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Salt (NaCl) tolerance in the Ni hyperaccumulator *Alyssum murale* and the Zn hyperaccumulator *Thlaspi caerulescens*

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Abstract

Many metal hyperaccumulating plants have to tolerate abiotic stresses in their native soils such as high metal concentrations, low nutrient status and drought. This paper tests the ability of the Ni-hyperaccumulator *Alyssum murale* and two races of the Zn-hyperaccumulator *Thlaspi caerulescens* (Prayon and Close House) to tolerate salinity. The plants were exposed to salt (NaCl) solutions ranging between 0 mM and 100 mM in conjunction with either high or low concentrations of Ni or Zn. *Alyssum murale* was most resistant to salt in terms of seedling emergence and survival of emerged seedlings. The two races of *T. caerulescens* and *T. arvense* were salt sensitive. High Ni or Zn concentrations did not have a clear effect on the salt tolerance of any of the plants tested. The implications of the findings are discussed for the development of metal phytoremediation/phytomining technologies on saline soils or where brackish water (e.g., mining wastewater) could be used to irrigate phytoremediation 'crops'.

Introduction

Metal-hyperaccumulating plants have received considerable investigation for their unusual physiology and genetics (Pollard et al., 2003). Such investigations have often been associated with the commercial prospect of using these species for remedial purposes e.g. to extract metals from contaminated land (phytoremediation) or to extract low-grade mineral ores (phytomining) (McGrath et al., 2002; Whiting et al., 2002). Further development of these technologies is required to optimize the amount of metals that can be removed. Phytomining is likely to be utilized on substrates that are certainly unfavorable for growth of conventional agricultural crops because of soil factors such as: 1. Presence of phytotoxic heavy metal ions; 2. Excessive salinity; 3. Poor soil structure with a low content of organic material; 4. Low water-holding capacity; and 5. Poor mineral nutrient status. Moreover, significant interactions can occur between these stress factors; for example, Cd uptake by wheat is increased at high salinities (Mühling and Läuchli, 2002).

The potential for large amounts of soluble salts to accumulate in the soils is a common problem in arid and semi-arid regions (Charman and Murphy, 2000). Thus, the toxic and osmotic effects of salinity will be critical issues affecting the growth and phytoextraction potential of hyperaccumulator particularly where the mineralized substrate is subject to mediterranean or dry tropical climates. Angle et al. (2003) and Whiting et al. (2003) demonstrated that several metal-hyperaccumulating species are able to tolerate moderate levels of drought. However, the hyperaccumulation of metals in the shoots did not confer or augment the drought resistance of these species at the whole plant scale as had been proposed by Severne (1974) and Baker and Walker (1989) (for review see Boyd and Martens, 1992).

High levels of salinity may be an issue for the growth of hyperaccumulator plants both *in situ* in their native sites, and *ex situ* where hyperaccumulator

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plants are used for phytoremediation or phytomining. Although sweet water irrigation will likely be an important part of management practices for remediation or phytomining such water is, itself, a valuable commodity with restricted availability in many areas. Indeed, much of the water available for secondary uses such as irrigating restoration projects around mineral extraction operations often consists of processing wastewater which is burdened with dissolved salts. If some metal-hyperaccumulating plants are also salt tolerant and able to maintain high levels of metal uptake under saline conditions then this offers promise for their commercial use on saline soil substrates, or even for the use of saline wastewater to irrigate remediation projects.

Here we report the first test of salt (NaCl) tolerance of the Ni hyperaccumulator *Alyssum murale* Waldst. & Kit., of the Zn hyperaccumulator *Thlaspi caerulescens* J. & C. Presl and of the non-metal accumulating species *Thlaspi arvense* L. (all Brassicaceae). Salt tolerance is very complex because salt stress can cause growth inhibition directly by ion toxicity, and indirectly, by a combination of osmotic stress and inhibition of nutrient uptake (Marschner, 1995; Neumann, 1997; Munns, 2002). In this paper, our three-fold aim was to determine (i) whether two selected hyperaccumulator species are salt tolerant, (ii) whether elevated Ni and Zn supply influences the degree of salt tolerance, and (iii) whether elevated salinity influences the concentrations of metals hyperaccumulated in the shoots. The effects of salinity on growth of the three species were tested at two developmental stages, a) during germination and b) during seedling development. All species were grown in nutrient solutions with or without additions of NaCl.

