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SHORT COMMUNICATIONS

Comparison of L-Histidine Effects on Nickel Translocation into the Shoots of Different Species of the Genus *Alyssum*

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Abstract—The work dealt with the influence of free L-histidine on nickel (Ni) translocation into the shoots of the hyperaccumulator plants *Alyssum murale, A. fallacinum, A. corsicum, A. tenium, A. lesbiacum, A. bertolonii, A. pintodasilvae,* and *A. obovatum* and of the closely related non-hyperaccumulator *Aurinia saxatilis* (formerly *Alyssum saxatile*). The Ni concentration in the xylem sap was determined by graphite furnace or flame atomic absorption spectrophotometry. If plants were not treated with L-histidine or L-alanine, the highest Ni concentration was found in the xylem sap of *A. murale* and *A. corsicum*. When the plants were pretreated with L-histidine, the Ni loading into the xylem vessels increased in only two hyperaccumulator species, *A. pintodasilvae* and *A. obovatum*, and in the non-hyperaccumulator *A. saxatilis*. The plant pretreatment with L-alanine did not increase the Ni level in the xylem sap. This indicates that the stimulation of Ni xylem loading is histidine-specific and not characteristic of any amino acid. Therefore, the role of histidine in the selective nickel accumulation in the shoots may considerably differ even in closely related plant species of one genus. This may presumably be accounted for by both different contents of endogenous histidine in the roots and specific patterns of the metal transport and distribution in different species.

Keywords: Alyssum, histidine, alanine, nickel, hyperaccumulators

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INTRODUCTION

One of key goals of modern ecological plant physiology is investigation of processes determining selective metal accumulation in plants of two contrasting groups: non-accumulators sequestrating metals predominantly in the root system and accumulators mainly storing metals in the aboveground organs [1]. Among the accumulators, a class of hyperaccumulators is of special attention. It includes the species that contain more than 1% zinc (Zn) or manganese (Mn) in dry shoot biomass. The threshold values are 0.1% for nickel (Ni), cobalt (Co), copper (Cu), and selenium (Se), and 0.01% for cadmium (Cd) and arsenic (As) [2]. Currently, approximately 500 hyperaccumulator species are known, and most of them belong to Ni hyperaccumulators [2–4]. Among them, nearly 50 species are representatives of the genus *Alyssum* [5] that makes them ideal model plants to study the phenomenon of hyperaccumulation [6]. The ability for nickel hyperaccumulation has emerged more than once in the evolution of the Alysseae tribe [6]. As a result, species of the *Alyssum* genus capable of accumulation from 1280 to 29400 mg Ni/kg dry wt can now be found in Russia, Mediterranean countries, Armenia, and Iraq [2].

Hyperaccumulators are very tolerant to one or several metals and metalloids. The capability for hyperaccumulation is based on the high effectiveness of metal root to shoot transport and detoxification. The essential constituent of these processes is metal binding to chelators characterized by high affinity to metals [4].

In addition to glutathione, phytochelatins, metallothioneins, organic acids, and nicotianamine, free histidine is among the most important chelators [7, 8]. The latter is involved in the processes of hyperaccumulation of Ni and Zn [9, 10] but not Cd [11] in *Noccaea caerulescens* (formerly *Thlaspi caerulescens*). There is some discrepancy on this subject in literature. Thus, the addition of L-histidine to the medium does not change the Ni xylem loading in the hyperaccumulator *Alyssum lesbiacum* [12] but stimulates Ni and Zn xylem loading in different accessions of the hyperaccumulator *N. caerulescens* in most cases [9, 10]. Effects of exogenous histidine supply on non-hyperaccumulators are also variable. The pre-treatment of *Alyssum montanum* [13] and *Brassica juncea* [12] with L-histidine elevates the Ni concentration in their xylem sap. However, neither Ni nor Zn levels increase in the

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xylem exudate of *T. arvense* after such a pre-treatment [9, 10]. It is reasonable to ask whether the differences in the effects of exogenous L-histidine on the metal translocation are due to the differences in experimental procedures or if the role of histidine in the selective Ni accumulation in the shoots may actually differ even in closely related non-hyperaccumulator or hyperaccumulator species. Generally, the content of metals in the plant tissues and organs depends to a large extent on metal concentrations in the growth medium, time of incubation, and conditions of plant growth [7]. This hinders the correct comparison of the results obtained by different authors in many cases. However, the solution to the question that we have put forward appears to be urgent for the further studies of the mechanisms of selective metal accumulation in plants. For the first time, this would allow us to understand how common the histidine-mediated mechanism of metal xylem loading is, and to estimate the contribution of histidine to the hyperaccumulation phenomenon in different plant species.

