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ACCUMULATION OF CESIUM IN LEAVES OF *LEPIDIUM SATIVUM* AND ITS INFLUENCE ON PHOTOSYNTHESIS AND TRANSPIRATION

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The study examines the transfer factor (TF) for cesium in a soil-plant system and cesium accumulation in cress *Lepidium sativum* L. plants grown in hydroponic culture and subjected to root and foliar application of 0.3 mM CsCl. The experiments showed a high TF for radiocesium: 2.97 (kBq/kg plant DW)/(kBq/kg soil DW). High accumulation of cesium was observed in leaves after both root and foliar treatments. A higher concentration of cesium (3 mM) caused significant disturbance in water uptake, tissue hydration (FW/DW) and production of biomass (DW). Accumulation of cesium in leaves affected gas exchange parameters. Stomatal conductance (C) and transpiration rate (E) were strongly inhibited but photosynthetic CO_2 assimilation (P) was disturbed to a lesser extent. As a result, photosynthetic water utilization efficiency (P/E) was unaffected by 3 mM cesium at photosynthetically active radiation (PAR) of 220 $\mu mol \times m^{-2} \times s^{-1}$. Increasing PAR from 220 to 450 $\mu mol \times m^{-2} \times s^{-1}$ stimulated the photosynthetic rate after 3 days, but no stimulation was observed after 5 days of cesium treatment, in comparison with potassium-grown plants. Changes in chlorophyll fluorescence, indicating maximal quantum yield of photosystem II (PSII) photochemistry, were observed only as a late stress effect. Decreased stomatal opening was an early effect of cesium stress in *Lepidium sativum*, which resulted in limitation of transpiration and water uptake. It is suggested that the decrease in tissue hydration is what limits photosynthetic CO_2 assimilation, synthesis of organic matter and light reactions of photosynthesis.

Key words: *Lepidium sativum* L., cesium accumulation, stress detection, stomatal conductance, transpiration, photosynthesis, chlorophyll fluorescence.

INTRODUCTION

Cesium (atomic number 55) is thought of as an element occuring in the form of radioactive isotopes (Cs-137, Cs-134) which circulate in the environment (Krajewski and Rosiak, 2001) and significantly increase naturally occurring radioactivity. However, most of the cesium in the natural environment is nonradioactive cesium-133. This isotope is released during the decomposition of pollucite; its concentration in the soil is about 5 $\mu g \times g^{-1}$ (Broadley et al., 2001).

Data on the ecotoxicological effects of stable cesium are scarce; this aspect is often overlooked as attention is directed to the behavior of radiocesium in living organisms (Zhu et al., 1999). The physiological similarity of cesium and potassium is frequently indicated in radioecological and physiological studies (Zhu et al., 1999; White and Broadley, 2000). Observations that cesium and potassium share the same transport pathways in plants have become almost a paradigm (White and Broadley, 2000; Urban and Bystrzejewska-Piotrowska, 2002). Despite some similarities in the chemistry of K and Cs, which belong to the same group in the periodic table, there are numerous differences. For example, cesium has a much higher atomic weight and ionic radius than potassium (Siekierski, 1998).

Environmental cesium is accumulated by various biota. Edible mushrooms have been shown to accumulate radiocesium to the highest extent (Mietelski et al., 1992; Ruhm et al., 1999). Plants also accumulate cesium (Mollah et al., 1998; Broadley et al., 1999; Zhu and Smolders, 2000; Ban-Nai and

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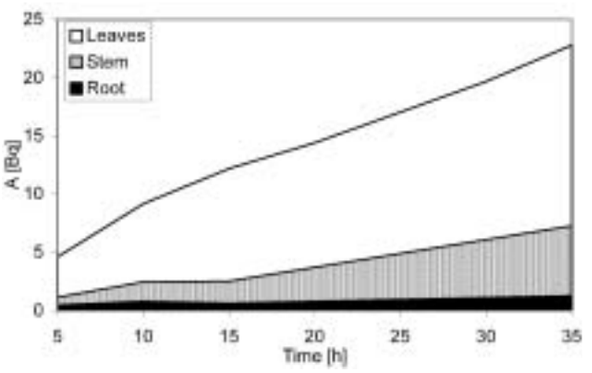

Fig. 1. Accumulation of ^{137}Cs in organs of 6-day-old cress over time. Plants were grown hydroponically, and during the experiment the roots were incubated in 0.3 mM CsCl plus tracer $^{137}CsCl$.