Materials and methods

Three experiments were performed. The first experiment determined the effects of salt on the germination of *A. murale*, *T. caerulescens* and *T. arvense*. The second experiment determined effects of salt on growth and Ni accumulation by *A. murale*. The third experiment determined the effects of salt on the growth and Zn accumulation by *T. caerulescens*. Two accessions of *T. caerulescens* were tested; one was the race ('Prayon') collected from the vicinity of a disused Zn/Cd smelter at Prayon, Belgium. Prayon has been tested extensively in studies of the Zn-hyperaccumulating ability of this species (e.g., Whiting et al., 2000; Meerts and Grommesch, 2002; Lombi et al., 2002; Meerts et al., 2003). The second accession of *T. caerulescens* was collected from shale on the bank of the tidal section of the River Tyne UK, near Close House, where the soils may have been salt impacted (Richards et al., 1989). Seeds of *Alyssum murale* were collected from a population growing on a drought-prone skeletal serpentine soil in Thessaloniki, Northern Greece. Seeds of *T. arvense* L. were collected from arable land that was not contaminated with metals (Suffolk, UK). All seeds were bulk accessions collected from many mother plants across the site.

Experiment 1: The effect of salt (NaCl) on seed germination of T. caerulescens *(Prayon),* T. arvense *and* A. murale.

The objectives of this study were to determine the sensitivity of *T. caerulescens, T arvense* and *A. murale* to salinity during seed germination, and during seedling development. Seeds were germinated in sterile 9-cm Petri dishes that contained 40 g of washed heat-sterilized sand. One hundred seeds of *T. caerulescens* (Prayon) were counted into each of 40 dishes. The sand was moistened with 20 ml of treatment solutions of 0, 25, 50 or 100 mM NaCl (equivalent to $\sim$0.2, 2.5, 5 and 10 dS m^{-1}) dissolved in tenth-strength Rorison's nutrient solution [tenth-strength Rorison's contains 200 μM $Ca(NO_3)_2.4H_2O$, 100 μM $MgSO_4.7H_20$, 100 μM $K_2HPO_4.3H_20$, 5 μM Fe-chelate, 1 μM $MnSO_4.4H_20$, 5 μM H_3BO_3, 0.1 μM $(NH_4)_6Mo_7O_{24}.4H_20$, 0.2 μM $ZnSO_4.7H_2O$ and 0.15 μM $CuSO_4.5H_2O$ in deionized water. For testing at high metal levels, the treatments were prepared as above except that the solutions used for *T. caerulescens* contained 100 μM $ZnSO_4.7H_20$. Five replicates were prepared for each treatment, giving a total of 40 Petri dishes. Another 40 dishes were prepared as above for each of *T. arvense* and *A. murale*. In this case, the high metal treatments contained 20 μM $ZnSO_4.7H_20$ for *T. arvense* and 20 μM $NiSO_4.6H_2O$ for *A. murale*. The lids were replaced, and the dishes placed in the shade in a controlled environment greenhouse. Temperature and relative humidity in the glasshouse were logged every five minutes throughout the study period using a HMP 35A relative humidity sensor (Vaisala, Helsinki, Finland) attached to a Datataker DT500 (Ohio, USA). Mean daytime temperature was 18.4 $\pm$ 0.9 °C ($\pm$1 SD); mean night temperature was 17.2 $\pm$ 0.4 °C mean daytime humidity was 54%; mean night-humidity

was 60%. Photosynthetically-active photon flux density (PPFD) was logged every 5 min using a Li-Cor LI-190SA quantum sensor (Nebraska, USA). Mean daytime PPFD was 76.5 ± 20.8 mol m^{-2} s^{-1} (± SD) over the 11-h photoperiod. Germination was determined by the emergence of a radicle and subsequent development of two cotyledons. Seedling germination was first recorded after 10 days, and the dishes were then checked regularly for a further period of 14 d until no more seedlings had germinated.