With consideration of the high Ni-accumulating potential of different species of the genus *Alyssum*, the goal of the work was the comparison of these species in the aspect of free L-histidine effects on Ni translocation into their shoots. In order to make a meaningful comparison of the results of this study with our previous data obtained on different accessions of the hyperaccumulator *N. caerulescens*, the experiments were carried out under the same conditions [9] and at the same concentration of the nickel salt (250 μM).

MATERIALS AND METHODS

Plant growth. The Ni hyperaccumulators species *Alyssum murale* Waldst. & Kit., *A. fallacinum* Hausskn., *A. corsicum* Duby, *A. tenium* Halácsy, *A. lesbiacum* (Candargy) Rech.f., *A. bertolonii* Desv., *A. pintodasilvae* Dudley (syn. *A. serpyllipholium* Desf. subsp. *lusitanicum* Dudley & Pinto da Silva), and *A. obovatum* (C.A. Mey.) Turcz. that usually grow on Ni-enriched serpentine soils as well as the closely related non-hyperaccumulator *Aurinia saxatilis* (L.) Desv. (formerly *Alyssum saxatile* L.) were examined. Their seeds were allowed to germinate on tap-water-moistened filter paper in Petri dishes at 20°C in a darkened thermostat for 2 weeks. The seedlings were transplanted to 1-L culture pots (four seedlings per pot) to be grown on half-strength Hoagland's solution in a growth chamber (20/15°C day/night, 14-h daily illumination). The medium consisted of the salts: 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM NH_4HPO_4, 0.5 mM $MgSO_4$, 1 μM KCl, 25 μM H_3BO_3, 2 μM $ZnSO_4$, 2 μM $MnSO_4$, 0.1 μM $CuSO_4$, 0.1 μM $(NH_4)_6Mo_7O_{24}$, and 20 μM Fe(Na)EDTA. The medium was adjusted to pH 5.25 with 2 mM Mes/KOH that prevented Ni and Zn binding to EDTA [14]. The nutrient solution was refreshed weekly.

Assay for nickel quantification in the xylem exudate. Prior to collecting the xylem sap, 8-week-old plants were incubated in 1 mM L-histidine or 1 mM L-alanine (pH 5.5, Mes/KOH) for 4 h. Unpretreated plants were used as control. After the pretreatment, the roots were rinsed in distilled water, the shoots were detached, and the roots were incubated in half-strength Hoagland's solution containing 250 μM $NiSO_4$. In the preliminary experiments, the Ni concentration was adjusted to be non-phytotoxic upon a short-term incubation. Xylem sap was collected overnight via silicon tubing connecting the stems with 2-mL Eppendorf-type microtubes. The samples were stored at −20°C until analyses that were performed by graphite furnace or flame atomic absorption spectrophotometry according to the standard protocol [9].

Statistics. The experiments were carried out in three independent replications with four plants per variant in each experiment. The quantitative determination of Ni in the xylem exudate was performed in three independent analytical replications. The data were statistically analyzed using one-way ANOVA and presented as means and their SEs.

RESULTS AND DISCUSSION

In the unpretreated plants, the highest Ni concentration was found in the xylem sap of *A. murale* and *A. corsicum*. Other hyperaccumulators exhibited lower metal concentrations in the saps, and the differences between the plant species were negligible. The lowest Ni concentration was detected in the non-hyperaccumulator *A. saxatilis.*

We earlier reported an uptake of exogenous L-histidine by root systems of both non-hyperaccumulators and hyperaccumulators [9]. Analysis of Ni concentration in the xylem sap of the histidine-pretreated plants of different *Alyssum* species clearly evidenced that this amino acid stimulated the Ni loading into the xylem vessels only in two hyperaccumulators: *A. pintodasilvae* and *A. obovatum*. Such a treatment did not significantly change the Ni concentration in the xylem exudate of the other hyperaccumulators (Fig. 1). The absence of L-histidine influence on Ni loading into the xylem of the hyperaccumulator *A. lesbiacum* was also reported by Kerkeb and Krämer [12]. The plant pretreatment with L-histidine enhanced the Ni level in the xylem sap of the non-hyperaccumulators *A. saxatilis* (Fig. 1), *Alyssum montanum* [13] and *Brassica juncea* [12] but not in the non-hyperaccumulator *Thlaspi arvense* [9]. The disclosed discrepancy in the L-histidine effects on Ni concentration in the xylem exudate of different species might be related to the different constitutive histidine contents in roots of these species. Further investigation is required to clear up this question.

