Chemosphere 80 (2010) 901–907

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS)

Erika Fässler [a,*], Michael W. Evangelou [a], Brett H. Robinson [b], Rainer Schulin [a]

[a] Institute of Terrestrial Ecosystems, ETH Zurich, Universitätstr. 16, 8092 Zurich, Switzerland
[b] Agriculture and Life Sciences Division, Lincoln University, Lincoln 7647, Canterbury, New Zealand

ARTICLE INFO

Article history:
Received 8 March 2010
Received in revised form 28 April 2010
Accepted 29 April 2010
Available online 26 May 2010

Keywords:
IAA
EDDS
Zn
Pb
Sunflower
Stress alleviation

ABSTRACT

The use of plants for phytoextraction of heavy metals from contaminated soil is limited by the ability of the plants to grow on these soils and take up the target metals, as well as by the availability of the metals for plant uptake in the soil solution. The hypotheses of this study were that the growth-promoting phytohormone auxin (indole-3-acetic acid, IAA) can alleviate toxic effects of metals on plants and increase metal phytoextraction in combination with the biodegradable chelating agent ethylene diamine disuccinic acid (EDDS). To test these hypotheses we performed two sets of experiments with sunflowers (*Helianthus annuus* L.) in hydroponic solution. In the first set of experiments, five IAA concentrations (0, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} M) were applied in combination with Pb (2.5 μM) or Zn (15 μM). In the second set of experiments we applied combinations of IAA (0 or 10^{-10} M) and EDDS (0 or 500 μM) to Pb or Zn-stressed sunflowers.

Root and shoot growth of metal-stressed plants were most effectively increased with 10^{-10} M IAA, and also the extraction of both metals was significantly increased at this treatment level. IAA reduced the negative metal effects, such as reduced shoot and root dry weight, root length, root volume and root surface area. EDDS significantly decreased metal uptake by the plants, thus reducing metal stress and promoting plant growth. The combined application of IAA with EDDS significantly increased Zn uptake in comparison to EDDS only treated plants. The experiments indicate that IAA can alleviate toxic effects of Pb and Zn on plant root and shoot growth and can in combination with chelants such as EDDS increase the phytoextraction potential of these plants.

1. Introduction

Phytoextraction potentially is an attractive strategy to clean up metal-polluted agricultural soils, provided that sufficiently high extraction rates can be achieved. Restrictions on phytoextraction are given by the plant's ability to grow in the polluted soil, to take up the target metals and by the availability of the target metals in the soil for plant uptake. Only metals that are in the soil solution can be taken up by plants. The uptake takes place at the interface between soil solution and root tissue. The larger the contact area between roots and soil solution, the higher the potential uptake. Due to the formation of special tissue layers with suberin incrustations in their cell walls, in particular the epidermis layer with the well-known casparian strips between root cortex and stele, there is a gradient in the uptake of water and solutes along the root axis, declining from the apex to the basal zones. These layers act as an efficient barrier against the uptake of aqueous solutes via the apoplast (Marschner, 1995). These barriers are not fully developed at the root tips and also become disrupted in the basal zones where emerging lateral roots penetrate the cortex, forming leaks for uncontrolled apoplastic uptake of solutes into the xylem of the roots (Haynes, 1980; Clarkson, 1996; Tandy et al., 2006a).

Increasing root surface area, in particular of those zones with incomplete or leaky endo- and exodermis layers, may be helpful to increase metal uptake in phytoextraction. The growth-promoting phytohormone auxin is known to induce root growth by enhancing cell division, cell extension and inducing lateral root growth (Taiz and Zeiger, 2000). Rhizosphere bacteria, such as some strains of *Pseudomonas* and *Acinetobacter*, were found to produce indole-3-acetic acid (IAA), the most common auxin in plants, and thereby stimulate root elongation and lateral root production (Lippmann et al., 1995). Root inoculation with rhizosphere bacteria strains had similar effects on root morphology as exogenous IAA application (Lippmann et al., 1995). In particular IAA increased root and sometimes also shoot growth of plants that were stressed by salinity or heavy metals (Chaudhry and Rasheed, 2003; Sheng and Xia, 2006; Egamberdieva, 2009). Leinhos and Bergmann

* Corresponding author. Tel.: +41 44 633 61 43; fax: +41 44 633 11 23.
E-mail addresses: erika.faessler@env.ethz.ch (E. Fässler), michael.evangelou@env.ethz.ch (M.W. Evangelou), Brett.Robinson@lincoln.ac.nz (B.H. Robinson), rainer.schulin@env.ethz.ch (R. Schulin).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.04.077

(1995) found that IAA alleviated drought stress and suggested that exogenously applied IAA may serve in mediating morphological reactions of plants in response to stresses, in particular by increasing root growth. The growth-promoting effect of auxin on stressed plants may be used for phytoextraction purposes.