Experiment 2: Salt tolerance of A. murale *seedlings in hydroponic culture*

The tolerance of *A. murale* seedlings to growth inhibition by salinity was tested in hydroponic solutions. Seeds of *A. murale* were placed on washed, heat-sterilized sand that had been wetted with distilled water. After 14 d, the seedlings were transferred to light-proof hydroponic vessels containing 2.5 L of aerated tenth-strength Rorison's nutrient solution described in Experiment 1. Each vessel held 12 plants supported by polyurethane foam plugs in bottomless Eppendorf tubes fitted through the lid. The plants were grown under greenhouse conditions as described in Experiment 1. The hydroponic solutions were replaced every third day to prevent nutrient depletion. Twenty-one days after transplanting, half of the number of vessels received a Ni treatment of nutrient solution plus 20 μM $NiSO_4.6H_2O$. The remainder of the vessels received unmodified nutrient solution. The Ni treatment continued for 7 days and the solutions were changed every third day. Three vessels of each plus or minus metal treatment were then exposed to salt treatments of 0, 25, 50 and 100 mM NaCl, which are representative of moderate to severe salt stress. The salt treatments continued for 21 days, with constant aeration, and the solutions were changed every third day.

The addition of millimolar NaCl to Rorison's solution resulted in increasing Na:Ca ratio, which might reduce plant growth. However, the Ca concentration was not increased with increasing NaCl supply because the intention was to simulate the effect of brackish/saline water irrigation to hyperaccumulators cultivated for phytoremediation/phytomining. Under this scenario the Ca concentration in a soil will be fixed; irrigation with saline water would therefore result in increasing Na:Ca ratio in the field, and thus the treatments here were considered valid. The ion activity in the high-Ni treatment solution was modeled using MINTEQA2 to quantitatively determine that effect of NaCl addition on Ca and Ni activity. The modeling indicated that the Ca^{2+} activity was 162, 102, 85 and 68 μM for the 0, 25, 50 and 100 mM NaCl treatments, respectively. The Ni activity in these NaCl treatments was predicted to be 12, 8, 7 and 6 μM, respectively.

At harvest, the roots were removed, the shoots rinsed in distilled water containing a trace of the surfactant Tween 80^{TM} and dried at 70 °C for 3 days. The dried samples were weighed, digested in 2 mL of concentrated HNO_3 and the Ni and Na contents of the sample solutions determined by Inductively-coupled plasma atomic emission spectroscopy (ICP-AES) on a Varian Vista AX (Varian Inc., Melbourne, Australia).

Experiment 3: Salt tolerance of T. caerulescens *and* T. arvense *seedlings in hydroponic culture*

The tolerance of *T. caerulescens* and *T. arvense* seedlings to growth inhibition by salinity was tested in hydroponic solutions. Seeds of *T. caerulescens* Prayon, *T. caerulescens* Close House and *T. arvense* were germinated and transferred to hydroponic solutions as described in Experiment 2 except that for the initial treatment period, half of the vessels supporting *T. caerulescens* received nutrient solution containing 100 μM $ZnSO_4.7H_2O$.

Similarly, half the vessels supporting *T. arvense* received 20 μM $ZnSO_4.7H_2O$ (note that *T. arvense* received a lower Zn concentration than *T. caerulescens* because this non-accumulator species is less tolerant to Zn). The plants were grown under greenhouse conditions as described in Experiment 1. The Zn pretreatment continued for 7 days (the solutions were changed every third day) and then 12 plants of each metal/species treatment were harvested randomly and analyzed for their Zn content as described in Experiment 2. Three vessels of each species/metal pretreatment were then exposed to salt treatments of 0 25 and 50 mM NaCl for 14 days as described in Experiment 2. This range of salt concentrations was selected because the highest concentration in Experiment 1 (100 mM) severely inhibited the germination of these *Thlaspi* species. For the same reasons as in Experiment 2, the Ca concentration was not changed with increasing NaCl supply. The ion activity in the high-Zn treatment solutions for *T. caerulescens* and *T. arvense* were modeled using MINTEQA2. In the 100 μM Zn treatment of *T. caerulescens*, the modeling indicated that the Ca^{2+} activity was 159, 102 and 84 μM for the 0, 25 and 50 mM NaCl treatments, respectively; the

Zn activity in these NaCl treatments was predicted to be 67, 46 and 38 μM, respectively. In the 20 μM Zn treatment of *T. arvense*, the modeling indicated that the Ca^{2+} activity was 162, 102 and 85 μM for the 0, 25 and 50 mM NaCl treatments, respectively; the Zn activity in these NaCl treatments was predicted to be 11, 8 and 7 μM, respectively.

