrhizal colonization intensity for all and individual mycorrhizal plant roots (M,% and m,% correspondingly) when compared to those of control plants (Fig. 4B).

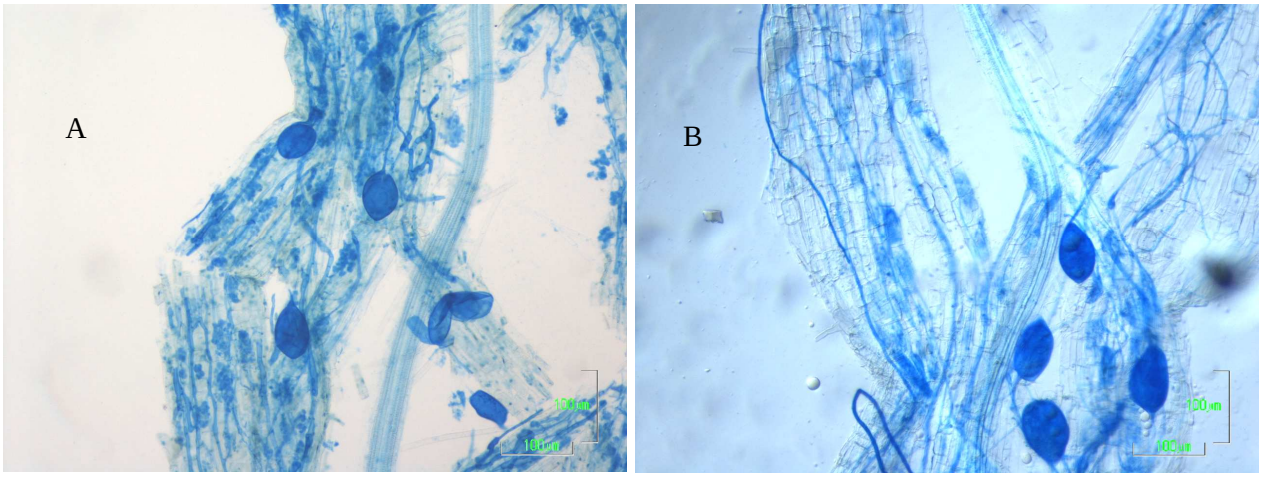

Fig. 3. Arbuscules and vesicles of *Glomus intraradices* within roots of *Plantago lanceolata* (A) and *Medicago runcatula* (B) cultivated on substrata spiked with ^{134}Cs (77 000 Bq·kg^{-1}).

