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Exogenous treatments with phytohormones can improve growth and nickel yield of hyperaccumulating plants

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HIGHLIGHTS

- Ni hyperaccumulating plants were treated with gibberellins, cytokinins and auxins.
- Two commercial products increased biomass in four hyperaccumulating plant species.
- Phytohormones application generally reduced plant Ni accumulation.
- IAA treatments increased phytoextraction efficiency in hyperaccumulator species.

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ABSTRACT

The application of plant growth regulators (PGRs) or phytohormones could be an interesting option for stimulating biomass production of hyperaccumulating plants and, consequently, their metal phytoextraction capacity. The effect of exogenous applications of phytohormones (PGR) on the Ni phytoextraction capacity of four Ni hyperaccumulating species (*Alyssum corsicum*, *Alyssum malacitanum*, *Alyssum murale* and *Noccaea goesingense*) was evaluated. Four different commercially available phytohormones (B, C, K and P) based on gibberellins, cytokinins and auxins were applied to the plant aerial tissues. Each product was applied at three different concentrations (B1-3, C1-3, K1-3 and P1-3). The effect on biomass production was dependent on the species, the PGR type and the concentration at which it was applied. Two of the four products (K and P) consistently increased biomass production compared to untreated control plants in all four plant species. On the other hand, all four products led to a significant increase in the number of branches (and leaves in the case of *N. goesingense*) of all four species compared to control plants. Application of phytohormones generally led to a reduction in shoot Ni concentration. Nonetheless, in some cases as a consequence of the increase observed in biomass after the application of phytohormones a significant increase in the Ni phytoextraction efficiency was also observed (but this was species- and PGR type-dependent). The results show that PGRs can be successfully used to improve the growth and biomass production of hyperaccumulating species such as *Alyssum* and *Noccaea*. However, an increase in biomass did not always lead to a higher Ni removal, and the most effective PGR for increasing Ni removal was the IAA-based product.

1. Introduction

Plant growth regulators (PGRs) are a group of naturally occurring organic compounds that at low concentrations regulate physiological processes in plants (Pazurkiewicz Kocot, 2003). Nowadays, PGRs are used in many areas in agriculture, horticulture and floriculture for a wide range of purposes, such as increasing plant growth, delaying or promoting ripening, induction of rooting, lateral branching, promotion of abscission or weed control (Emongor, 1995). Numerous studies have shown positive effects on the growth and development of a wide range of forage, cereal or fruit crops after the application of PGRs (Nickell, 1982; Weaver, 1972). Kefeli and Kalevitch (2003) have classified PGRs into seven different groups: auxins, cytokinins, gibberellins, abscisic acid, brassinosteroids, salicylic acid and jasmonates. Each of these phytohormones is involved in different processes and affects the plant in a specific way. For example, auxins (such as indoleacetic acid, IAA) are known to stimulate cell elongation, growth of roots and shoots, and suppress the development of lateral buds (apical dominance) (Thimann and Skoog, 1934). On the other hand, cytokinins have been identified by Miller et al. (1955) for their ability to induce plant cell division, and gibberellins (GAs; such as GA_3, GA_4, GA_7) to regulate stem growth and elongation, induction of seed germination, and fruit setting and growth, etc. (Jones, 1973; Salisbury and Ross, 1992; Taiz and Zeiger, 2006). Cytokinins (CKs; such as kinetin, benzyladenine) have also been shown to play an important role in the regulation of

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plant response to environmental stress (Ha et al., 2012). Their effects on the plant vary according to the applied concentration, environmental factors influencing their absorption, and on the physiological status of the plant at the time of application (Carey, 2008).

Due to the fact that PGRs may stimulate plant growth or reduce abiotic stress their use has also been considered as a means of enhancing the efficiency of remediation techniques such as phytoextraction (Barbafieri and Tassi, 2010; Cabello-Conejo et al., 2013; Cassina et al., 2011). Phytoextraction is based on the cultivation of plants to accumulate trace metals from contaminated soils and transport them to the shoots which can then be harvested. Metal-hyperaccumulating plants are ideal candidates due to their extraordinary capacity to absorb and accumulate metals in their harvestable parts (Baker et al., 1994). In those cases where the economic value of the recovered metal is the primary motive the process is known as phytomining (Chaney, 1983). The technique of phytomining involves growing a crop of a metal-hyperaccumulating plant species, harvesting the biomass and burning it to produce a bio-ore. To be used in phytoextraction technologies, hyperaccumulators must be highly metal tolerant, able to accumulate large concentrations of the targeted trace elements in harvestable shoots, and have a reasonable biomass production so that metal removal from the site is economic (Li et al., 2003; Vangronsveld et al., 2009). The efficiency of the process can be limited by poor plant survival and growth, metal phytotoxicity or restricted soil metal bioavailability, making the application of PGRs a potential means of overcoming some of these bottlenecks. Three groups of PGRs have been proposed as being useful for phytoextraction purposes: auxins, cytokinins, and gibberellins (Bulak et al., 2014). Several studies have demonstrated that the application of IAA can increase shoot metal accumulation, resulting in a higher metal removal yield which is the primary objective of phytoextraction. Hadi et al. (2010) have observed that applying a foliar spray of IAA at a concentration of 0.175 mg L^{-1} significantly increases the total Pb accumulation in the plant. Liphadzi et al. (2006) have applied IAA on *Helianthus annuus* and observed an increased Pb and Cd accumulation in leaves. Recent studies have tested the effects of PGRs on Ni phytoextraction by hyperaccumulators within the *Alyssum* genus (Cabello-Conejo et al., 2013; Qiu et al., 2009). Cassina et al. (2011) have demonstrated that applications of cytokinins (Cytokin) to *Alyssum murale* grown in serpentine soil improves Ni phytoextraction (mg Ni pot^{-1}) due to a higher biomass production in treated plants compared to control plants. Likewise, Cabello-Conejo et al. (2013) have observed a positive effect on biomass production and Ni phytoextraction efficiency of *Alyssum corsicum* after applying a combination of gibberellins and cytokinins (Promalin), although the effects are not statistically significant. These studies highlight the need to study more types of PGRs for application in phytoextraction processes, as well as the optimum application method, concentration and timing of exogenous treatments.

