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Intra-specific variation in Ni tolerance, accumulation and translocation patterns in the Ni-hyperaccumulator *Alyssum lesbiacum*

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HIGHLIGHTS

- A hydroponic experiment performed using the Ni hyperaccumulator *Alyssum lesbiacum*.
- Different populations show significant variation in Ni tolerance and accumulation.
- *A. lesbiacum* populations differed in Ni translocation from roots to shoots.
- Seed Ni concentration was significantly correlated to shoot Ni accumulation.
- There was a significant positive relationship between tolerance and accumulation.

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ABSTRACT

A hydroponic experiment was conducted to investigate inter-population variation in Ni tolerance, accumulation and translocation patterns in *Alyssum lesbiacum*. The in vitro results were compared to field data (soil bioavailable and leaf Ni concentrations) so as to examine any potential relationship between hydroponic and natural conditions. Seeds from the four major existing populations of *A. lesbiacum* were used for the cultivation of plantlets in solution cultures with incrementally increasing Ni concentrations (ranging from 0 to 250 μmol L^{-1} $NiSO_4$). Ni accumulation and tolerance of shoots and roots, along with initial seed Ni concentration for each population were measured. The ratio of root or shoot length of plantlets grown in $NiSO_4$ solutions to root or shoot lengths of plantlets grown in the control solution was used as tolerance index. For the range of metal concentrations used, *A. lesbiacum* presented significant inter-population variation in Ni tolerance, accumulation and translocation patterns. Initial seed Ni concentration was positively correlated to shoot Ni accumulation. A significant positive relationship between tolerance and accumulation was demonstrated. Initial seed Ni concentration along with physiological differences in xylem loading and Ni translocation of each population, appear to be the determining factors of the significant inter-population variation in Ni tolerance and accumulation. Our results highlight the inter-population variation in Ni tolerance and accumulation patterns in the Ni-hyperaccumulator *A. lesbiacum* and give support to the suggestion that the selection of metal hyperaccumulator species with enhanced phytoremediation efficiency should be considered at the population level.

1. Introduction

Hyperaccumulators are plants that can accumulate exceptionally high amounts of metals in their above-ground biomass; metal concentrations in their dry mass are up to 100 times higher than in normal plants (Reeves and Baker, 2000). Presently over 450 Ni-hyperaccumulator species have been recorded (van der Ent et al., 2013), corresponding to about 2% of serpentine species worldwide. Although Ni is the most commonly hyperaccumulated metal (Baker and Brooks, 1989), the rarity of the Ni-hyperaccumulation trait in nature is apparent. The property of hyperaccumulation has acquired much interest not only for its biochemical and physiological uniqueness (Kazakou et al., 2008) but also for the potential use of the hyperaccumulator species in the remediation of heavy metal-polluted soils and in phytomining technology (Chaney et al., 2005, 2007).

Alyssum (Brassicaceae) is the genus with the greatest number of hyperaccumulator species (Baker and Brooks, 1989). Among them, *Alyssum lesbiacum* is a well-known Ni-hyperaccumulator (Brooks et al., 1979; Reeves et al., 1997; Kazakou et al., 2010) endemic to the serpentine soils of Lesbos Island Greece (Strid and Tan, 2002).

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It is one of the few Ni-hyperaccumulators for which significant progress has been made in identifying the mechanism of Ni uptake and hyperaccumulation (Krämer et al., 1996; Kerkeb and Krämer, 2003; Ingle et al., 2005) and the cellular compartmentation of Ni in its tissues (Krämer et al., 1997; Küpper et al., 2001; Smart et al., 2007). Furthermore, it has been proposed that its phytoextracting efficiency is not significantly reduced even in soils marginally contaminated with polycyclic aromatic hydrocarbons (PAHs) (Singer et al., 2007). In phytoremediation technology the ability of a species to tolerate and hyperaccumulate metals is more important than the trait of producing high biomass yields (Chaney et al., 1997).

Recently, Kazakou et al. (2010) demonstrated that different populations of *A. lesbiacum* living in their natural habitats present differences in Ni-hyperaccumulation related to soil Ni concentration. Intra-specific variation in Ni-hyperaccumulation has been also presented for other Ni-hyperaccumulating species (e.g. *Alyssum murale* (Massoura et al., 2004; Bani et al., 2009, 2010) and *Alyssum bertolonii* (Galardi et al., 2007a)). However, *A. lesbiacum* (Kazakou et al., 2010) and *A. bertolonii* (Galardi et al., 2007a) are the first 'micro-edaphic' endemic Ni-hyperaccumulating species (i.e. endemic species with populations that diverge in their ability to hyperaccumulate metals; Galardi et al., 2007a) for which the micro-edaphic factors are being studied in detail. This inter-population differentiation in Ni-hyperaccumulation showed by both species is comparable or, in some cases, higher to the inter-specific differences shown among the various hyperaccumulator species of the genus *Alyssum* (Kazakou et al., 2010). The previous observation supports the proposal that studies towards the selection of species with the highest phytoextraction ability should be conducted at the population level (Kazakou et al., 2010). In this context, the most efficient populations could be used both directly as phytoremediation crops themselves (Bani et al., 2009) and indirectly for the improvement of plant traits through selective breeding or as sources of genes for improvement of other remediation crops (Pollard et al., 2002).