The plants were harvested after 14 days exposure to salt, the solutions having been changed every third day. The plants were harvested, weighed and analyzed for Zn and Na contents as described in Experiment 2.

Statistical analysis

Statistical analyses were performed using Minitab v.11 (Minitab Inc., Pennsylvania, USA). General Linear Model (GLM) was used to determine the effects of metal and salt treatments on germination (Experiment 1) and on root length, shoot mass and shoot metal contents (Experiments 2 and 3). The percentages reported in Experiment 1 were arcsine square-root transformed before analysis.

Results

The effects of NaCl and metals on germination

The emergence of *A. murale* seedlings was not inhibited by the either the high Ni or high salt treatments, as compared to the no-salt no-Ni control (Figure 1). The statistical analysis indicated that there were no significant effects of either the increase in salt concentration ($P > 0.05$), the addition of Ni ($P > 0.05$) or their interaction ($P > 0.05$). High germination rates (80–95%) were seen for all treatments, even the most severe treatment of 20 μM Ni plus 100 mM NaCl. For *T. caerulescens* Prayon (Figure 1), there was a highly significant effect of salt ($P < 0.001$), but not of Zn treatment or the Zn-salt interaction term (both $P > 0.05$). 50 mM NaCl caused a small but statistically significant reduction in the germination of *T. caerulescens* and 100 mM NaCl almost totally inhibited germination. The addition of 100 μm Zn had no effect on this response to salt. For the non-accumulator species *T. arvense*, there was a significant effect of salt treatment on the germination of *T. arvense*, but not of Zn treatment or their interaction (both $P > 0.05$). The germination success of *T. arvense* was reduced by ~10% in the 25 and 50 mM NaCl treatments, and by 60% in the 100 mM treatment.

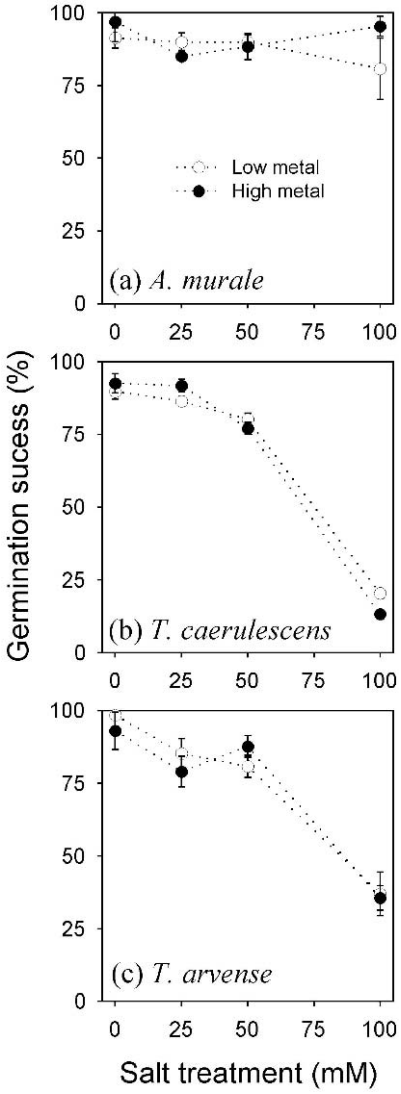

Figure 1. Effect of salt stress and metal supply on the germination success of *A. murale*, *T. caerulescens* (Prayon) and *T. arvense.* Seeds were germinated on sand wetted with the treatment solution; germination was defined as the emergence of the radicle and subsequent development of the cotyledon leaves. Low metal treatments (open circles) contained 1 μM Zn^{2+} and 0 μM Ni^{2+}; high metal treatments (blck circles) were 20 μM Ni^{2+} for *A. murale*, 100 μM Zn^{2+} for *T. caerulescens* and 20 μM Zn^{2+} for *T. arvense.*

Effect of NaCl and metals on hydroponically grown Alyssum murale

Shoot growth was used as the indicator of plant performance under salt stress. Most individuals of the nickel hyperaccumulator *A. murale* survived exposure to 100 mM salt, but their shoot growth at this concentration was inhibited (Figure 2). Statistical analysis indicated that there were significant inhibitory effects

