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RESEARCH PAPERS

Cadmium Tolerance and Accumulation in Excluder *Thlaspi arvense* and Various Accessions of Hyperaccumulator *Noccaea caerulescens*

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Abstract—Cadmium (Cd) accumulation and tolerance were analyzed in hyperaccumulator *Noccaea caerulescens* F.K. Mey and excluder *Thlaspi arvense* L.. Five accessions of *N. caerulescens* (La Calamine (LC, Belgium), Saint Félix de Palliéres (SF, France), Col du Mas de l'Aire (CMA, France), Ganges (GA, France) from metalliferous soils and Lellingen (LE, Luxembourg) from nonmetalliferous soils) were grown in half-strength Hoagland solution for 8 weeks in the presence of 1, 5, 25, and 50 μM $Cd(NO_3)_2$ and *T. arvense* in the presence of 0.1, 0.2, 1.0, and 5.0 μM $Cd(NO_3)_2$. The toxic effect of Cd was assessed by changes in root and shoot dry weight. The content of Cd in roots and shoots was determined by atomic absorption spectrophotometry and expressed in mg/kg dry weight of plant material and calculated per plant root system or shoot. The tolerance of *N. caerulescens* to Cd was higher than that of *T. arvense* and increased in various accessions of *N. caerulescens* in the row GA < CMA < LE < SF ≈ LC. The ability to accumulate Cd in roots of *N. caerulescens* accessions increased in the row LC < LE ≈ GA < CMA ≈ SF, while that in shoots were in the row LC < LE ≈ GA < SF < CMA. Reduction in accumulation of root biomass of hyperaccumulator *N. caerulescens* started at lower Cd content in them compared with that in shoots, while an opposite pattern was observed for excluder *T. arvense*. Thus, accessions of hyperaccumulator *N. caerulescens*, having a higher tolerance to Cd compared with excluder *T. arvense*, differed significantly from each other not only in their capacity to accumulate heavy metals but also in tolerance to them. LC accession from calamine soils accumulated less Cd and, possibly, this was the reason why it was more tolerant than the other accessions. SF accession, also growing on calamine soils, was characterized both by high Cd tolerance and accumulation, which is probably due to more efficient mechanisms of Cd detoxification. The results obtained suggest that there are differences in the mechanisms and causes of tolerance to Cd in various accessions of hyperaccumulator *N. caerulescens*.

Keywords: Noccaea caerulescens, Thlaspi arvense, cadmium, growth, tolerance
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INTRODUCTION

Environmental pollution by heavy metals (HM) as a result of human activities is a serious worldwide problem. Cadmium (Cd) is among the most toxic elements exerting multiple toxic effects on plants [1, 2]. At the same time, the necessity of Cd was shown for the marine diatom *Thalassiosira weissflogii*, in which Cd-dependent carbonic anhydrase isoform was discovered [3]. However, the biological role of Cd in higher plants has not yet been reliably established.

Plants are able to accumulate HM in large quantities in various tissues and cell compartments [4]. The ability to accumulate HM and tolerance to their excess in plant tissues can vary for different species [5]. There are two contrasting groups of plants: excluders, accumulating metals mostly in root systems, and accumulators, accumulating metals mainly in the aboveground organs [6, 7]. More than 450 different species of HM hyperaccumulators have been found among the latter group so far; only nine of them are hyperaccumulators of Cd, accumulating more than 100 mg Cd/kg dry wt [8, 9].

Plants-hyperaccumulators combine high tolerance to one or more metals and the ability to accumulate them in shoots. The content of metals in shoots of these species often exceeds manyfold the content of metals in the root system of excluders and in the environment [10, 11]. It is believed that the genes that determine the ability of HM hyperaccumulation and tolerance are not species-specific, but they are rather differently expressed in hyperaccumulators and excluders [5, 8]. It is known that high Cd tolerance and accumulation are

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Abbreviations: HM—heavy metals; accessions of hyperaccumulator *Noccaea caerulescens*: CMA—Col du Mas de l'Aire; GA—Ganges; LC—La Calamine; LE—Lellingen; MP—Monte Prinzera; PR—Prayon; SF—Saint Félix de Palliéres.

inherited independently, being the result of recent microevolutionary processes [8, 12, 13].

Not only different species of hyperaccumulators, but different accessions of the same species can also vary considerably in their capacity for HM accumulation and tolerance [8, 14, 15]. Taking into account that accessions belong to the same species, it is important to understand the mechanisms that determine various extent of their tolerance and capacity to accumulate HM. Much attention is paid to the comparison of accessions of hyperaccumulator *Noccaea caerulescens* (former *Thlaspi caerulescens*), which is represented by isolated populations growing on soils enriched in HM and capable to accumulate Ni, Zn, and Cd [8, 11]. It was shown that the accession of *N. caerulescens* originating from nonmetalliferous soils (LE) accumulated more Zn but was less tolerant to it compared with SF and LC accessions, growing naturally on calamine soils rich in Zn, Cd, and Pb. At the same time, MP accession originating from serpentine soils rich in Ni, Co, and Cr, exhibited not only significantly greater tolerance to Ni compared with other accessions studied but also the highest accumulation of Ni in its tissues [15].