We previously observed an increase in the Ni and Zn concentrations in the xylem sap after the pretreatment with exogenous L-histidine of four accessions of

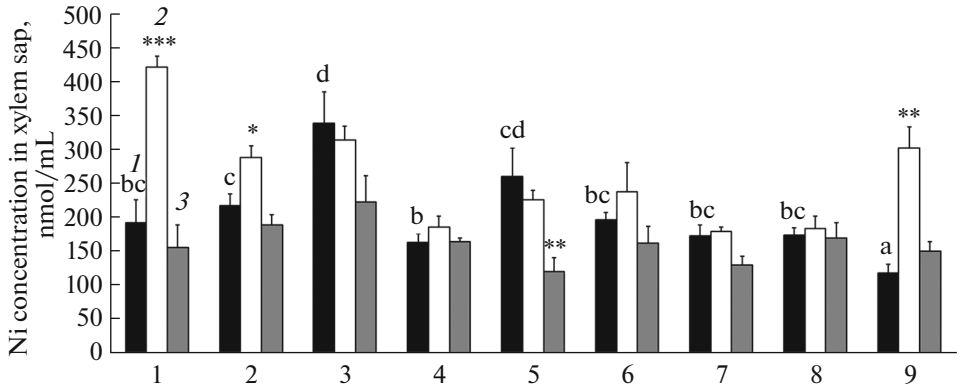

Fig. 1. Effects of the plant pretreatment with L-histidine or L-alanine on the Ni concentration in the xylem sap of different species of the *Alyssum* genus: (1) *A. obovatum,* (2) *A. pintodasilvae*, (3) *A. murale,* (4) *A. fallacinum,* (5) *A. corsicum,* (6) *A. tenium,* (7) *A. lesbiacum,* (8) *A. bertolonii,* as well as (9) *Aurinia saxatilis* (formerly *Alyssum saxatile*). Kind of treatment: (*1*) plants not treated with amino acids (unpretreated); (*2*) plants pretreated with L-histidine; (*3*) plants pretreated with L-alanine. The statistically significant differences between the aminoacid-pretreated and unpretreated plants are indicated with *, **, or *** for $P < 0.05$, $P < 0.01$, or $P < 0.001$, respectively. The statistically significant differences between the unpretreated plants of different species are indicated with different letters.

the hyperaccumulator *Noccaea caerulescens*: La Calamine (LC, Belgium), Saint Félix de Palliéres (SF, France), Monte Prinzera (MP, Italy), and Lellingen (LE, Luxembourg) [9, 10]. The Zn xylem loading was stimulated much stronger than that of Ni. This may be explained by the fact that Zn xylem loading was preceded by its mobilization and redistribution, since it had been present in Hoagland's solution and accumulated in the roots prior to histidine pretreatment [9, 10]. At low Ni concentration in the medium (25 μM), the pretreatment with histidine did not affect the Ni concentration in the xylem sap of the LC plants, which typically accumulate the lowest metal amounts in their shoots. The strongest effect, even at low Ni concentration, was observed in the MP accession whose evolution occurred on serpentine soils rich in nickel. At higher Ni concentration (250 μM), the effect of histidine clearly manifested itself in all the studied *N. caerulescens* accessions [9].

When tonoplast vesicles were isolated from the roots and incubated in the medium containing Ni or Zn complexes with histidine, the metal uptake was less intensive in the vesicles from hyperaccumulator *N. caerulescens* than in those from the non-hyperaccumulator *T. arvense* [9, 10]. Moreover, the Zn content in the vacuoles was 2.4 times higher in non-hyperaccumulator compared to hyperaccumulator, as the direct quantitative analysis showed. Besides, the rate of Zn efflux from the vacuoles in *N. caerulescens* approximately twofold exceeded that in *T. arvense* [15]. It is known that some hyperaccumulators are characterized by higher levels of endogenous histidine in the roots and lower metal levels in the vacuoles of the cortical cells than non-hyperaccumulators. Therefore, it may be suggested that metal binding to histidine restricts the metal translocation into the vacuoles of the root cortical cells. This promotes the metal transport to the xylem in hyperaccumulators, while non-hyperaccumulators accumulate Ni and Zn in the cortical cells [9, 10, 12, 13, 16–18]. However, our data indicate that the involvement of histidine into the selective accumulation of Ni in the shoots is much more variable even among closely related species.

