

## CHARACTERIZATION OF CADMIUM UPTAKE BY ROOTS OF DURUM WHEAT PLANTS ХАРАКТЕРИСТИКА НА ПОГЛЪЩАНЕТО НА КАДМИЙ ОТ КОРЕНИТЕ НА РАСТЕНИЯ ОТ ТЪВРДА ПШЕНИЦА

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### ABSTRACT

Root Cd uptake of durum wheat plants (cv. Beloslava) was characterized in hydroponics conditions. The uptake experiments have been performed in Cd concentration range of 0 – 2  $\mu\text{M}$  adjusted by both stable Cd and radiolabeled ( $^{109}\text{Cd}$ ) tracer. Cd removal from the solution over duration of 1 hour reached 50%. The part of loosely adsorbed Cd ions on root surface accounted for about 20%. Over 30% of absorbed Cd at 0.5  $\mu\text{M}$  Cd treatment was retained in root cell walls. The apparent root Cd accumulation showed concentration-dependant tendency with the highest accumulation value of 7.45 nmol Cd g FW<sup>-1</sup>.

KEY WORDS: cadmium,  $^{109}\text{Cd}$ , durum wheat, uptake

### РЕЗЮМЕ

Поглъщането на Cd от корените на растения от твърда пшеница (сорт Белослава) е характеризирано в условия на хидропонни опити. Опитите са проведени в концентрационен интервал 0 – 2  $\mu\text{M}$  Cd, създаден чрез стабилен Cd и радиоактивен маркер ( $^{109}\text{Cd}$ ). Извличането на Cd от разтвора за период от 1 час достига 50%. Частта на слабо свързаните (адсорбирани) Cd йони с кореновата повърхност представлява около 20%. Клетъчните стени на корените при варианта с 0.5  $\mu\text{M}$  Cd задържат над 30% от акумулирания Cd. Действителната Cd акумулация в корените показва концентрационно-зависима тенденция като най-високата стойност е 7.45 nmol Cd g FW<sup>-1</sup>.

КЛЮЧОВИ ДУМИ: кадмий,  $^{109}\text{Cd}$ , твърда пшеница, поглъщане

**INTRODUCTION**

Parts of the agricultural soils all over the world are slightly to moderately contaminated by Cd due to large-scale use of super phosphate fertilizers, sewage sludge application as well as atmospheric deposition of smelters dust. In Bulgaria metal contaminated area stretch over nearly 19 500 ha of arable land and a part of these soils has elevated Cd levels. Due to high Cd mobility in the soil-plant system it can easily enter into food chain and can create risk for human and environmental health [2]. Therefore, significant research attention addresses the mechanisms of Cd uptake, translocation and grain accumulation, especially in crops having ability to accumulate higher Cd, such as durum wheat, sunflower and some others [3].

In fact, plant Cd uptake has been studied for more than 30 years and the basic characteristics have been determined [12]. Nevertheless, the increasing international concern about the risks associated with long-term consumption of Cd-containing food stimulates both applied and fundamental research on plant-Cd interactions. As a result a huge germplasm has been screened for low grain Cd accumulation. Significant cultivar variation in many crops has been observed [7; 8; 9] as well as new breeding programs for low grain Cd consisting genotypes initiated [10]. At the same time the information about cultivar differences in grain Cd accumulation within the most important crops is very scant.

Model studies revealed that grain Cd accumulation in durum wheat cultivars correlated with root-to-shoot Cd transfer [4; 6]. Lower Cd translocation to leaves diminishes grain Cd accumulation directly during grain filling or indirectly maintaining lower Cd pools in the leaves that could be remobilized. According to Stolt et al. [13] low grain Cd accumulating wheat genotypes have low shoot Cd content, which is detectable even in the early vegetative stages. A seedling-based bioassay based on root / shoot <sup>109</sup>Cd accumulation ratio has been suggested as a rapid and cost-effective way of screening large numbers of seedlings for low Cd-accumulating phenotype [1]. Nevertheless, more fundamental research is needed to characterize in detail both Cd uptake and translocation pattern in low Cd accumulating genotypes. The problem for grain Cd accumulation in cereals is not well addressed in Bulgaria. Therefore, we initiated studies on Cd uptake, translocation and grain accumulation within Bulgarian durum wheat cultivars. Here we report the first results obtained from a hydroponics study aimed to characterize the unidirectional influx of radiotracer-labeled cadmium (<sup>109</sup>Cd) by roots of durum wheat plants.