The objective of this study was to evaluate a wider array of PGRs and to test these at a range of concentrations in contrasting Ni-hyperaccumulating species (*A. corsicum*, *Alyssum malacitanum*, *A. murale* and *Noccaea goesingense*). Plants were grown in serpentine soil and treated with four commercial products (based on combinations of indoleacetic acid, cytokinins and/or gibberellins). Effects on plant growth and biomass production, nutrient status and Ni phytoextraction efficiency were determined.

2. Materials and methods

2.1. Soil collection, analysis and preparation of experimental pots

The soil used in this experiment was collected from the serpentinitic region of Barazón, located in Galicia (NW Spain). Soil was air-dried, sieved through a 2-mm stainless steel sieve and mixed for pot preparation and soil analysis. The general properties of the soil used are given in Table 1. The soil had a pH_{H_2O} of 6.7 and as expected for a serpentine soil, presented high concentrations of Ni, Co and Cr (2092, 175 and 1346 mg kg^{-1}, respectively), and a Ca/Mg ratio <1. Soil organic C and N were 1.97% C and 0.15% N. Basal fertilisers were added to the soil and thoroughly mixed to obtain a homogenous mixture. Nitrogen was added at 100 kg ha^{-1} as NH_4NO_3, phosphorus and potassium were added as K_2HPO_4 at 100 kg ha^{-1} and 125 kg ha^{-1}, respectively. In addition the soil was mixed with perlite in the ratio of 10:1 (v/v) to improve aeration and drainage. After mixing, approximately 700 g of soil was weighed into each pot. A total of 312 pots of 12.5 cm diameter were used.

Table 1
Physicochemical characteristics of the serpentine soil used in the experiment.

Soil properties	
pH_{H_2O}	6.7 ± 0.0
% C	1.97 ± 0.04
% N	0.15 ± 0.01
CEC (cmol kg^{-1})	4.48 ± 0.62
Ca/Mg	0.32 ± 0.02
$Sr(NO_3)_2$-extractable Ni (mg kg^{-1})	3.63 ± 0.02
Available P (Olsen) (mg kg^{-1})	11.49 ± 0.98
Pseudo-total metal concentration (mg kg^{-1})	
Co	174.6 ± 4.7
Cr	1346 ± 9.5
Cu	16.9 ± 0.2
Mn	1884 ± 44
Ni	2092 ± 16
Zn	28.4 ± 1.1

2.2. Germination and plant growth conditions

Four Ni-hyperaccumulating plant species were used: *A. corsicum*, *A. malacitanum*, *A. murale* and *N. goesingense* (previously named as *Thlaspi goesingense*). Seeds of *A. corsicum* were collected from Turkey (Koycegiz), and *A. murale* 'Kotodesh' from Albania. *A. malacitanum* is endemic to the Iberian Peninsula (Asensi et al., 2004; Brooks et al., 1981) and seeds were collected from Sierra Bermeja, Málaga (S Spain). Seeds of *N. goesingense* were collected from Redlschlag (E Austria). Seeds were germinated on a perlite:quartz sand mixture (2:1 v/v) in a growth chamber under controlled conditions (temperature 22–25 °C, PPFD of 190 mmol m^{-2} s^{-1}, under a 16/8 h light/dark cycle). Seeds were watered daily with deionised water until germination and then twice per week with a serpentine-like macro-nutrient solution which consisted of 2 mM $MgSO_4$, 0.8 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 0.1 mM K_2HPO_4, 20 μM FeEDDHA, 10 μM H_3BO_3, 2 μM $MnCl_2$, 1 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.2 μM Na_2MoO_4 and 300 μM $NiSO_4$ (Chaney et al., 2008). Three-month-old seedlings (2–3 cm tall) were transferred into the pots containing the serpentine soil.

2.3. Treatments with plant growth regulators

Seedlings were grown in pots for one month before applying any treatments so as to allow them to adapt to the new substrate and recover from transplantation. After this adjustment period, four different commercially available phytohormones were applied: B (Berelex-L® purchased from Kenogard, Barcelona, Spain), C (from Daymsa, Zaragoza, Spain), K (from Daymsa, Zaragoza, Spain) and P (Promalin® purchased from Kenogard, Barcelona, Spain). Berelex is based on gibberellic acid (also called GA_3) (16,000 mg L^{-1}). C is based on natural seaweed extracts and has a cytokinic activity equivalent to 400 mg L^{-1} kinetin. K is also derived from marine algae extracts and contains 11 mg L^{-1} auxins (IAA, indoleacetic acid). Both C and K may contain small quantities of other plant growth promoting substances, such as polysaccharides, micronutrients and/or vitamins, but are free of heavy metals. Both products are certified treatments for use in organic agriculture (Council Regulation (EC) No 834/2007). Promalin is a mixture of cytokinins (benzyladenine) and gibberellins (GA_4 and GA_7) in a 1:1 ratio.