Histidine complexes with metals may represent a form in which metals are loaded into the xylem [19], but there is no direct evidence of this yet. Zinc xylem loading involves HMA4, a P-type ATPase localized in the plasma membrane. High level of expression of *HMA4* gene is achieved owing to multiplication of its copies in hyperaccumulators [4, 20, 21]. Thus, the high level of the *AhHMA4* gene expression in the Zn hyperaccumulator *Arabidopsis halleri* results from the presence of three copies of this gene under one promoter. In contrast, the non-hyperaccumulator *A. thaliana* has only one copy of *AtHMA4* gene [21]. The expression level of the *NcHMA4* gene in *N. caerulescens* is even higher than that of the *AhHMA4* in *A. halleri* in both roots and shoots [22]. The mechanism of loading of Ni or its complexes into the xylem vessels remains unstudied so far. Presumably, the effectiveness of its functioning may differ not only between hyperaccumulators and non-hyperaccumulators but also between representatives of one plant group.

In the xylem vessels, the metal-histidine complexes appear to partially dissociate, since they are less stable at the xylem sap pH (approximately 5.5–6.2) than at the cytoplasmic pH (approximately 7.2–7.5) because of the protonation of the nitrogen of histidine imidazole group [23, 24]. Nickel travels by xylem vessels for a long distance not only as a complex with histidine [13] but also as a complex with organic acids [25], nicotianamine [26, 27], or in an ionic form [28].

Complexes of nickel with alanine are fivefold less stable than those with histidine [29]. In our experiments, pretreatment with alanine did not influence the Ni concentration in the xylem sap of most of the tested species, but decreased it only in *A. corsicum* (Fig. 1). A similar absence of increase in Ni and Zn concentrations in the xylem sap of *N. caerulescens* after pretreatment with alanine [9, 10] points to the fact that the stimulative effect is specific to histidine and is not a common property of amino acids.

Therefore, the discrepancy of literature data concerning the effects of exogenous L-histidine on Ni translocation into the shoots of different plant species is not accounted for by differences in experimental procedures. Histidine participation in the selective Ni accumulation in plant shoots may significantly differ even in closely related species of one genus. This is presumably a consequence of different levels of endogenous histidine in the roots and of some specific features of the metal transport in different species.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

1. Brooks, R.R., Lee, J., Reeves, R.D., and Jaffré, T., Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants, *J. Geochem. Explor.*, 1977, vol. 7, pp. 49–57.
2. Reeves, R.D. and Baker, A.J.M., Metal-accumulating plants, in *Phytoremediation of Toxic Metals Using Plants to Clean Up the Environment*, Raskin, I. and Ensley, B.D., Eds., New York: John Wiley and Sons, 2000, pp. 193–229.
3. Verbruggen, N., Hermans, C., and Schat, H., Molecular mechanisms of metal hyperaccumulation in plants, *New Phytol.*, 2009, vol. 181, pp. 759–776.
4. Krämer, U., Metal hyperaccumulation in plants, *Annu. Rev. Plant Biol.*, 2010, vol. 61, pp. 517–534.
5. Koch, M. and Al-Shehbaz, I.A., Phylogeny of *Brassica* and wild relatives, in *Biology and Breeding of Crucifers*, Gupta, S.K., Ed., Boca Raton: Taylor and Francis Group, 2009, pp. 1–19.
6. Cecchi, L., Gabbrielli, R., Arnetoli, M., Gonnelli, C., Hasko, A., and Selvi, F., Evolutionary lineages of nickel hyperaccumulation and systematics in European Alysseae (Brassicaceae): evidence from nrDNA sequence data, *Ann. Bot.*, 2010, vol. 106, pp. 751–767.
7. Seregin, I.V. and Kozhevnikova, A.D., Physiological role of nickel and its toxic effects on higher plants, *Russ. J. Plant Physiol.*, 2006, vol. 53, pp. 257–277.
8. Leitenmaier, B. and Küpper, H., Compartmentation and complexation of metals in hyperaccumulator plants, *Front. Plant Sci.*, 2013, vol. 4, pp. 1–13.
9. Richau, K.H., Kozhevnikova, A.D., Seregin, I.V., Vooijs, R., Koevoets, P.L.M., Smith, J.A.C., Ivanov, V.B., and Schat, H., Chelation by histidine inhibits the vacuolar sequestration of nickel in roots of the hyperaccumulator, *Thlaspi caerulescens, New Phytol.*, 2009, vol. 183, pp. 106–116.
10. Kozhevnikova, A.D., Seregin, I.V., Erlikh, N.T., Shevyreva, T.A., Andreev, I.M., Verweij, R., and Schat, H., Histidine-mediated xylem loading of zinc is a species-wide character in *Noccaea caerulescens, New Phytol.*, 2014, vol. 203, pp. 508–519.
11. Kozhevnikova, A.D., Seregin, I.V., Verweij, R., and Schat, H., Histidine promotes the loading of nickel and zinc, but not of cadmium, into the xylem in *Noccaea caerulescens, Plant Signal. Behav.*, 2014, vol. 9: e29580. http://www.tandfonline.com/doi/full/10.4161/psb.29580
12. Kerkeb, L. and Krämer, U., The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea, Plant Physiol.*, 2003, vol. 131, pp. 716–724.
13. Krämer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, A.J.M., and Smith, A.C., Free histidine as a metal chelator in plants that accumulate nickel, *Nature*, 1996, vol. 379, pp. 635–638.
14. Assunção, A.G.L., Bookum, W.M., Nelissen, H.J.M., Vooijs, R., Schat, H., and Ernst, W.H.O., Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types, *New Phytol.*, 2003, vol. 159, pp. 411–419.
15. Lasat, M.M., Baker, A.J.M., and Kochian, L.V., Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens, Plant Physiol.*, 1998, vol. 118, pp. 875–883.
16. Ingle, R.A., Mugford, S.T., Rees, J.D., Campbell, M.M., and Smith, J.A.C., Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants, *Plant Cell*, 2005, vol. 17, pp. 2089–2106.
17. Seregin, I.V. and Kozhevnikova, A.D., Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium, *Russ. J. Plant Physiol.*, 2008, vol. 55, pp. 1–22.
18. Baklanov, I.A., Seregin, I.V., and Ivanov, V.B., Histochemical analysis of nickel distribution in the hyperac-