**MATERIAL AND METHODS**

The experiments were conducted with a durum wheat cultivar Beloslava. The seeds were surface sterilized

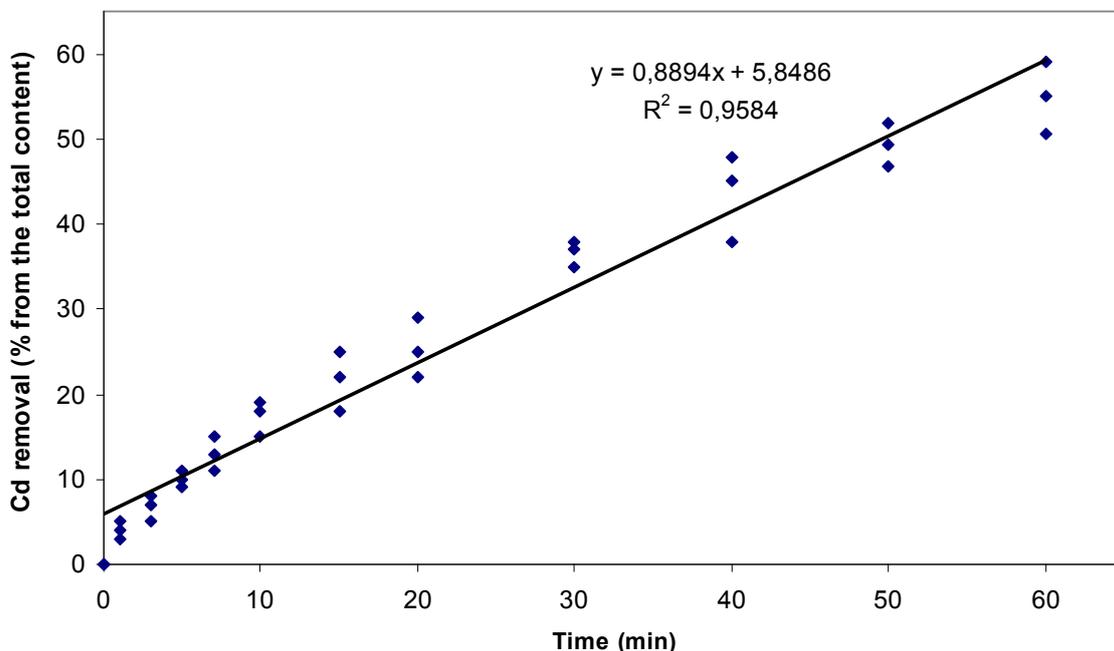


Figure 1. Time-course Cd removal from uptake solution by wheat plants  
 Фигура 1. Извличане на Cd от хранителния разтвор от пшеничени растения

in  $H_2O_2$ , rinsed, and germinated in dark on moistened paper in Petri dishes.

Three-day-old seedlings were covered by 2-cm elastic band above root-shoot junction and positioned in holes of pots filled by  $\frac{1}{2}$  strength modified Hoagland solution. Plants were grown for 15 days in a control environment with a 14 h / 24 °C day and 10 h / 20 °C night, photosynthetically active radiation of  $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and relative air humidity of 55 - 60%. The solution was regularly aerated and changed every third day.