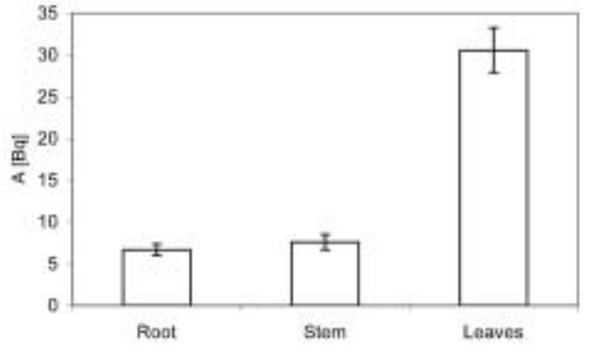

Fig. 2. Distribution of ^{137}Cs among organs of cress 48 h after contamination of leaves of 6-day-old seedlings with 0.3 mM CsCl plus tracer $^{137}CsCl$ (means ± SD for 10 seedlings).

Muramatsu, 2002). Cesium is also transferred to animals through ecological cycling, resulting in elevated ^{137}Cs content in meat (Andersson et al., 2001; Bostedt et al., 2001). Thus, plants are an important link in the cesium transfer chain in trophic systems.

Many plant species reach enormous values of the soil-to-plant transfer factor for cesium (Mollah et al., 1998; Skarlou et al., 1999). Cesium is not distributed evenly among different organs (Gerzabek et al., 1998; White et al., 2003). In onion we observed the highest cesium level in leaves (Bystrzejewska-Piotrowska and Urban, 2003). Here the radiocesium distribution within the plant vegetative organs of cress (*Lepidium sativum* L.) was assessed.

Cress, a herbaceous plant, is recognized as a pollution bioindicator (Hafner, 1989). Since cesium may be accumulated in aboveground parts (Ban-Nai et al., 1999) it was expected that the accumulation of extreme (millimolar) cesium concentrations would affect such physiological processes as stomatal conductance, transpiration (E), photosynthetic CO_2 assimilation (P) and light reactions of photosynthesis. These parameters were measured, and cesium-induced inhibition of growth was evaluated versus the results of potassium overdosing.

TABLE 1. Transfer factors for cress plants grown on ^{137}Cs-contaminated soil for 7 days (means ± SD for 16 values and additionally total uncertainty indicated)

Unit	TF
(kBq/kg)/(kBq/kg)	2.97 ± 0.28
(kBq/kg)/(kBq/m^2)	2.07 ± 0.20

MATERIALS AND METHODS

PLANT CULTURE

Cress seeds (*Lepidium sativum* L., producer: PNOS, Ożarów Mazowiecki) were germinated in distilled water at 20°C in darkness. Then the seedlings were transferred to perlite and supplied with a modified Mayer nutrient solution: 1 mM KNO_3, 1 mM KH_2PO_4, 1 mM KCl, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 1 mM FeEDTA and microelements (Mayer et al., 1959), with CsCl or KCl added. The potassium concentration of the basal solution was 3 mM. The surplus CsCl and KCl concentrations were in the range of 2–10 mM. The seedlings were grown for 3 to 7 days at 20°C under a photoperiod of 16 h (PAR intensity 220 µmol × m^{-2} × s^{-1}). At the end of the experiment the seedlings were weighed, and after drying at 105°C their dry mass was determined.