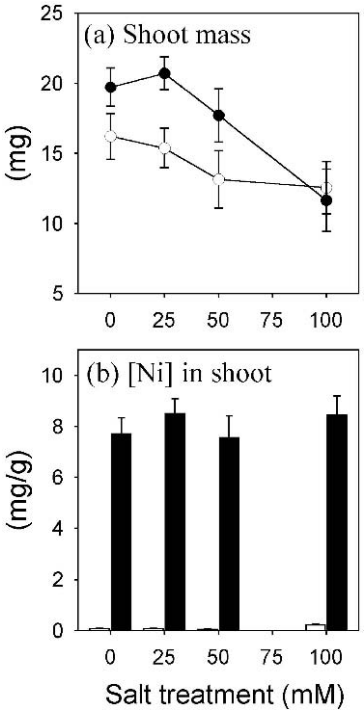

Figure 2. Effect of salt stress and metal supply on (a) the shoot dry weight and (b) the Ni concentration in the shoots of *A. murale* seedlings grown in solution culture. Low Ni treatments (open circles and open bars) contained 0 μM Ni^{2+}; high Ni treatments (black circles and black bars) contained 20 μM Ni^{2+}.

of the salt treatments ($P < 0.05$), together with stimulatory effects of the Ni treatments ($P < 0.05$), and no effects of their interaction ($P > 0.05$). In the 0, 25, and 50 mM NaCl treatments, the plants treated with Ni had a significantly greater shoot mass at harvest than those that had received no Ni, irrespective of the salt treatment. Shoot growth in *A. murale* was not affected by the 25 or 50 mM NaCl treatments. It was however, was significantly reduced in the 100 mM treatment. Importantly, there was no significant effect of the NaCl treatments on the concentration of Ni in the shoots of *A. murale* at either the low or high Ni treatments (Figure 2; $P > 0.05$). The Na concentration in the shoots of *A. murale* (Table 1) was 5, 16, 28 and 34 mg g^{-1} dry weight in the 0, 25, 50 and 100 mM NaCl treatments.

The effects of NaCl and metals on hydroponically grown T. caerulescens *(Prayon and Close House) and* T. arvense

Before the salt treatments commenced, there was no significant difference between the shoot mass of the low Zn and high Zn plants of *T. caerulescens* Prayon

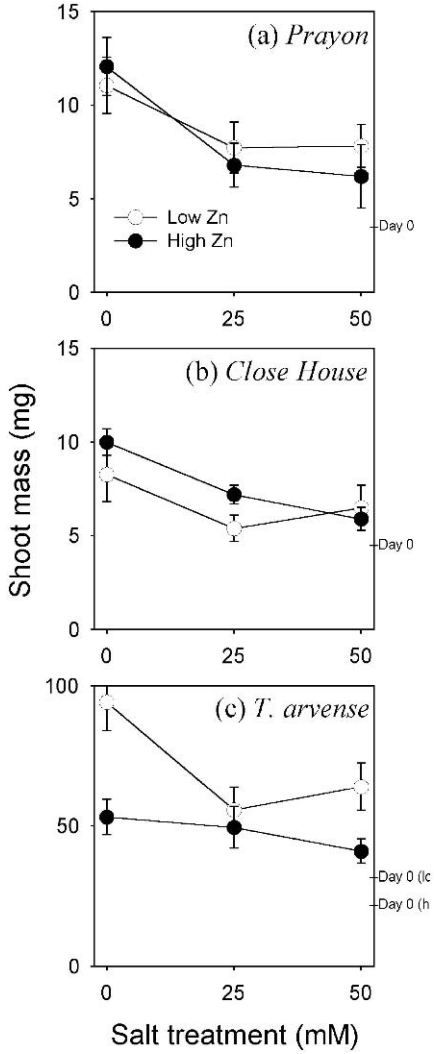

Figure 3. Effect of salt stress and metal supply on the shoot dry weight of (a) *T. caerulescens* Prayon, (b) *T. caerulescens* Close House and (c) *T. arvense* seedlings grown in solution culture. Low Zn treatments (open circles) contained 1 μM Zn^{2+}; high Zn treatments (black circles) contained 100 μM Zn^{2+} for the *T. caerulescens* races and 20 μM Zn^{2+} for *T. arvense.* 'Day 0' indicates the shoot mass of *T. caerulescens* plants ($-/+$ Zn) harvested just before the salt treatments began. 'Day 0 (low Zn)' and 'Day 0 (high Zn)' are shown independently for *T. arvense* because in contrast to the other species, the high-Zn pretreatment had significantly ($P < 0.05$) impeded the shoot growth of *T. arvense* compared to the low-Zn treatment.

or of *T. caerulescens* Close House. There was a significant difference between the low and high Zn treatments for the mass of shoots of *T. arvense* before the salt treatments commenced (*t*-test, $P < 0.05$); the shoots of the plants treated with Zn were smaller than those receiving low Zn.