GA accession, which was found in the South of France, can accumulate up to 3600 mg Cd/kg dry wt under field conditions [16] and 10000 mg Cd/kg dry wt under hydroponic conditions [17, 18]. Cadmium is required for optimum plant growth of this accession. That, along with the increased activity of carbonic anhydrase in the presence of Cd (up to 6000 mg Cd/kg dry wt), allows us to suggest a possible physiological role of Cd for the representatives of this accession [19, 20]. Due to the high Cd content in the aboveground organs, GA accession was less attractive to thrips *Frankliniella occidentalis* compared with PR accession with significantly lower Cd content [21].

Studying the differences between the populations of hyperaccumulators is of interest not only from the evolutionary point of view and from geobotanical aspects but it is also important for the assessment of the mechanisms of metal transport and plant tolerance to metal excess at the physiological and molecular levels. In addition, it is promising to compare HM tolerance and capacity to accumulate HM in different species of hyperaccumulators and excluders, which can vary significantly according to these parameters, not only among themselves but also within these plant groups. Therefore, the main aim of this work was to perform a comparative analysis of tolerance and capacity to accumulate Cd by different accessions of hyperaccumulator *N. caerulescens* and excluder *Thlaspi arvense*. These findings will be related to the data on Ni and Zn obtained previously under the same growth conditions [15].

MATERIALS AND METHODS

Plant Growing and Evaluation of the Toxic Effect of Cadmium

Seeds of five accessions of hyperaccumulator *Noccaea caerulescens* F.K. Mey (former *Thlaspi caerulescens* J. & C. Presl), La Calamine (LC, Belgium), Saint Félix de Palliéres (SF, France), Col du Mas de l'Aire (CMA, France), Ganges (GA, France) from metalliferous soils, and Lellingen (LE, Luxembourg) from nonmetalliferous soils, as well as the seeds of excluder *Thlaspi arvense* L., were germinated in Petri dishes on filter paper moistened with tap water for two weeks at 20°C in a dark thermostat. Seedlings at the stage of cotyledon emergence were transplanted into 1-L pots (four seedlings per pot) with half-strength Hoagland solution and grown in a climate chamber (23°/18°C day/night temperature, 14-h photoperiod, 200 μM/(m^2 s) photosynthetically active radiation, relative humidity 70%).

The Hoagland medium contained KNO_3 (3 mM); $Ca(NO_3)_2$ (2 mM); NH_4HPO_4 (1 mM); $MgSO_4$ (0.5 mM); KCl (1 μM); H_3BO_3 (25 μM); $ZnSO_4$ (2 μM); $MnSO_4$ (2 μM); $CuSO_4$ (0.1 μM); $(NH_4)_6Mo_7O_{24}$ (0.1 μM); Fe(Na)EDTA (20 μM). The medium was adjusted to pH 5.25 using Mes (2 mM)/KOH [22]. During the first week, plants were grown in the absence of exogenously added Cd, which allowed them to adapt to the water culture conditions. During the next eight weeks, the test plants of *N. caerulescens* were grown in the presence of 1, 5, 25, and 50 μM $Cd(NO_3)_2$, and *T. arvense* was grown in the presence of 0.1, 0.2, 1.0, and 5.0 μM $Cd(NO_3)_2$. *T. arvense* plants died at 25 μM $Cd(NO_3)_2$. Thus, this concentration was not used for this species in the subsequent experiments. Control plants were grown in half-strength Hoagland medium in the absence of Cd. The nutrient medium was replaced weekly. All plants developed a rosette of leaves by the end of the experiment. The toxic effect of Cd was assessed by changes in dry weight of roots and shoots.

Determination of Cadmium Content

The content of Cd was determined in roots and shoots. Prior to analysis, the roots were washed sequentially with 20 mM EDTA for 10 min at room temperature and then with distilled water. Plant material was collected after 8 weeks of incubation, dried in an oven to a constant weight at 80°C for 24 hours, and then weighed. Samples dried to a constant weight were subjected to wet-ashing at 140°C for 7 hours in a teflon bombs in the mixture of 65% HNO_3 and 37% HCl added to the samples in a ratio of 4 : 1 (v/v). Quantitative analysis was performed by the standard method using a Perkin Elmer 1100V atomic absorption spectrophotometer (Perkin Elmer, Netherlands).

The analysis was conducted in three independent analytical replicates. Each plant was analyzed individ-

ually. That allowed us to calculate not only the Cd content in mg/kg of dry wt but also Cd content per root system or shoot. Based on these data, the ratio of Cd content in shoots to the Cd content in roots was calculated in mg/kg of dry wt, as well as per root system or shoot.

Statistical Analysis of the Data

The experiments were performed in four independent replicates. Each replicate consisted of four plants per variant. Quantitative data were processed using one-way and two-way ANOVA. Data are presented as mean values and their standard errors. In assessing correlation between the content of the metal in plant organs and its growth inhibiting effect, we calculated the mean values, their standard errors, and coefficients of correlation.

RESULTS AND DISCUSSION

All studied accessions of *N. caerulescens* continued to grow even at the highest studied concentrations of Cd in solution (50 μM). Reduction in root dry weight was observed only for LE accession at 50 μM $Cd(NO_3)_2$ and GA accession at 25 and 50 μ $Cd(NO_3)_2$. Shoot growth inhibition was observed for LE accession at 50 mM $Cd(NO_3)_2$ and for CMA and GA accessions at 25 and 50 μM $Cd(NO_3)_2$. LC and SF accessions appeared to be the most tolerant to Cd: no root or shoot growth inhibition was recorded at any Cd concentrations. Thus, the tolerance of *N. caerulescens* accessions to Cd under these growth conditions increases in the row GA < CMA < LE < SF ≈ LC. Shoot growth of excluder *T. arvense* was inhibited at lower Cd concentration in the solution (5 μM) (Fig. 1) than in *N. caerulescens*.