cumulator and excluder in the genus *Alyssum* L., *Dokl. Biol. Sci.*, 2009, vol. 429, pp. 548–550.

19. Haydon, M.J. and Cobbett, C.S., Transporters of ligands for essential metal ions in plants, *New Phytol.*, 2007, vol. 174, pp. 499–506.
20. Hussain, D., Haydon, M.J., Wang, Y., Wong, E., Sherson, S.M., Young, J., Camakaris, J., Harper, J.F., and Cobbett, C.S., P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis, Plant Cell*, 2004, vol. 16, pp. 1327–1339.
21. Hanikenne, M., Talke, I.N., Haydon, M.J., Lanz, C., Nolte, A., Motte, P., Kroymann, J., Weigel, D., and Krämer, U., Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4, Nature*, 2008, vol. 453, pp. 391–395.
22. Bernard, C., Roosens, N., Czernic, P., Lebrun, M., and Verbruggen, N., A novel CPx-ATPase from the cadmium hyperaccumulator *Thlaspi caerulescens, FEBS Lett.*, 2004, vol. 569, pp. 140–148.
23. Salt, D.E., Prince, R.C., Baker, A.J., Raskin, I., and Pickering, I.J., Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy, *Environ. Sci. Technol.*, 1999, vol. 33, pp. 713–717.
24. Monsant, A.C., Kappen, P., Wang, Y., Pigram, P.J., Baker, A.J., and Tang, C., In vivo speciation of zinc in *Noccaea caerulescens* in response to nitrogen form and zinc exposure, *Plant Soil*, 2011, vol. 348, pp. 167–183.
25. Kersten, W.J., Brooks, R.R., Reeves, R.D., and Jaffre, T., Nature of nickel complexes in *Psychotria douarrei* and other nickel-accumulating plants, *Phytochemistry*, 1980, vol. 19, pp. 1963–1965.
26. Vacchina, V., Mari, S., Czernic, P., Marquès, L., Pianelli, K., Schaumlöffel, D., Lebrun, M., and Łobiński, R., Speciation of nickel in a hyperaccumulating plant by high-performance liquid chromatography-inductively coupled plasma mass spectroscopy and electrospray MS/MS assisted by cloning using yeast complementation, *Anal. Chem.*, 2003, vol. 75, pp. 2740–2745.
27. Kim, S., Takahashi, M., Higuchi, K., Tsunoda, K., Nakanishi, H., Yoshimura, E., Mori, S., and Nishizawa, N.K., Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants, *Plant Cell Physiol.*, 2005, vol. 46, pp. 1809–1818.
28. Sagner, S., Kneer, R., Wanner, G., Cosson, J.-P., Deus-Neumann, B., and Zenk, M.H., Hyperaccumulation, complexation and distribution of nickel in *Sebertia acuminata, Phytochemistry*, 1998, vol. 47, pp. 339–343.
29. Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research*, Oxford: Clarendon, 1986.

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