Table 1. The concentration of Na in the shoots (mg g^{-1}) of plant in Experiments 1 and 2. Values are means ± standard errors

	Salt treatment (mM)			
	0	25	50	100
Experiment 2				
A. murale				
Low Ni	2.1 ± 0.6	21.3 ± 3.1	22.4 ± 3.3	34.5 ± 3.8
High Ni	9.3 ± 0.8	11.4 ± 1.0	34.1 ± 9.8	32.8 ± 8.4
Experiment 3				
T. caerulescens Prayon				
Low Zn	2.1 ± 0.6	19.6 ± 3.6	57.8 ± 11.0	NT
High Zn	1.7 ± 0.4	48.4 ± 8.2	52.6 ± 9.6	NT
T. caerulescens Close House				
Low Zn	5.3 ± 0.7	21.9 ± 3.4	47.2 ± 7.9	NT
High Zn	2.6 ± 0.3	21.5 ± 3.5	46.5 ± 3.8	NT
T. arvense				
Low Zn	1.9 ± 0.3	19.9 ± 5.1	34.3 ± 10.1	NT
High Zn	4.2 ± 1.0	21.3 ± 4.0	36.1 ± 5.3	NT

NT – not tested.

The salt treatments strongly inhibited the shoot growth of both accessions of the zinc hyperaccumulator *T. caerulescens* (Prayon and Close House) and also of *T. arvense* (Figure 3). For *T. caerulescens* Prayon a statistically significant effect of the salt treatment ($P < 0.05$) was indicated, but not of the Zn treatment ($P > 0.05$) or of Zn-NaCl interactions. Similarly, only the salt treatment had a statistically significant effect on the shoot mass of *T. caerulescens* from the Close House population (tidal river). For both accessions, the 25 and 50 mM salt treatments inhibited shoot growth. The shoot mass of the non-hyperaccumulator *T. arvense* was reduced by the salt treatments ($P < 0.05$) and strongly reduced by the metal treatment ($P < 0.001$), but the interaction term was not statistically significant ($P > 0.05$). The 25 and 50 mM salt treatments both inhibited the growth of *T. arvense* shoots by ∼60% in the absence of Zn during the 14 d treatment period (Figure 3). The addition of Zn strongly inhibited the growth of the shoots *T. arvense* in the absence of salt. The combination of Zn and 50 mM salt treatment almost totally inhibited the shoot growth of *T. arvense.*

Salt effects on metal accumulation

The salt treatments reduced the concentration of Zn accumulated in the shoots of the *Thlaspi* species (Figure 4). Thus, in the high Zn treatments, both the 25 and the 50 mM salt treatments significantly reduced the concentration of Zn that was hyperaccumulated in the shoots of *T. caerulescens* Prayon and Close House (both $P < 0.05$). For *T. caerulescens* from both Prayon and Close House, the 50 mM NaCl treatment caused the greatest reduction in Zn concentration in the shoots (44%, $P < 0.05$). In the case of Close House the Zn concentration in the shoots of the 50 mM NaCl treatment was only 25% of that in the no-salt control (4,220 mg kg^{-1} compared with 17,990 mg kg^{-1}). *Thlaspi arvense* is a non-accumulator of Zn and had much lower concentrations of Zn in its shoots than *T. caerulescens* and the salt treatments had lesser effects on the concentration of Zn in the shoots of the Zn-treated *T. arvense* than on the hyperaccumulator species (Figure 4). There was no significant effect of the 25 mM salt treatment on the concentration of Zn in the shoots.