The assessment of patterns of variation in hyperaccumulation and tolerance (sensu Baker, 1987) may enable us to elucidate the relationship between these two phenomena. The exact relationship between metal tolerance and hyperaccumulation has not been decoded and it is not yet known if this relationship is similar for all metals and/or species (reviewed by Pollard et al. (2002)). In general, tolerance is regarded as an adaptive trait in response to high soil metal concentrations (Pollard et al., 2002; Agrawal et al., 2012). The relationship between tolerance and hyperaccumulation has been mostly addressed for metallicolous and non-metallicolous populations (Wu et al., 2009; Mohtadi et al., 2012). However, the existence of micro-edaphic, endemic metal hyperaccumulators offers ideal model plant species for investigating the micro-evolution of inter-population differences in tolerance and hyperaccumulation due to adaptation in micro-edaphic conditions. Furthermore, as metals affect seed germination and seedling growth (reviewed by Kranner and Colville (2011)), the seed Ni concentration could be another factor influencing the plant Ni concentration (Reeves and Baker, 1984). However, the hypothesis that the accumulation capacity of the different populations of a metal hyperaccumulating species is related to initial seed Ni concentration has not been tested so far.

The present study aimed to evaluate the inter-population variation in Ni tolerance, accumulation and translocation patterns among the four populations of *A. lesbiacum*. Moreover it aimed to investigate any significant relationship between initial seed Ni concentration and Ni accumulation, to determine if any significant relationship between tolerance and accumulation exists, and to reveal any significant relationship between these two phenomena and soil bioavailable Ni and/or Ni hyperaccumulation from a previous field survey.

2. Materials and methods

2.1. Hydroponic culture

Seeds were collected randomly from more than 50 individuals in each of the four populations of *A. lesbiacum* giving coverage of the altitudinal and geographic ranges shown by this species on Lesbos. The four distant localities chosen support the only four large populations of *A. lesbiacum* (LO, Loutra; VA, Vatera; OL, Olympos and AM, Ampeliko) and are described in detail in Kazakou et al. (2010) and in Adamidis et al. (in press). Seeds were germinated for 4 d on floating trays in vessels containing a nutrient solution, pH 5.5 ± 0.1 with the following composition: KNO_3 60 μmol L^{-1}, $Ca(NO_3)_2{\cdot}4H_2O$ 30 μmol L^{-1}, $NH_4H_2PO_4$ 10 μmol L^{-1}, $MgSO_4{\cdot}7H_2O$ 20 μmol L^{-1}, $FeSO_4{\cdot}7H_2O$ 0.18 μmol L^{-1}, tartaric acid 0.9 μmol L^{-1}, H_3BO_3 4.6 μmol L^{-1}, $MnCl_2{\cdot}4H_2O$ 0.92 μmol L^{-1}, $CuSO_4{\cdot}5H_2O$ 0.03 μmol L^{-1}, $ZnSO_4{\cdot}7H_2O$ 0.077 μmol L^{-1}, and H_2MoO_4 0.06 μmol L^{-1} (Barzanti et al., 2011). A metal chelator was not included in the medium since Kerkeb and Krämer (2003) showed that Ni^{+2} is taken up by the roots of *A. lesbiacum* as the free aqueous cation, independent of the chelators and histidine presence. After germination, the trays with seedlings were placed in vessels with continuously aerated fresh nutrient solutions spiked with $NiSO_4$ solution to form increasing Ni concentrations (0-control-, 50, 100, 175 and 250 μmol L^{-1} $NiSO_4$). Ni concentration range was in line with that of other hydroponic cultures studying *Alyssum* species (e.g. Galardi et al. (2007b), Ghasemi and Ghaderian (2009), Ghasemi et al. (2009)). Four replicates were used for each population-by-treatment combination and the total number of vessels used was 80. After 12 d of growth, the floating trays were removed from the hydroponic solutions and the seedlings harvested and prepared for the appropriate measurements. The experiment was performed in a glasshouse with temperatures ranging from 18 to 23 °C between night and day.

2.2. Ni tolerance

After harvest the root and shoot lengths of at least 15 plantlets from each treatment were used as a measurement of the Ni toxic effect on the different populations (Baker and Walker, 1989). To compare the four populations we used the same tolerance index (TI) as that proposed by Galardi et al. (2007b), namely the ratio of root or shoot length of plantlets grown on $NiSO_4$ solutions to the root or shoot lengths of plantlets grown on the control solution.

2.3. Ni accumulation

The harvested plantlets were rinsed with distilled water and washed carefully with 10×10^3 μmol L^{-1} $CaCl_2$ at 4 °C for 10 min to remove any adsorbed metals (Gonnelli et al., 2001). The plantlets were separated into roots and shoots, pre-frozen at −20 °C and then freeze-dried for 48 h in a LabconcoFreeZone 4.5 laboratory apparatus, at −40 °C collector temperature under <5 mBar vacuum. This was followed by pulverization of the freeze-dried samples in a laboratory mixer-mill. The pulverized samples were digested with conc. HNO_3 in a Mars Xpress system (CEM), according to the US EPA's method 3051A (2007). Ni determination was performed by Flame Atomic Absorption Spectrometry (Perkin–Elmer 5100ZL). Ni concentrations in plant tissues were calculated on a dry weight basis. For simplicity the term Ni accumulation is used for describing both shoot and root Ni concentrations, although we acknowledge that the term Ni accumulation is used in case of Ni storage and that root Ni concentration is constituted by an unknown combination of both stored and transited Ni (Coinchelin et al., 2012).