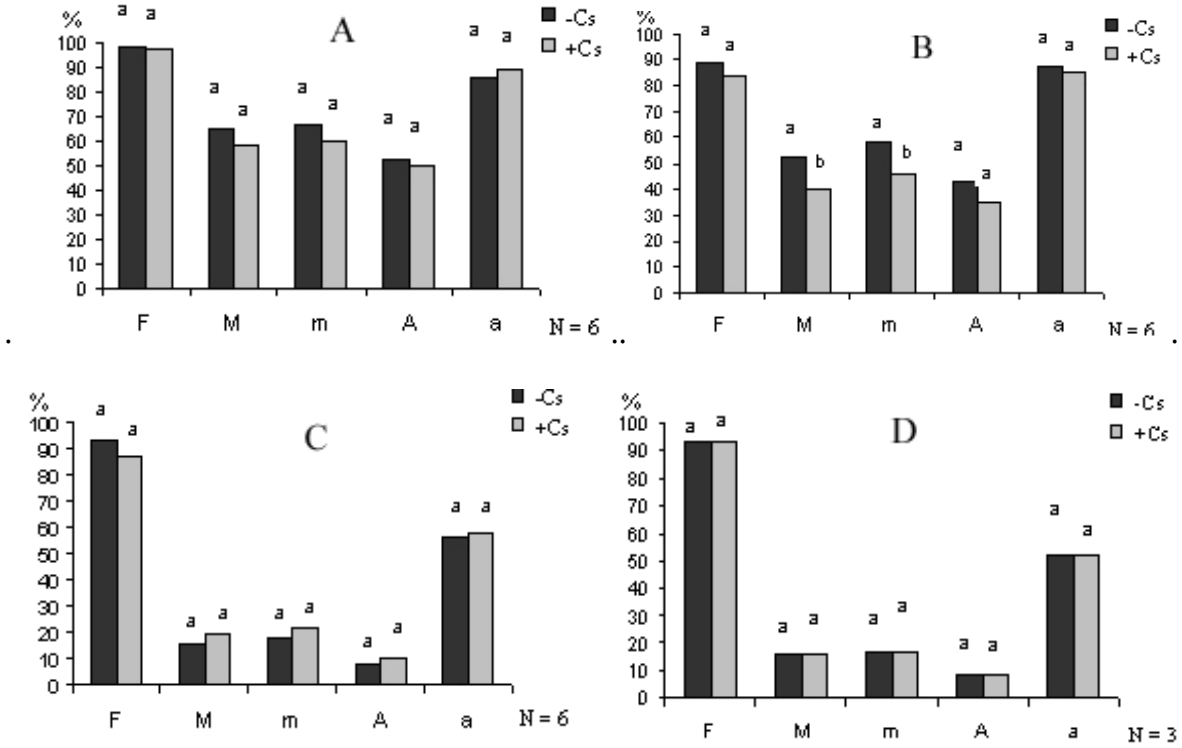

Fig. 4. Arbuscular mycorrhizal colonization parameters of *Plantago lanceolata* (A), *Medicago truncatula* (B), *Lolium perenne* (C) and *Helianthus annuus* (D) inoculated with *Glomus intraradices*: F% - frequency of mycorrhiza; M% - mycorrhizal colonization intensity for all roots; m% - mycorrhizal colonization intensity within individual mycorrhizal roots; A% - arbuscular richness for all roots; a% - arbuscular richness in root fragments where the arbuscules were present, medians. Plants were cultivated on unspiked substrata (- Cs) and substrata treated with ^{134}Cs (+ Cs). The different letters above bars mean statistically significant differences ($p < 0.05$).

The principal goals of the research were to compare the possible influence of mycorrhiza on various AM fungal symbionts cultivated on the same substrata under the impact of ^{134}Cs. Our results suggest that inoculation with AMF changed substantially the uptake of ^{134}Cs by studied plant species and influenced the translocation of caesium isotopes within the plants. The arbucular mycorrhiza resulted in considerable decrease of ^{134}Cs activity concentration in shoots of *P. lanceolata*, *M. truncatula* and *L. perenne* when compared to nonmycorrhizal ones. The most significant (about threefold) reduction of ^{134}Cs activity concentration was found in shoots of mycorrhizal *L. perenne*.

This result contradicts to the data obtained by [9] who found that inoculation with arbuscular mycorrhiza significantly enhanced uptake of ^{137}Cs by ryegrass.

The exception in our study was *H. annuus* where the AM colonization led to nearly tenfold increase of ^{134}Cs activity concentration both in plant roots and shoots. The sunflower was previously shown to be an effective hyperaccumulator of ^{137}Cs and ^{60}Co [10], although the ability of this plant to form mycorrhiza has not been studied. In our case *H. annuus* revealed its ability of ^{134}Cs hyperaccumulation only in the presence of the mycorrhiza. Also, *H. annuus* was only plant species in our experiment whose shoot biomass was significantly affected by the impact of AM fungus. Such contradictory findings demonstrate that basic knowledge of Cs potential uptake mechanisms are needed to facilitate the design of countermeasures to reduce or enhance the transfer of radiocaesium into plants.

Conclusions

In summary, *M. truncatula* was suggested to be the most sensitive plant species relative to the radiocaesium impact. Due to considerable reduction of caesium in their shoots this plant species as well as *P. lanceolata* and *L. perenne* could not be applied in phytoremediation, but they may be potentially used in phytostabilization of the radioactively polluted ecosystems.