The tolerance of populations of angiosperms to HM under natural conditions and the ability of plants to accumulate these metals depend not only on the biological characteristics of the species but also on the source of contamination, as well as on the concentration of bioavailable forms of metal in the soil, which, in turn, is determined by many factors and, primarily, by soil acidity [1]. Therefore, it is quite difficult to compare results obtained by the authors due to the differences in plant growth conditions and exposure time.

In our previous work, we compared the tolerance of *T. arvense* and different accessions of *N. caerulescens* to Ni and Zn under the same conditions [15] that allows us to compare the results. Overall tolerance of hyperaccumulator *N. caerulescens* to all studied metals was significantly higher than that for excluder *T. arvense*, and Cd tolerance was much lower for both species than Ni or Zn tolerance. The tolerance of different accessions of *N. caerulescens* to Ni increased in the row LE < LC < SF < MP, while that for Zn increased in the row LE < SF < LC, which confirms similar patterns for all three metals. However, it should be noted that the order may be different for other growth conditions. For example, it is known that the ability of GA accession to uptake Cd increased significantly with iron deficiency, which was not observed for PR accession [23].

The content of Cd (mg/kg dry wt) in the roots and shoots was similar in many treatments and elevated with increasing Cd concentration in solution. The content of Cd in roots exceeded considerably its content in shoots of *T. arvense* at all studied concentrations and in shoots of SF, LE, LC, and Ga accessions of *N. caerulescens* only at 1–25, 25–50, 1, and 5 μM $Cd(NO_3)_2$, respectively (Figs. 2, 3). The shoot-to-root ratio of Cd content increased in LC and GA accessions along with metal concentration in the solution, practically did not change in SF and CMA accessions, and decreased in LE accession, as well as in *T. arvense* (Fig. 3).

Identified patterns of Cd accumulation were significantly different from those of Ni and Zn. All studied accessions of *N. caerulescens* accumulated Ni and Zn preferably in shoots, whereas *T. arvense* did it mainly in roots [15]. These differences could be partly explained by limited Cd translocation into the central cylinder through the endodermal barrier in mature parts of the root, while Ni and Zn passed freely through the endodermis, entered the xylem, and then the aboveground organs [1, 4, 24].

Various accessions of *N. caerulescens* differed in their ability to accumulate Cd, which depended on the concentration of metal in the environment. For example, the content of Cd in roots and shoots increased in the row LC < SF < CMA ≈ GA < LE at a concentration of 1 μM $Cd(NO_3)_2$, and increased in the row LC < LE ≈ GA < CMA ≈ SF at a concentration of 25 μM $Cd(NO_3)_2$ (Fig. 2). The lowest content of Ni and Zn was also observed for LC accession from calamine soils while the highest content of Ni was found in MP accession from serpentine soils, while that of Zn was detected in LE accession from nonmetalliferous soils [15].

The content of Cd in roots of LC and SF accessions at 1 μM $Cd(NO_3)_2$ and in all accessions of *N. caerulescens*, except for GA accession, was lower than that for the excluder *T. arvense* at 5 μM $Cd(NO_3)_2$ in solution. The content of Cd in shoots of *T. arvense* was lower than that of *N. caerulescens* at 1 μM $Cd(NO_3)_2$, except for LC and SF accessions. The content of Cd in shoots of *T. arvense* at 5 μM $Cd(NO_3)_2$ was lower than that for GA and LE accessions, did not differ significantly from that in CMA accession, and was even higher than in LC and SF accessions. This indicates that its content in the shoots of excluders may be even higher than that in hyperaccumulators at low concentrations of Cd in the medium (Fig. 2). Quantitative analysis of Cd content in an individual plant with known biomass allowed us to calculate the content of the metal per root system or shoot. The results shown in Fig. 4 led to the conclusion that the content of Cd per root system or shoot did not significantly change in LE and GA accessions of *N. caerulescens* with increasing Cd con-