RADIOCESIUM UPTAKE

Pot cultures were prepared to study the phytoextraction of soil cesium. Soil (pH 5–6, Kronen Blumenerde, ISO 9002, Eugen Stohp GmbH, Tiste, Germany) was contaminated with $^{137}CsCl$ (in 0.3 mM CsCl) and 16 cress seeds were allowed to germinate and grow for 7 days. The transfer factor (TF) was estimated by a standard procedure (Mollah et al., 1998). TF was calculated as follows:

TF = (^{137}Cs concentration in plant DW [Bq/kg]) / (^{137}Cs concentration in soil DW [Bq/kg])

TF = (^{137}Cs concentration in plant DW [Bq/kg]) / (^{137}Cs concentration in soil DW [Bq/m^2])

Six-day-old seedlings were used in hydroponic studies to determine radiocesium uptake. The plant

roots were put in eppendorf tubes filled with 0.3 mM CsCl with ^{137}Cs (2.5 kBq/ml). This value is suggested to be the upper concentration for K transport in plants (Zhu and Smolders, 2000). Seedlings were sampled every five h (10 seedlings in each group), washed, divided into three parts (roots, stems, leaves) and measured by a standard procedure described previously (Urban and Bystrzejewska-Piotrowska, 2002). In the leaf uptake study, $^{137}CsCl$ (25 kBq/ml in 0.3 mM CsCl) solution was spread three times over the leaf surface with a piece of cellulose before the measurements were made.

PHOTOSYNTHESIS AND RESPIRATION MEASUREMENTS

To determine photosynthetic disturbances, 3-day-old seedlings grown on nutrient solution were given nutrient solution enriched with 3 mM CsCl or KCl. Such a high Cs concentration has been used in other studies (Willey and Martin, 1997). The other group of seedlings was collected after a further two days (5 days of treatment). Each group consisted of 4 seedlings. The photosynthetic parameters were measured with a CO_2 infrared analyzer (CI-301PS CO_2 Gas Analyzer, CID, Inc., U.S.A.). The measured parameters were PAR, conductance (which indicates the level of stomatal opening), and rates of transpiration and photosynthesis. Measurements with a camera with PAR and temperature sensors (supplied with the instrument; CID, Inc., U.S.A.; flow rate 0.5 dm^3/min, window 6.25 cm^2) were performed for 20 min following a 10 min adaptation period (average of 20 values). All the experiments were performed in three replicates.

WATER UPTAKE

The effects cesium on water uptake were assayed by fresh weight determination. Three-day-old seedlings were treated with nutrient solutions supplemented with 3 mM CsCl or KCl for a further 3 days, then were put in 1.5 ml eppendorf tubes filled with nutrient solution (with the addition of 3 mM CsCl or KCl; 10 seedlings per group). After 12 h of incubation at PAR intensity 220 $\mu mol \times m^{-2} \times s^{-1}$ the tubes with plants were weighed and the water loss estimated. The tube openings were protected with plasticine to avoid evaporation.

LIGHT REACTIONS OF PHOTOSYNTHESIS

The plants were treated with CsCl or KCl for 3 and 5 days. Chlorophyll *a* fluorescence ratios F_v/F_m and fluorescence half-life $t_{1/2}$ were measured using a time-resolved fluorescence apparatus (Plant Stress Meter, Biomonitor S.C.I., Umea, Sweden) according to Oquist and Wass (1998) and Maxwell and Johnson (2000). Leaves adapted to laboratory conditions were placed in darkness for 30 min to ensure that all energy-dependent quenchings had relaxed (Poskuta et al., 1998). Twenty repetitions were performed for each variant.

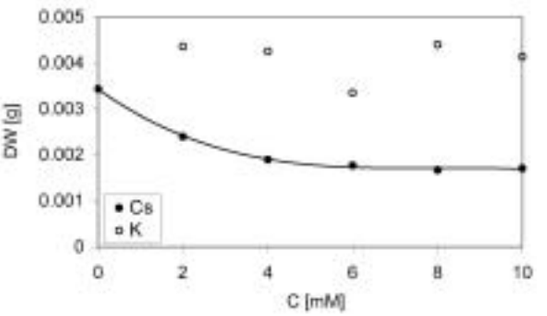

Fig. 3. Changes in dry weight of cress seedlings exposed to various cesium and potassium concentrations for 7 days in a hydroponic system. Basal nutrient solution contained 3 mM K.