Before Cd uptake experiments plants were removed from the nutrient solution, transferred to plastic tubes (5 plants per tube) filled by pretreatment solution (0.2 mM  $\text{CaSO}_4$  and  $12.5 \mu\text{M H}_3\text{BO}_3$  at pH 5.5) and left for several hours for adaptation. Boron ions were included in the pretreatment solution [5] to better maintain the integrity of selective ion transport. Then, the pretreatment solution was replaced with Cd uptake solution containing 0.2 mM  $\text{CaSO}_4$ ,  $12.5 \mu\text{M H}_3\text{BO}_3$  and various Cd concentrations (0.5, 1.0, 1.5 and 2.0  $\mu\text{M}$ ) given as  $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ . An aliquot of  $^{109}\text{Cd}$ -labelled  $\text{CdCl}_2$  solution (specific activity  $106 \text{ MBq mg}^{-1} \text{ Cd}$ ) was added to Cd treatments ranging within 150 - 600  $\text{Bq ml}^{-1}$ . Plants were maintained on the uptake solution for up to 1 hour.

After the uptake period plants were removed from the solution and transferred to cool ( $5^\circ\text{C}$ ) desorption solution containing 5 mM  $\text{CaSO}_4$ , 12.5 M  $\text{H}_3\text{BO}_3$  and 100  $\mu\text{M } 3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$  at pH 5.5 for different time exposure. This procedure allowed removing loosely adsorbed Cd

ions on root surface that could overestimate total root Cd accumulation [5].

Cd binding to root cell walls was estimated after treating roots with methanol:chloroform mixture (2:1, v/v). Plants from each treatment were divided in 2 groups and one part was immersed in the mixture for 3 days to remove cellular content, followed by a deionized water rinse for several hours. Then, the roots were blotted dry with tissue paper. Both roots and shoots were excised (about 1 cm above and below the root-shoot junction), cut into pieces, weighted and measured by  $\gamma$  spectrometry [Canberra 85 with a Ge (Li) detector].

Statistical analysis was performed using one way ANOVA (for  $P < 0.05$ ). Based on the ANOVA results, a Tukey test for mean comparison was performed, for a 95% confidence level, to test for significant differences among treatments. In the table, different letters (a, b, c, d) express significant differences, with a representing the highest value. A regression analysis was applied to Cd removal data.

## RESULTS AND DISCUSSION

The experimental design used in our study was set up with relatively low external Cd concentrations. Selected Cd concentrations were in the range of 0 to 2.0  $\mu\text{M}$ , which are low enough to prevent Cd phytotoxicity problems and, on the other hand, to be environmentally relevant. The apparent root Cd uptake by wheat plants