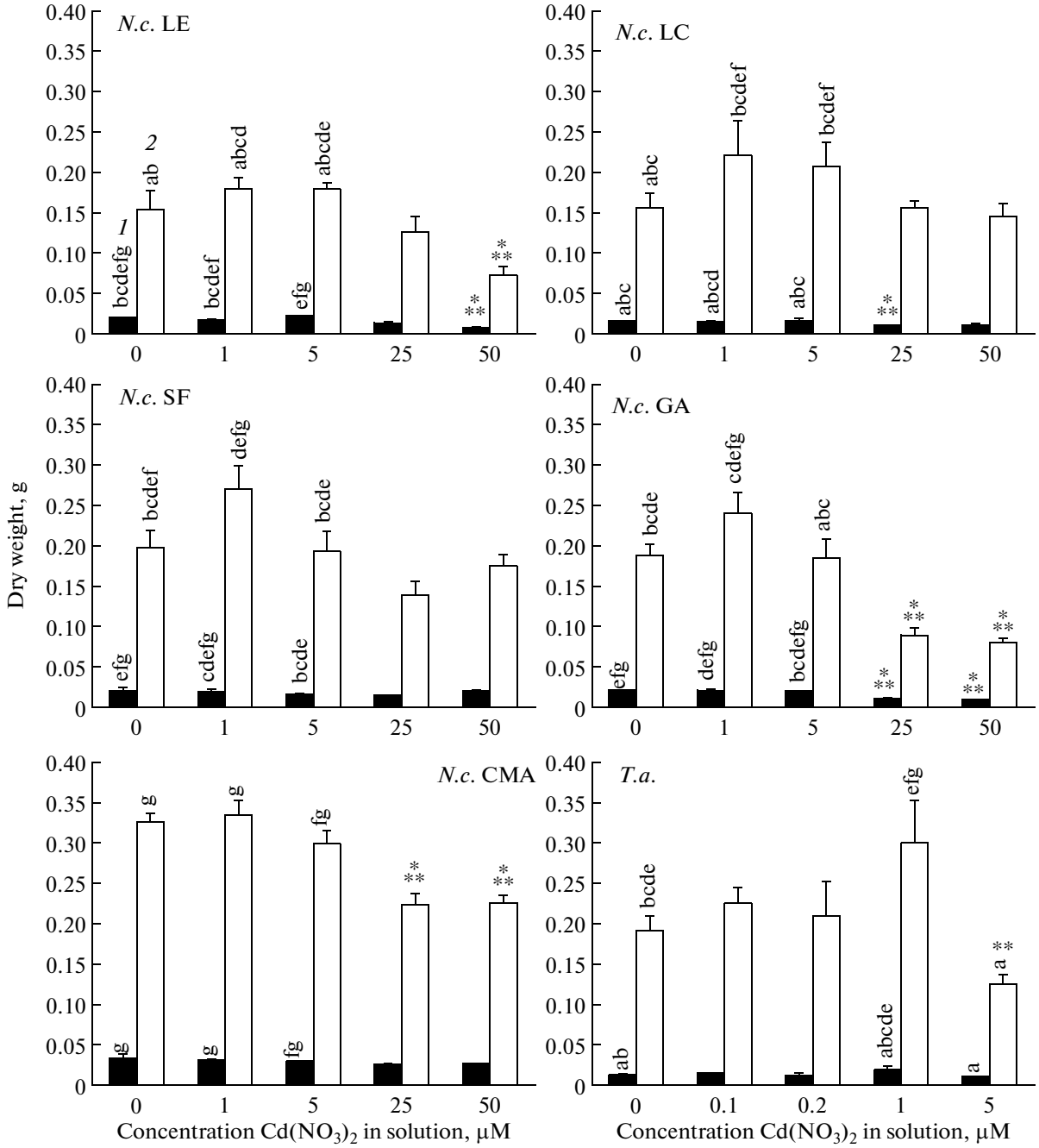

Fig. 1. Effect of Cd on accumulation of root and shoot dry weight in excluder *Thlaspi arvense (T.a.)* and in five accessions of hyperaccumulator *Noccaea caerulescens (N.c.)* (LE, LC, SF, GA, and CMA). (*1*) roots and (*2*) shoots. Mean values and their standard errors are shown for four independent replicates (four plants per replicate). Two-way ANOVA was performed in order to compare root or shoot dry weight in *T. arvense* and in various accessions of *N. caerulescens* at concentrations of 0–5 μM $Cd(NO_3)_2$. Significantly differing values ($p < 0.001$) are marked with different letters. One-way ANOVA was performed in order to clarify the significant reduction in biomass of Cd-treated plants compared with control ones. Statistically significant differences are shown with asterisks: $^{**}p < 0.01$ for *T. arvense*, $^{***}p < 0.001$ for LE, GA, LC, and CMA. Statistical analysis was performed separately for roots and shoots.

centrations in solution, whereas it rose substantially in LC, SF, and CMA accessions. The capacity of *N. caerulescens* accessions to accumulate Cd increased in roots in the following order LC < LE ≈ GA < CMA ≈ SF and in shoots in a row LC < GA ≈ LE < SF < CMA, which is broadly consistent with the data obtained by the analysis of metal content calculated per mg/kg of dry wt (Fig. 2).

Analyzing the relationship between the Cd accumulation and decrease in root and shoot biomass, we can find out the following pattern: the reduction in