RESULTS

Cress plants grown on ^{137}Cs-contaminated soil for 7 days showed a high soil-to-plant transfer factor (TF), expressed on the basis of weight or surface measures (Tab. 1). In hydroponic culture, cress took up cesium, which was transported from the roots to the stem and leaves (Fig. 1). At 48 h after foliar contamination, transport of cesium to roots and stem was low, but the relative amounts of ^{137}Cs in roots, stem and leaves were roughly similar to those after ^{137}Cs was applied to the roots, suggesting the movement of cesium in the phloem (Fig. 2).

Seedling dry weight (DW) measurements after 7-day hydroponic culture indicated that cesium concentrations above 4 mM reduced DW significantly, whereas potassium concentrations did not cause significant changes in DW (Fig. 3). The FW/DW ratio of plants growing on solutions containing more than 4 mM CsCl was practically unchanged (Fig. 4). Water uptake in the cesium-treated plants was lower than in plants growing on nutrient solution with additional K^+ (Fig. 5).

Measurements of gas exchange parameters showed greatly reduced stomata conductance under cesium stress (Tab. 2). After 3 days of growth on CsCl

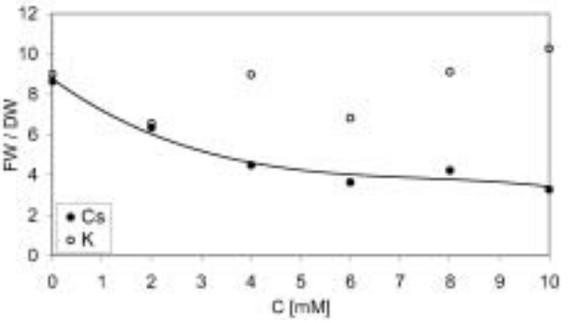

Fig. 4. Changes in FW/DW ratio of cress seedlings exposed to various cesium and potassium concentrations for 7 days in a hydroponic system. Basal nutrient solution contained 3 mM K.

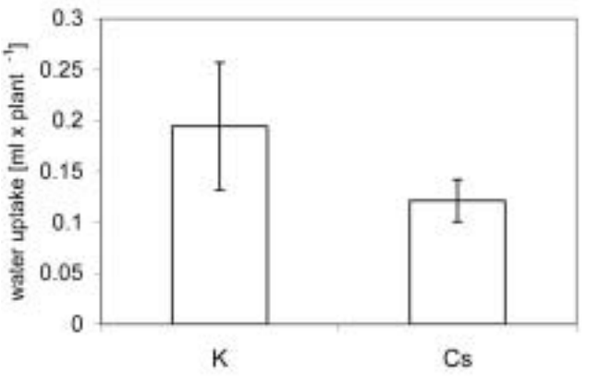

Fig. 5. Water uptake by cress seedlings incubated in nutrient solution containing 3 mM cesium or 3 mM additional potassium for 12 h (means ± SD for 10 seedlings).

medium under irradiance of 220 µmol × m^{-2} × s^{-1}, stomatal conductance was about 20% lower than in plants growing on KCl medium. Partial closure of stomata as a result of cesium application was accompanied by reduced transpiration, but not by a decrease in the photosynthetic CO_2 assimilation rate. After 2 more days of growth on CsCl, stomatal CO_2 conductance was more than 50% lower than in seedlings grown on KCl solution. Transpiration was reduced to a comparable extent, and the CO_2 assimilation rate was decreased.

Increasing PAR intensity from 220 to 450 µmol × m^{-2} × s^{-1} did not affect photosynthesis in seedlings treated with cesium for 3 days (Tab. 2), whereas in seedlings grown on potassium a significant elevation (over 50%) in photosynthesis was observed.