In order to enhance phytoextraction it has been proposed to increase limited availability of polluting soil metals for plant uptake by adding chelating agents such as ethylene diamine tetraacetic acid (EDTA), nitrilotriacetic acid (NTA) or ethylene diamine disuccinic acid (EDDS) to the soil (Blaylock et al., 1997; Huang et al., 1997; Kulli et al., 1999; Tandy et al., 2006b). Increased metal availability may, however, also increase metal stress to plants. Tandy et al. (2006b) showed that the application of EDDS to soil, while increasing the uptake of Cu and Pb by sunflowers, decreased plant dry weight in pot experiments. Liu et al. (2007) found that the adverse effect of chelating agents on plant growth could be reduced by the application of IAA. López et al. (2005) furthermore found that the addition of IAA together with EDTA increased Pb uptake by alfalfa.

Based on these findings, the aim of this study was to test the hypotheses that: (1) auxin is capable of alleviating metal stress on plants, and (2) to test the potential of auxin to increase metal phytoextraction in combination with a chelant. As experimental system we chose sunflowers grown in hydroponic solution, treated with the heavy metals Pb and Zn, the auxin IAA and the chelating agent EDDS. Hydroponics were chosen because we were interested in the effects of these chemicals in solution on plant growth and metal uptake, separating them from effects of the treatments on metal partitioning between soil solution and solid phases.

2. Materials and methods

2.1. Experimental setup

In preliminary experiments we screened sunflower growth at Pb concentrations of 125, 62.5, 31, 15.5, 7.8, 3.9, 2.5 and 1 μM and Zn concentrations of 122, 61, 30.5 and 15 μM in nutrient solution to determine at which concentrations the metals stunted plant growth without killing them. On the basis of these experiments we chose treatments with 2.5 μM Pb and 15 μM Zn for this study.

We performed two sets of experiments (Table 1). In the first set of experiments we applied IAA concentrations between 10^{-9} and 10^{-12} M as well as no IAA (control), with and without metal stress (15 μM Zn, 2.5 μM Pb or no metal). The treatments were replicated four times. In the second set of experiments we grew seedlings in solutions with Zn, Pb or no metal, and applied IAA (0 or 10^{-10} M) and EDDS (0 or 500 μM) in four combinations to each metal treatment. Each treatment was replicated five times.

All experiments were carried out in a climate chamber at a 16 h (22 °C)/7 h (15 °C) day/night cycle with 0.5 h transition times between day and night phases. Seeds of *Helianthus annuus* L. (cv. Sanluca) were germinated in silica sand. Six days old seedlings were transferred into aerated brown 1-L bottles (one seedling per bottle) containing nutrient solution. Two different nutrient solutions were prepared. The first was a modified 10% Hoagland nutrient solution in which NaFe(II)EDTA was replaced by $FeSO_4 \cdot 7H_2O$ to avoid interference between EDTA and EDDS. The other solution was the treatment solution containing metal contaminant, auxin and chelant according to the before-mentioned treatments. Here, all micronutrients and KH_2PO_4 were omitted to avoid competition between metals and metal precipitation, in particular of Pb phosphate. To maintain a solution pH of 6, 2 mM 2-(N-morpholino)ethanesulfonic acid was added as buffer to both solutions. The solutions were always freshly prepared before application and alternately applied for periods of 3 d for a total of 18 d, starting with the Hoagland solution, so that the two solutions were each applied three times during the experiment. This alternation maximized the treatment time while avoiding P and micronutrient deficiency stress.

2.2. Root morphometry

At harvest, plants were immediately separated into roots and shoots and weighed.