Metal effects on Na accumulation

The sodium concentrations in the shoots of the *Thlaspi* species were very variable between replicates within a treatment as indicated by the large standard errors (Table 1). However, all NaCl treatments significantly increased the concentration of Na in the shoots of *T. caerulescens* and *T. arvense*. The Na concentrations in the shoots of *T. caerulescens* (both Prayon and Close House) increased from 2–5 mg g^{-1} in the no-salt treatment (note that the no-salt treatments received a moderate Na concentration from the Fe(III)-Na-

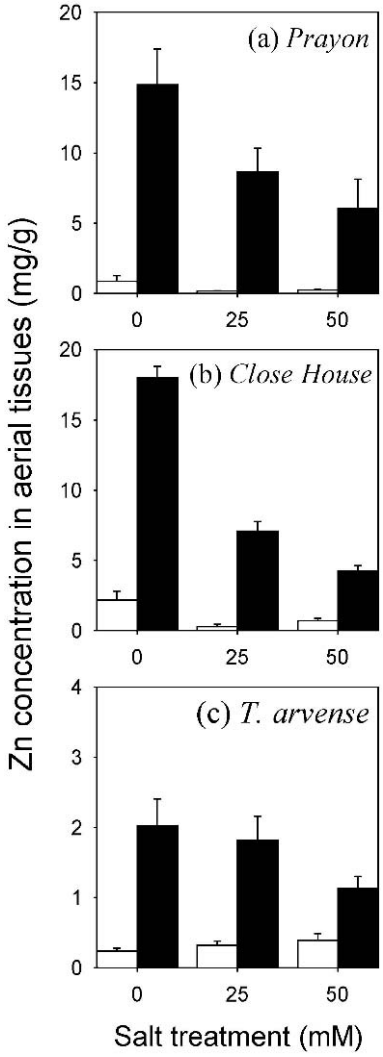

Figure 4. Effect of salt stress and metal supply on the concentration of Zn in the shoots of (a) *T. caerulescens* Prayon, (b) *T. caerulescens* Close House and (c) T. arvense seedlings grown in solution culture. Low Zn treatment solutions (open bars) contained 1 μM Zn^{2+}; high Zn treatment solutions (black bars) contained 100 μM Zn^{2+} for the *T. caerulescens* races and 20 μM Zn^{2+} for *T. arvense.*

EDTA in the nutrient medium) to almost 60 mg g^{-1} (6%) in the 50 mM NaCl treatment. Notably, the Na concentration in the shoots of *T. caerulescens* exposed to 25 and 50 mM NaCl were approximately double those in *A. murale* exposed to the same NaCl concentrations. *Thlaspi arvense* had similar Na concentrations to *T. caerulescens* in the 0 and 25 mM NaCl treatments, but reached only 35 mg g^{-1} in the 50 mM treatment (Table 1).

Discussion

This research appears to be the first test of salt tolerance in metal-hyperaccumulating species. Of the three species tested, *A. murale* proved to be the most tolerant to salt during seedling emergence (germination). In contrast, *T. caerulescens* and the non-accumulator *T. arvense* tended to be salt sensitive, which may be due to the inability of their seeds to overcome external osmotic potential and take up water for embryo expansion (Al-Niemi et al., 1992; Croser et al., 2001). This raises interesting questions about the contrasting ability of *A. murale* to germinate under the same stress. Perhaps this is an indication of adaptation to the xeric Mediterranean environments to which *A. murale* is native.

In the hydroponic experiments (early seedling-development), *Alyssum murale* was more tolerant to NaCl than *T. caerulescens* and *T. arvense*, which is consistent with Whiting et al. (2003) who found that *A. murale* was more tolerant to drought than *T. caerulescens* (osmotic stress applied using polyethylene glycol as an osmoticum in the growth medium). Similarly, Angle et al. (2003) found that *A. murale* was tolerant to drought stress when grown in soil culture. Despite being salt tolerant, the tolerance to only 100 mM NaCl showed that *A. murale* is not a halophyte. A review by Greenway and Munns (1980) indicated that halophytes can tolerate over 400 mM NaCl; salt tolerant crop species such as barley or cotton can tolerate 100 mM NaCl.