The photosynthesis-to-transpiration intensity ratio (P/E) did not differ between the two groups of plants at PAR level of 220 µmol × m^{-2} × s^{-1} (Tab. 2), but at higher irradiance of 450 µmol × m^{-2} × s^{-1} it was lower in CsCl-treated than in KCl-treated plants.

After 5 days of cesium treatment, intensity of photosynthesis was lower than in seedlings exposed to cesium for 3 days. The effect was noted even at low PAR level of 220 µmol × m^{-2} × s^{-1} (Tab. 2). Intensity of photosynthesis was more than 40% lower in CsCl-exposed than in KCl-exposed seedlings. Photosynthetic water utilization efficiency (P/E) was comparable in the two treatments.

Chlorophyll *a* fluorescence measurements did not reveal any significant differences in the F_v/F_m ratio between the cesium- and potassium-treated seedlings (Tab. 3). There were no great differences in the $t_{1/2}$ parameter after 3 days of treatment, but $t_{1/2}$ was lower in plants exposed to CsCl after 5 days of treatment.

DISCUSSION

Normally, transfer factor values for cesium range from 0.01 to 1 (Menzel, 1965). The observed TF in cress, 2.97 (kBq/kg plant DW)/(kBq/kg soil DW) (Tab. 1), seems very high compared to other plant species. In Poaceae it is only 0.20 (Papastefanou et al., 1999) and in other plants grown in tropical climates it ranges from 0.18 to 0.79 (Mollah et al., 1998). Under conditions similar to those in the present study, onion *Allium cepa* had a TF for cesium of 2.87 (unpublished data). In most cases, such hyperaccumulating plants may be considered for possible application in phytoremediation (Dushenkov, 2003). In our study of cress seedlings, irrespective of the manner of application (roots and leaves), cesium was accumulated mostly in the leaves (Figs. 1, 2). This corresponds to the results obtained for other plants (Fogh and Andersson, 2001; Ban-Nai and Muramatsu, 2002).

Many papers point to the similarity of the potassium and cesium transport pathways in plants (e.g., Zhu et al., 1999; White and Broadley, 2000; Urban and Bystrzejewska-Piotrowska, 2002), but the role of cesium in mineral nutrition is not known (Marschner, 1995). Cesium (3–4 mM solutions) seems to disturb water uptake (Fig. 5) and tissue hydration (FW/DW ratio; Fig. 4). Thus even low cesium concentrations are toxic, in contrast to sodium, for which only high (150 mM) concentrations disturb the water balance (Amzallag, 2001; Ghoulam et al., 2002; Bystrzejewska-Piotrowska and Urban, unpublished data). Cesium concentrations of 2–4 mM also evoked osmotic stress in *Arabidopsis* (Zhu et al., 2002). In cress, accumulation of biomass

TABLE 2. Comparison of gas exchange parameters in cress seedlings grown on Mayer solution containing additional cesium or potassium. C – stomatal conductance; E – transpiration; P – photosynthesis; ΔP – photostimulation of photosynthesis; P/E – efficiency of photosynthetic water utilization (Means ± SD for 20 values)

		Gas exchange parameters				
		C [mmol × m^{-2} × s^{-1}]	E [mmol × m^{-2} × s^{-1}]	P [mol × m^{-2} × s^{-1}]	ΔP [%]	P/E
3 days, PAR 220	K	153.0 ± 7.0	2.8 ± 0.1	6.3 ± 2.3		2.3 ± 0.8
	Cs	122.3 ± 4.4	2.4 ± 0.1	6.1 ± 2.6		2.5 ± 1.1
3 days, PAR 450	K	251.7 ± 17.4	4.7 ± 0.3	10.0 ± 2.9	58.7 ± 27.4	2.1 ± 0.6
	Cs	169.3 ± 6.7	3.7 ± 0.1	6.2 ± 2.6	4.0 ± 4.1	1.7 ± 0.7
5 days, PAR 220	K	268.0 ± 50.4	3.8 ± 0.5	6.2 ± 1.8		2.1 ± 0.5
	Cs	109.2 ± 2.8	2.1 ± 0.0	3.6 ± 2.0		1.7 ± 0.9
5 days, PAR 450	K	228.2 ± 23.0	4.3 ± 0.4	10.4 ± 1.7	69.0 ± 22.7	2.4 ± 0.4
	Cs	89.7 ± 6.3	2.3 ± 0.1	3.7 ± 3.0	3.7 ± 3.6	1.6 ± 1.3