Table 1
Root growth parameters. Mean values of root growth parameters (dry weight, volume, surface area, length, diameter and density) of sunflowers grown in the IAA screening experiment with Pb or Zn (set 1) and in the IAA + EDDS with Pb or Zn (set 2). Standard errors of the means are in parentheses (set 1: $n = 4$, set 2: $n = 5$). Different letters indicate significant differences between the treatments ($p < 0.05$).

Experiment	Treatment	Volume (cm^3)	Surface area (cm^2)	Length (m)	Diameter (mm)	Density (mg cm^{-3})
1, Pb	Control	3.26 (0.15) a	303 (18) a	2.27 (0.19) a	0.45 (0.01) a	35 (1) a
	2.5 μM Pb	1.92 (0.16) b	188 (15) b	1.48 (0.11) b	0.42 (0.00) b	40 (4) ab
	10^{-10} M IAA	5.03 (0.19) d	530 (30) c	4.49 (0.36) c	0.39 (0.01) b	35 (0) a
	2.5 μM Pb + 10^{-10} M IAA	2.58 (0.24) c	245 (26) a	1.87 (0.24) ab	0.44 (0.02) a	42 (1) b
1, Zn	Control	2.46 (0.16) ab	394 (24) a	5.08 (0.39) a	0.26 (0.01) a	76 (3) a
	15 μM Zn	1.63 (0.27) c	260 (39) b	3.32 (0.49) b	0.25 (0.01) a	63 (5) b
	10^{-10} M IAA	2.80 (0.34) a	419 (63) a	5.04 (0.91) a	0.27 (0.01) a	70 (0) ab
	15 μM Zn + 10^{-10} M IAA	1.75 (0.13) bc	265 (17) b	3.22 (0.19) b	0.27 (0.00) a	78 (2) a
2, Pb	Control	2.83 (0.30) ac	283 (33) a	2.27 (0.29) a	0.41 (0.01) ad	35 (1) abc
	2.5 μM Pb	2.35 (0.23) a	230 (26) a	1.79 (0.23) a	0.41 (0.01) a	38 (1) c
	10^{-10} M IAA	3.08 (0.27) ab	300 (26) ac	2.34 (0.21) a	0.41 (0.00) a	33 (1) a
	2.5 μM Pb + 10^{-10} M IAA	3.92 (0.48) bc	414 (54) bc	3.50 (0.50) b	0.39 (0.01) cd	37 (0) cb
	500 μM EDDS	4.13 (0.41) b	492 (40) b	4.70 (0.34) b	0.34 (0.01) be	35 (1) abc
	500 μM EDDS + 2.5 μM Pb	3.97 (0.48) b	437 (42) b	3.84 (0.27) b	0.36 (0.01) cf	33 (2) ab
	500 μM EDDS + 10^{-10} M IAA	3.61 (0.35) bc	411 (34) b	3.75 (0.25) b	0.35 (0.01) ef	36 (1) abc
	500 μM EDDS + 2.5 μM Pb + 10^{-10} M IAA	4.12 (0.56) b	458 (58) b	4.07 (0.48) b	0.36 (0.01) c	34 (1) abc
2, Zn	Control	3.78 (0.43) ac	397 (48) ae	3.34 (0.44) ad	0.39 (0.00) a	37 (1) ac
	15 μM Zn	2.78 (0.29) b	286 (27) bd	2.36 (0.19) b	0.36 (0.03) a	40 (1) c
	10^{-10} M IAA	2.52 (0.29) b	255 (29) bd	2.06 (0.24) b	0.40 (0.00) a	35 (1) ad
	15 μM Zn + 10^{-10} M IAA	2.85 (0.24) bc	306 (28) ad	2.62 (0.26) bd	0.38 (0.00) a	41 (1) c
	500 μM EDDS	4.34 (0.32) a	535 (33) c	5.26 (0.27) c	0.33 (0.00) a	34 (1) ad
	500 μM EDDS + 15 μM Zn	3.69 (0.41) ac	411 (40) ae	3.66 (0.32) a	0.36 (0.01) a	34 (1) ad
	500 μM EDDS + 10^{-10} M IAA	3.44 (0.35) ab	399 (43) a	3.69 (0.42) a	0.35 (0.00) a	35 (1) ad
	500 μM EDDS + 15 μM Zn + 10^{-10} M IAA	4.31 (0.27) a	514 (25) ce	4.88 (0.17) c	0.24 (0.06) b	33 (1) bd