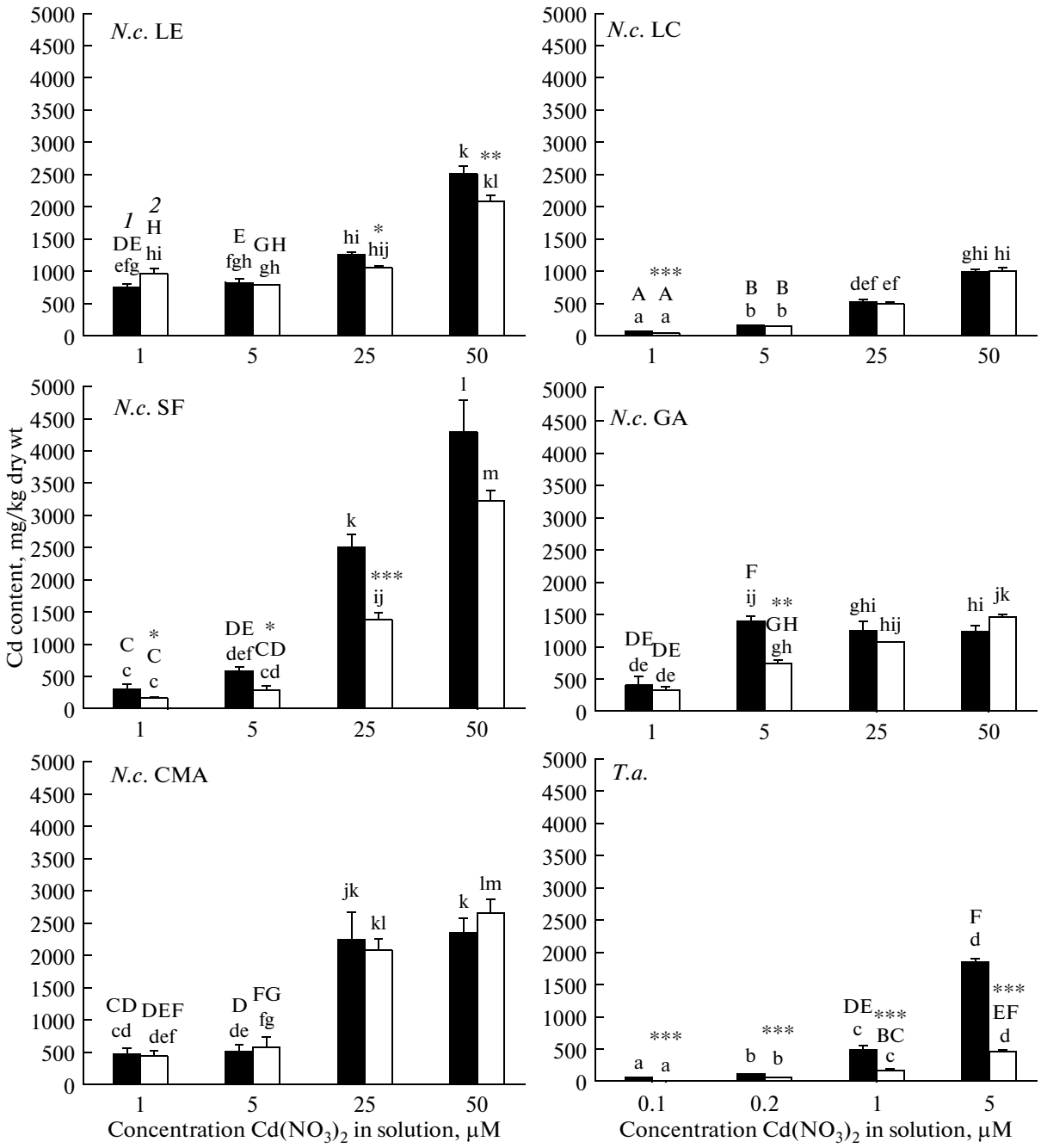

Fig. 2. Cd content in roots and shoots of excluder *Thlaspi arvense (T.a.)* and five accessions of hyperaccumulator *Noccaea caerulescens (N.c.)* (LE, LC, SF, GA, and CMA). (*1*) roots and (*2*) shoots. Mean values and their standard errors are shown for four independent replicates (four plants per replicate). Analytical repetition was triple. The content of Cd in control plants did not exceed 2 mg/kg dry wt. Two-way ANOVA was performed in order to compare the content of Cd in accessions of *N. caerulescens* at concentrations of 1–50 μM $Cd(NO_3)_2$. One-way ANOVA was performed in order to compare the content of Cd in *T. arvense* at concentrations of 0.1–5 μM $Cd(NO_3)_2$. Statistical analysis was performed separately for roots and shoots. Significantly differing values ($p < 0.001$) are marked with small letters. Statistically significant differences between Cd content in roots and shoots of one variant are shown with asterisks: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (one-way ANOVA). Two-way ANOVA was performed separately for roots and shoots in order to clarify the differences in Cd content in *N. caerulescens* and *T. arvense* at 1 and 5 μM $Cd(NO_3)_2$. Statistically significant differences ($p < 0.001$) are shown in capital letters.