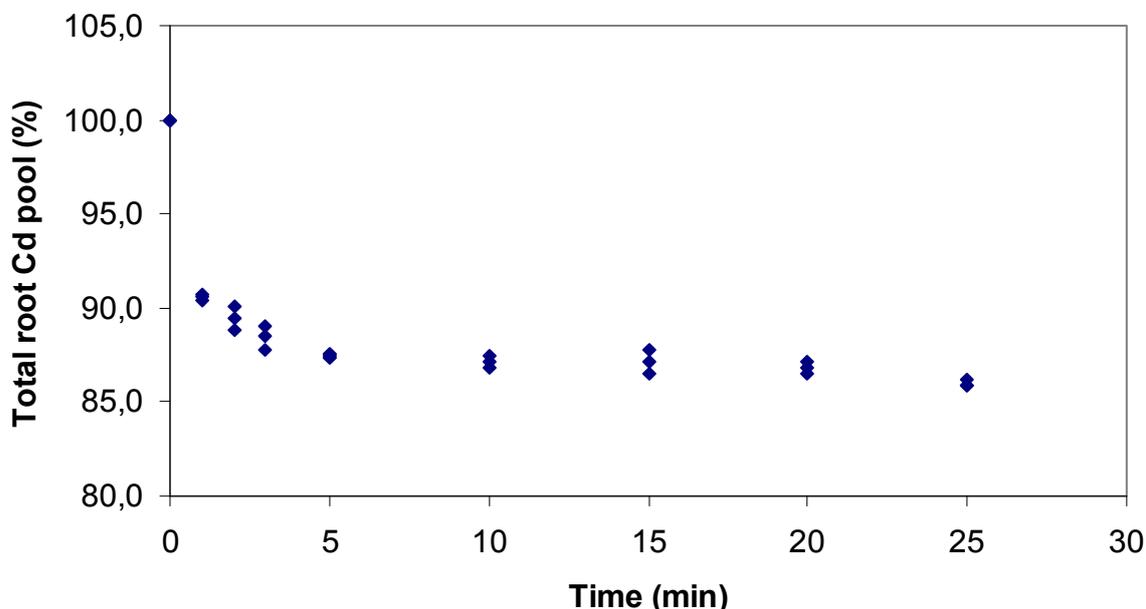


Figure 2. Time-course Cd desorption from roots of wheat plants

Фигура 2. Отделяне на слабо свързания Cd от корените на пшеничени растения

**Table 1.** Root Cd concentrations of wheat plants and Cd removal from the uptake solution  
**Таблица 1.** Съдържание на Cd в корените на пшеничени растения и процент на извличане на Cd от хранителния разтвор

Treatment	Root Cd concentrations (nmol Cd g <sup>-1</sup> FW)	Cd removal (% from the total content in solution)
Control (0)	not detectable	-
0.5 μM Cd	2.23 ± 0.14 <sup>a</sup>	26.5 ± 1.4 <sup>a</sup>
1.0 μM Cd	5.27 ± 0.29 <sup>b</sup>	29.1 ± 4.0 <sup>b</sup>
1.5 μM Cd	7.25 ± 0.09 <sup>c</sup>	24.7 ± 0.8 <sup>c</sup>
2.0 μM Cd	7.45 ± 0.21 <sup>d</sup>	20.3 ± 0.6 <sup>d</sup>

In the columns, data followed by different letters (*a, b, c, d*) express significant differences at P=0.05.

Представените в колоните данни следвани от различни букви (*a, b, c, d*) представят достоверните разлики при P=0.05.

might be determined after taking into account of several issues, namely: (1) rate of Cd removal from the solution; relative parts of both (2) loosely adsorbed Cd ions and (3) Cd ions retaining in cell wall.

Cd removal from the solution containing 2 μM Cd over duration of 1 hour followed strong linear trend (Figure 1; R<sup>2</sup> = 0.96), which did not suppose appearing of significant Cd efflux. At the end of the exposure period Cd removal from the uptake solution reached about 50%. Total Cd removal was associated only with roots as we did not detect any shoot Cd accumulation. Based on a significant Cd removal from the uptake solution, in the following concentration-dependant-uptake experiments we averaged Cd concentrations over the course of exposure to account for Cd depletion.

Root Cd pool contains both absorbed and loosely adsorbed Cd ions. The results presented in Figure 2 showed that the used desorption procedure was effective at rapidly removing <sup>109</sup>Cd from the root surface. In fact, most of Cd was removed during the first two minutes, but some Cd was released thereafter reaching about 15% of the total Cd pool after 20 minutes (100% root Cd concentration represent 5.58 nmol g FW<sup>-1</sup>). The results obtained showed that over 80% of Cd was bound to reactive sites within the apoplast. Of course, some has entered into the cytoplasm of root cells. The results obtained corresponded to the desorption kinetics of Cd from the roots of other species, such as maize [11] and wheat [5].

To maintain Cd homeostasis, the root cells should store Cd ions in less active compartments, such as cell wall and vacuole. According to Hart et al. [5] the root treatment by methanol-chloroform mixture removed cellular content and yielded morphologically intact root cell wall preparations. Comparing Cd content of both non-treated roots and methanol-chloroform treated roots at 0.5 μM Cd treatment we found that over 30% of Cd accumulated in roots was bound in cell walls.

Root Cd concentrations increased with increasing external Cd concentrations showing concentration-dependant tendency (Table 2). The apparent root Cd accumulation for 1 hour duration exposure reached 7.45 nmol Cd g FW<sup>-1</sup> at the highest Cd treatment (2 μM). These results are in a good correspondence with other studies on Cd uptake by durum wheat [5].

In conclusion, the results obtained in this study showed that durum wheat plants removed Cd from the uptake solution containing up to 2 μM Cd very effective; loosely adsorbed Cd ions on root surface accounted for about 20% and over 30% of absorbed Cd was retained in root cell walls at 0.5 μM Cd treatment.

#### ACKNOWLEDGEMENT

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