Exposing germinating seeds and early-growth seedlings of *A. murale* to salt indicated different threshold concentrations for growth inhibition. We hypothesized that the germinating seeds might be the most susceptible to the effects of NaCl, but this proved not to be true. One explanation is that the germinating seeds are self-reliant in terms of resources, the energy and mineral nutrients being supplied by the cotyledons, and are therefore not as dependent on their environment. In contrast, the growing seedlings are reliant on their roots to take up mineral nutrients and water. High concentrations of salt can inhibit these processes directly via toxic effects of the Na^+ and Cl^- ions on the root cells, or indirectly via osmotic effects, salt induced reductions in hydraulic conductivity or via competition at the sites of nutrient uptake in the cell membrane resulting in insufficient nutrient ion availability (Evlagon et al., 1990; Marshner, 1995;

Neumann, 1995, 1997). This study confirms the importance of defining growth stage when investigating plant salt tolerance.

The concurrent supply of high concentrations of Ni or Zn with the salt treatments had no effect on the germination rates of the seedlings of any of the species. However, Ni treatment of early-growth seedlings of *A. murale* resulted in more shoot growth at all NaCl concentrations than in the absence of Ni supply. It might, at first, appear that the high Ni uptake is improving plant resistance to salt (osmotic) stress as proposed by Severne (1974) and Baker and Walker (1989). Whiting et al. (2003) recently rejected this hypothesis because hyperaccumulation of Ni in *A. murale* and of Zn in *T. caerulescens* did not augment resistance to drought stress. In the current study, it is more probable that the Ni treatment directly stimulated the growth rate of non-salinized *A. murale*. This stimulatory effect was progressively lost as external concentrations of NaCl increased, despite the high levels of accumulated nickel. Growth enhancements of metal-hyperaccumulating plants by elevated metal supply have been noted in the past. For example, Ni supply enhanced the biomass of the Ni-hyperaccumulator *Alyssum lesbiacum* (Krämer et al., 1996), and Zn supply enhanced the shoot biomass of the Zn hyperaccumulator *T. caerulescens* (e.g., Tolrà et al., 1995; Shen et al., 1997).

A key component of this study was to determine the effect of high salt concentrations in the growth medium on the hyperaccumulation of metals in the shoots. The hyperaccumulation of Ni by *A. murale* exposed to salt contrasted strongly with the hyperaccumulation of Zn by *caerulescens.* Salt had no effect on the concentration of Ni in the shoots of *A. murale*, which suggests that Na^{+} did not compete or interfere with Ni^{2+} uptake at the root cell membrane of *A. murale.* The opposite was seen for both races of *T. caerulescens*, with reduced Zn accumulation under salt stress. Although it has been shown that some metals will compete with Zn for uptake by *T. caerulescens* (e.g., Ni and Cd; Lloyd-Thomas, 1995), it is unlikely that the reduction in concentration seen here is due to direct competition of the Na for the Zn transporter in the root cell membrane. Indeed, the 25 mM Na supply is 250 times greater than the concentrations of Zn supplied and would be expected to be a far stronger competitor for uptake if Na and Zn both used the same transporters. The reduction in Zn accumulation by *T. caerulescens* is more probably a result of either direct damage of the NaCl to the root cells and/or of the strong osmotic and ionic effects exerted by the salt.

The accumulation of Na in the shoots was highly variable between replicates within treatments (large standard errors), between treatments, and also between species. The variation between replicates might indicate that the plants could not regulate the Na accumulation at these high external concentrations. Notably, Na accumulation by *A. murale* was only 50–60% of that in the two races of *T. caerulescens* in the 50 mM NaCl treatment, perhaps indicating that *A. murale* roots are better able to control Na uptake. There were visible symptoms of stress on the leaves of the *Thlaspi* species, including chlorosis and necrotic areas.

In conclusion, *A. murale* was the most salt resistant of the three species studied in terms of seedling emergence and survival of emerged seedlings, which might be due to the adaptation of *A. murale* to surviving in harsh serpentine soils (metal toxicity) in Mediterranean environments. *Alyssum murale* maintained high concentrations of Ni in its shoots irrespective of the NaCl addition, which will be important for phytoremediation/phytomining of Ni on soils with a salt burden. It also indicates that brackish water (e.g., mining wastewater) could be used to irrigate crops of *A. murale* intended for phytomining of Ni. The next experimental step is to test these theories in soil culture, using salt impacted serpentine soils and/or watering with saline water. It will also be instructive to determine the NaCl tolerance of these species at higher Ca supply (adding Ca to saline irrigation water might be a practical way to relieve salinity effects by reducing harmful Na uptake into hyperaccumulator plants when used on low Ca heavy metal polluted soils).

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