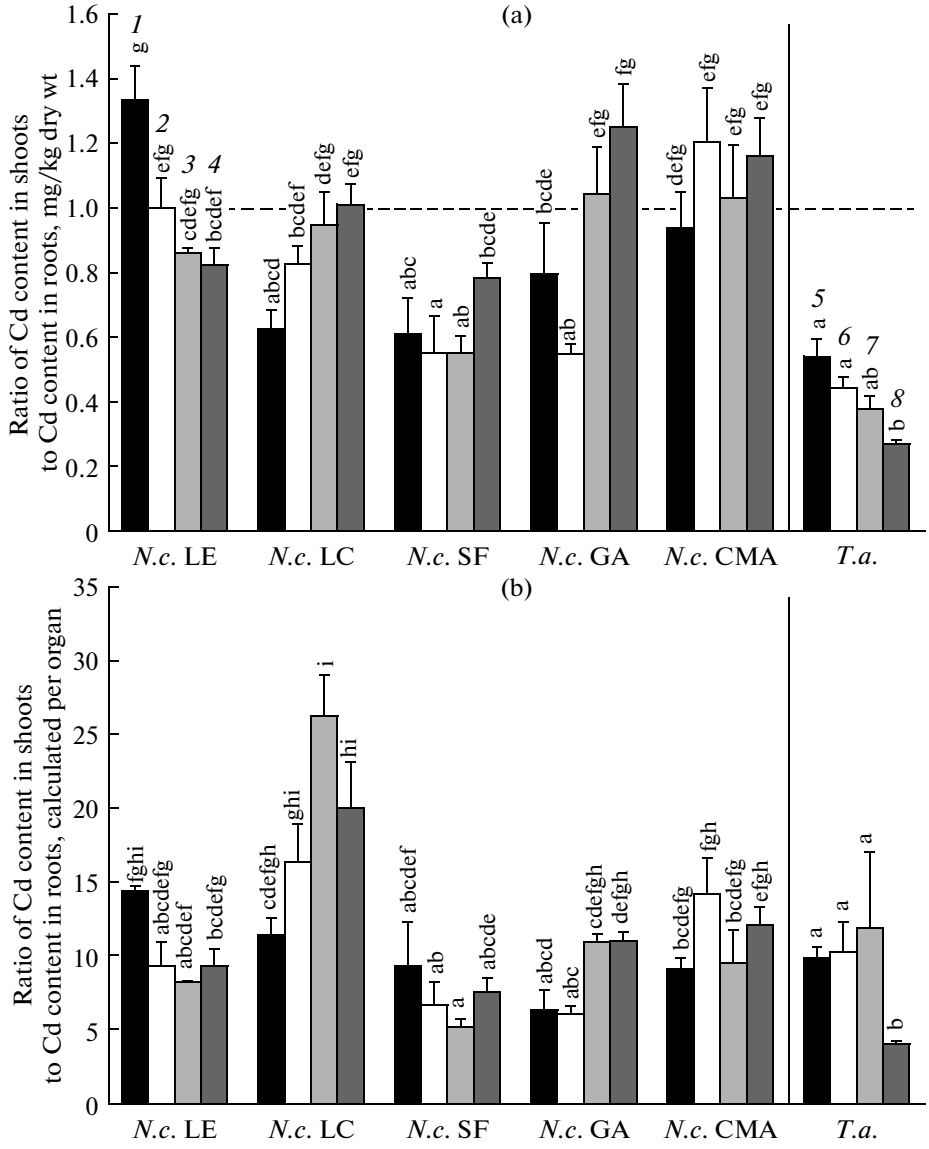

Fig. 3. Ratio of Cd content in shoots to Cd content in roots (a) expressed in mg/kg dry wt and (b) calculated per root system and shoot in excluder *Thlaspi arvense (T.a.)* and five accessions of hyperaccumulator *Noccaea caerulescens (N.c.)* (LE, LC, SF, GA, and CMA). Concentrations of $Cd(NO_3)_2$ in solution: *1*–1 μM, *2*–5 μM, *3*–25 μM, *4*–50 μM (*N. caerulescens*); *5*–0.1 μM, *6*–0.2 μM, *7*–1 μM, *8*–5 μM (*T. arvense*). Mean values and their standard errors are shown for four independent replicates (four plants per replicate). One-way (for *T. arvense*) and two-way (for the accessions of *N. caerulescens*) ANOVA were performed separately for roots and shoots. Significantly differing values ((a, b) $p < 0.001$ for *N. caerulescens* and (a) *T. arvense*; (b) $p < 0.05$ for *T. arvense*) are designated by different letters.

root biomass accumulation in hyperaccumulator *N. caerulescens* starts at a lower Cd content compared to shoots, whereas shoot growth in excluder *T. arvense* is, in contrast, inhibited at relatively lower Cd content than root growth (Fig. 5). Similar patterns were also found in the case of Ni and Zn [15]. However, high correlation between the inhibition of growth and Cd accumulation was found only for roots and shoots of LE and CMA accessions, for shoots of LC and GA, as well as roots of *T. arvense* (Fig. 5).

Comparing Cd tolerance and accumulation orders, it can be concluded that the highest tolerance of LC accession is at least partly determined by the lowest Cd accumulation in the plant, while a considerable tolerance, as well as the greatest ability to accumulate Cd, was observed for SF accession (Figs. 1, 2, and 4). In this regard, one can assume that, in this case, the tolerance is determined by effective mechanisms of Cd detoxication.

Various capacities of species and accessions to accumulate HM can be determined by the differences in metal uptake and transport. The molecular mechanisms that determine Cd transport into the cell and its intracellular translocation are being actively studied.