TABLE 3. Changes in chlorophyll *a* fluorescence in cress seedlings after application of 3 mM cesium or potassium (Means ± SD for 20 values)

	F_v/F_m	t1/2 [ms]
K, 3 days	0.77 ± 0.03	72.6 ± 6.2
Cs, 3 days	0.77 ± 0.03	73.2 ± 8.7
K, 5 days	0.77 ± 0.02	78.5 ± 13.2
Cs, 5 days	0.73 ± 0.04	66.4 ± 11.9

was also disturbed by 4 mM Cs, since DW decreased (Fig. 3). However, higher Cs concentrations (6–10 mM) did not further decrease biomass production. In bean even 0.2 mM cesium caused a decrease in dry weight (Cline and Hungate, 1960).

Cesium accumulation in the leaves may have disturbed their basic physiological functions. The first observed reaction in the cesium-treated plants was decreased stomatal opening. The control function of stomata in respect to photosynthetic CO_2 assimilation and transpiration was modified by the presence of cesium. Decreased stomatal opening limited transpiration and the uptake of water by roots (Fig. 5), but photosynthetic CO_2 assimilation did not change during short-term exposure to CsCl (Tab. 2). Stomatal closure in the presence of cesium may be a result of a decrease in osmotic potential (due to Cs accumulation in vacuoles), the role of calcium (Chen et al., 2003), potassium and ABA (Zhu et al., 2002) in the regulation of stomatal opening, or disturbance of the signal transduction pathway. Possibly Cs inhibits the channels responsible for K transport into guard cells, which might lead to impaired stomatal opening. It is known that there is no dependence between photosynthetic assimilation of CO_2 and the degree of stomatal opening (Bystrzejewska et al., 1971). After 3 days of treatment, cesium did not affect photosynthetic CO_2 assimilation rate, but after 5 days it did.

Photosynthetic water utilization efficiency is the result of a compromise between maximization of photosynthesis and minimization of transpiration, as described by Lambers et al. (1998). In our study the reduction of photosynthesis by CsCl was greater than transpiration when the seedlings were grown for a longer period (5 days of treatment) and exposed to light of higher intensity (Tab. 2). Due to this, photosynthetic water utilization efficiency went down, limiting the growth process. The mechanism of photosynthetic efficiency as a response to water stress, called "down-regulation," is not fully understood.

No differences in the F_v/F_m ratio, indicating the efficiency of the PSII photosystem, were observed between cesium- and potassium-treated plants after 3 days (Tab. 3). Only after 5 days did 3 mM cesium treatment cause a small decrease in F_v/F_m and in the half-life of chlorophyll *a* fluorescence, which could inhibit CO_2 assimilation. In cress seedlings, chlorophyll fluorescence and photosynthetic CO_2 assimilation are relatively insensitive to Cs treatment, and therefore, non-indicative for primary stress effects.

The results suggest a scheme for the effects of cesium on physiological processes in cress seedlings, with both primary and secondary consequences. Apparently, decreased stomatal opening is the primary cause of cesium stress effects in leaves. It is accompanied by reduced transpiration and water uptake. A consequence of decreased tissue hydration might be lowered photosynthetic CO_2 reduction efficiency,

but also restricted production of assimilates important to dry mass accumulation, and thus reduced growth. Although photosynthesis and transpiration were affected by cesium, photosynthetic water utilization efficiency was rather stable. The lowering of chlorophyll fluorescence seems to be a secondary effect of disturbed biosynthesis. It might further limit CO_2 assimilation and carbon reduction processes of photosynthesis.

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