On the other hand, the use of *H. annuus* with its Cs hyperaccumulation properties conditioned by mycorrhiza for the phytoremediation is also questionable. In our study, the evaluated total activity of ^{134}Cs accumulated in biomass of sunflowers grown in one pot (two plants) during three months was 221 Bq. This is only 2.2 % from total radiocaesium activity in the pot (100 000 Bq). Extrapolating these data for a longer term and assuming the plant active growth period is about 6 months per year, we can roughly estimate that nearly two decades are needed to remove radiocaesium completely from the soil. This assumption does not take into consideration the natural factors, such as the radiocaesium migration, inhomogeneous distribution in soil and possible leaching of the radionuclide below the 30 – 40 cm (i.e. outside of root zone) as well as potential impact of another AM fungi and various soil microorganisms on the radionuclide uptake by plants.

The number of authors proposed using of AM fungi in phytoremediation strategies for radiocaesium contaminated areas to enhance radionuclide removal by plant biomass ([1, 11, 12]). On the other hand, the effects of AM fungi on Cs accumulation could be applied in strategies to develop crops with smaller soil-to-plant transfer factors which accumulate less Cs ([8, 12, 13]). Such plant species may be potentially grown within areas with moderate radiocaesium contamination levels and further used in agricultural purposes, if the radionuclide content in their biomass does not exceed the prescribed permissible levels. Our results demonstrated the capacity of AM fungi to influence the acquisition and accumulation of caesium isotopes by plants by immobilizing, transporting and affecting the root-to-shoot translocation. Nevertheless the AM fungal ability to take part in phytoremediation strategies still remains questionable and needs for further researches.

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С. В. Дубчак

ВПЛИВ АРБУСКУЛЯРНОГО МІКОРИЗНОГО ГРИБА *GLOMUS INTRARADICES* НА НАКОПИЧЕННЯ РАДІОЦЕЗІЮ РОСЛИНАМИ

Досліджено роль арбускулярного мікоризного гриба *Glomus intraradices* у накопиченні ізотопу ^{134}Cs різними видами рослин. Розглянуто вплив радіоцезію на розвиток мікоризи та функціонування фотосинтетичного апарату рослин. Проаналізовано можливість застосування мікоризного симбіозу у фіторемедіації радіаційно забруднених територій. Установлено, що колонізація рослин арбускулярним мікоризним грибом призвела до суттєвого зменшення концентрації радіоцезію в їхній надземній частині й водночас не мала значного впливу на надходження радіонукліда до кореневої системи рослин.

Ключові слова: радіоцезій, радіоактивне забруднення довкілля, арбускулярна мікориза, арбускулярні мікоризні гриби, фотосинтетичний апарат рослин, мікоризна колонізація, фіторемедіація.

С. В. Дубчак

ВЛИЯНИЕ АРБУСКУЛЯРНОГО МИКОРИЗНОГО ГРИБА *GLOMUS INTRARADICES* НА НАКОПЛЕНИЕ РАДИОЦЕЗИЯ РАСТЕНИЯМИ

Исследована роль арбускулярного микоризного гриба *Glomus intraradices* в накоплении изотопа ^{134}Cs различными видами растений. Рассмотрено влияние радиоцезия на развитие микоризы и функционирование фотосинтетического аппарата растений. Проанализирована возможность применения микоризного симбиоза в фиторемедиации радиоактивно загрязненных территорий. Установлено, что колонизация арбускулярным микоризным грибом привела к существенному уменьшению концентрации радиоцезия в их надземной части и одновременно не имела значительного влияния на поступления радионуклида в корневую систему растений.

Ключевые слова: радиоцезий, радиоактивное загрязнение окружающей среды, арбускулярная микориза, арбускулярные микоризные грибы, фотосинтетический аппарат растений, микоризная колонизация, фиторемедиация.

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