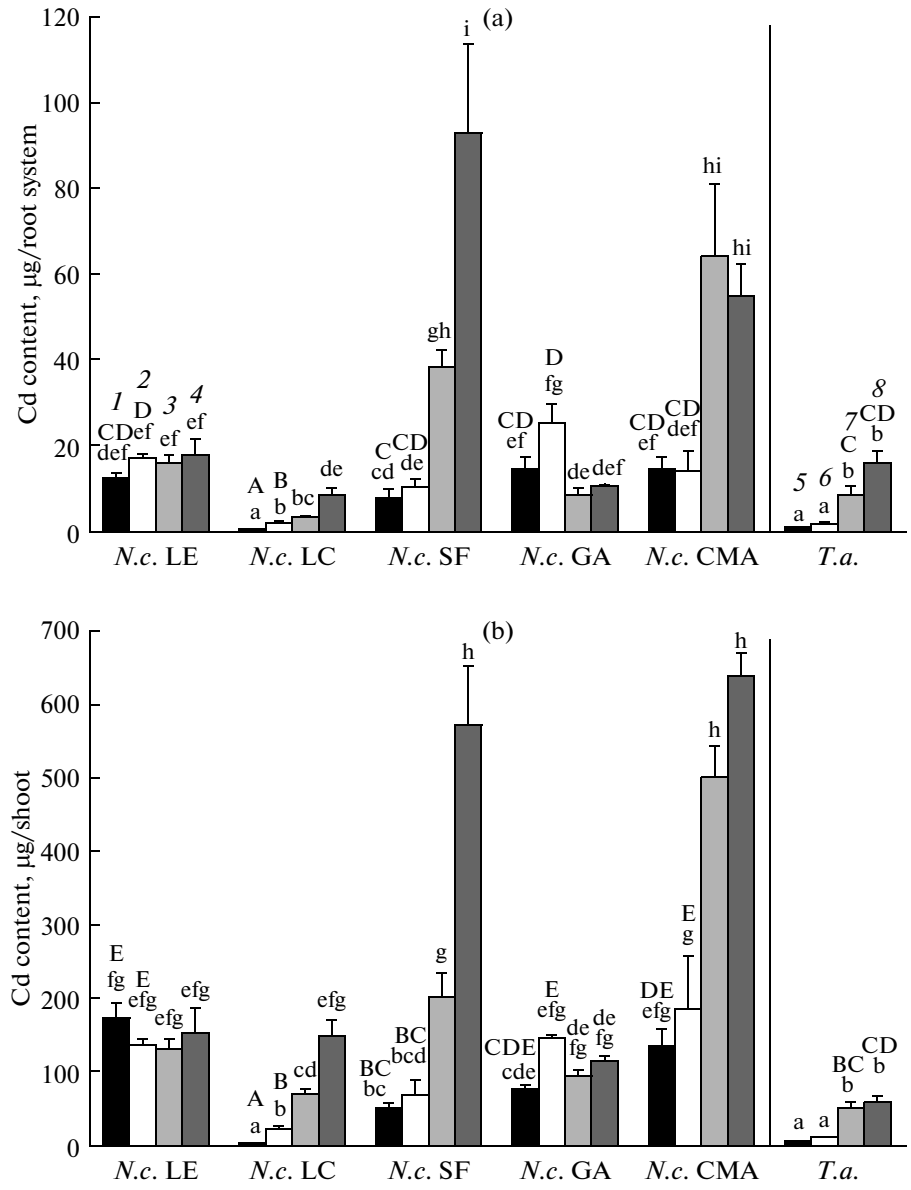

Fig. 4. Content of Cd in (a) roots and (b) shoots of excluder *Thlaspi arvense (T.a.)* and five accessions of hyperaccumulator *Noccaea caerulescens (N.c.)* (LE, LC, SF, GA, and CMA) calculated per root system or shoot. Concentrations of $Cd(NO_3)_2$ in solution: *1*–1 μM *2*–5 μM, *3*–25 μM, *4*–50 μM (*N. caerulescens*); *5*–0.1 μM, *6*–0.2 μM, *7*–1 μM, *8*–5 μM (*T. arvense*). Mean values and their standard errors are shown for four independent replicates (four plants per replicate). Two-way ANOVA was performed in order to compare the content of Cd in the accessions of *N. caerulescens* at concentrations of 1–50 μM $Cd(NO_3)_2$. One-way ANOVA was performed in order to compare the content of Cd in *T. arvense* at concentrations of 0.1–5 μM $Cd(NO_3)_2$. Significantly differing values ($p < 0.001$) are marked with small letters. Two-way ANOVA was performed in order to compare the content of Cd in *T. arvense* and *N. caerulescens* at concentrations of 1 and 5 μM $Cd(NO_3)_2$. Statistically significant differences ($p < 0.001$) are shown in capital letters. Statistical analysis was performed separately for roots and shoots.

However, no specific mechanisms of Cd transport in plants have been shown so far. This may be partly associated with the smallest, compared to other accessions, accumulation of Cd, Zn, and Ni in LC accession. Other authors also mention the presence of a correlation between the accumulation of Zn and Cd [11]. This supports the idea that Cd and Zn accumulation capacities are based on the same mechanisms [8].

Cd can be transported across the plasma membrane into the cell via the transporters of ZIP family having high affinity for Zn or Fe and considerably lower for Cd [25], as well as with the involvement of NRAMP1 transporter [26]. HMA3, a P_{1B}-type ATPase, is involved in Cd translocation into the vacuole [27], while the transport of Cd complexes with phytochelatins can be carried out by the transporters

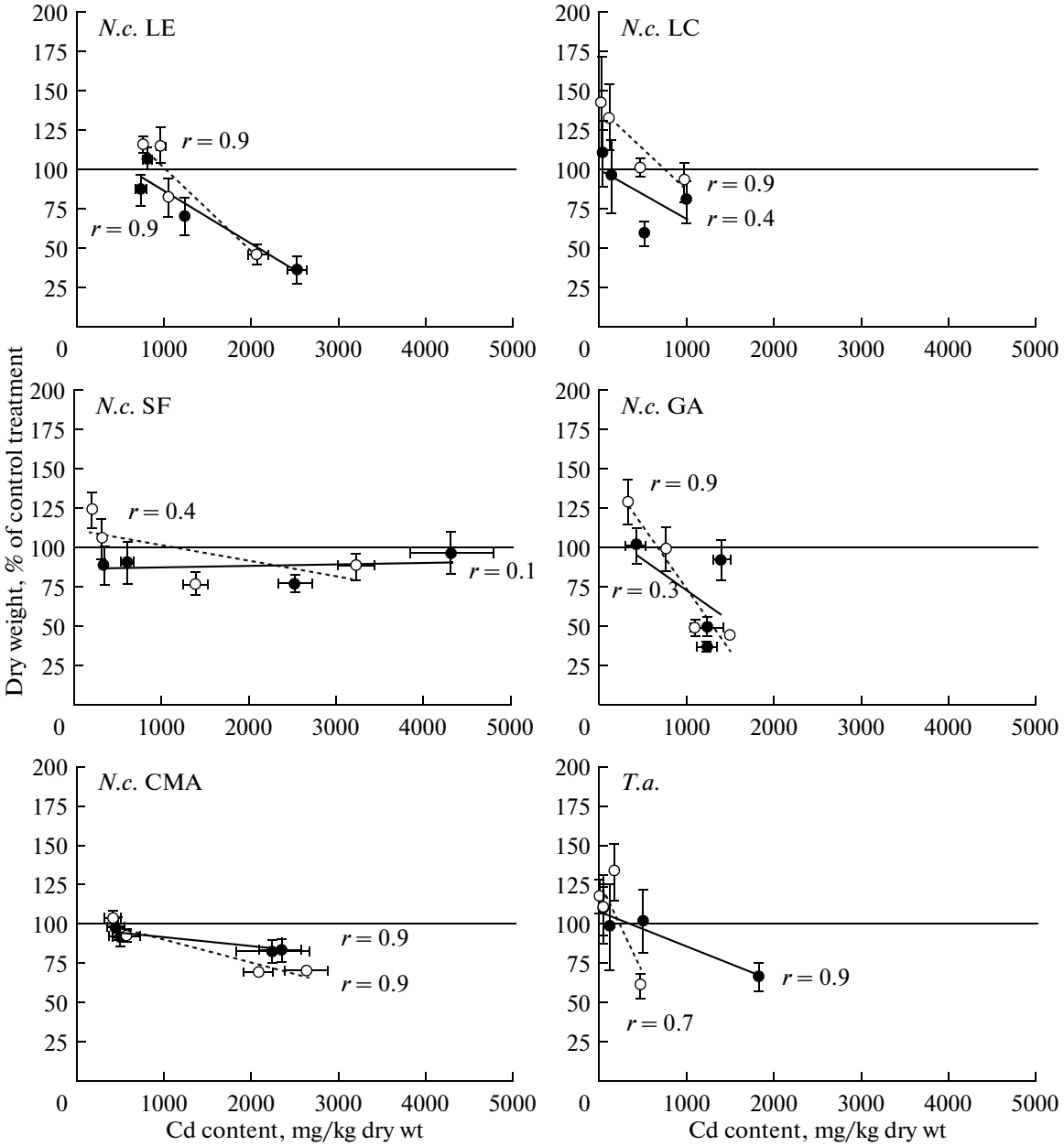

Fig. 5. Relationship between Cd content in roots and shoots of excluder *Thlaspi arvense (T.a.)* and five accessions of hyperaccumulator *Noccaea caerulescens (N.c.)* (LE, LC, SF, GA, and CMA) and the effect of Cd on growth, expressed as dry weight changes on a percentage basis. (●) roots, (——) regression line and (○) shoots (·····) regression line. Mean values and their standard errors are shown for four independent replicates (four plants per replicate). The values of correlation coefficients are shown near the curves.

of ABC family [1, 28]. HMA4, a P_{1B}-type ATPase localized in plasma membrane, participates in Cd root-to-shoot translocation, providing Zn and Cd loading into the xylem vessels and metal translocation to the aboveground organs [29]. In general, the expression level of genes encoding these transporters, is significantly higher in hyperaccumulators compared with nontolerant plant species, and this is often associated with the duplication of genes in hyperaccumulators during evolutionary processes [5, 8].

There are only limited data concerning the mechanisms that determine the differences in tolerance and the ability to accumulate HM between the accessions of one species. Obviously, these differences cannot be determined by a single mechanism. In this respect, GA accessions of *N. caerulescens* (Southern France) and PR (Belgium) are the most studied ones. It has been shown that the ability of GA accession to accumulate significant amounts of Cd is determined by higher levels of *HMA3* [27, 30] and *HMA4* [9] gene

expression, but no difference was found in Cd accumulation in the apoplast of two populations [31]. These accessions differ in the rate of Cd uptake and the effectiveness of its detoxification [23, 31], but the mechanisms underlying these differences are not fully clear yet.

A higher level of chelators in tolerant species makes some contribution to HM plant tolerance [32, 33]. However, it was shown that the phenomenon of Cd hyperaccumulation neither involves the phytochelatins, whose content in hyperaccumulators is even lower than that in excluders [34], nor the increased level of histidine in hyperaccumulators [32], probably due to low affinity of Cd to N-containing ligands [35]. Therefore, it is obvious that further research is needed in order to reveal the physiological mechanisms that determine different tolerance and capacity to accumulate HM, in particular Cd.

Thus, accessions of hyperaccumulator *N. caerulescens*, possessing higher tolerance to Cd compared with excluder *T. arvense*, greatly differ not only in their capacity to accumulate HM but also in tolerance to them. The causes of these differences, however, may be different. LC accession from calamine soils accumulates less Cd and, possibly, for this reason is more tolerant to it compared with other accessions. SF accession that grows also on calamine soils, was characterized not only by high tolerance to Cd but also by its highest accumulation, which was probably due to more efficient mechanisms of Cd detoxication. The obtained results suggest that there are species- and accession-specific differences in the mechanisms and causes of Cd tolerance, the study of which is an important task for future research.

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