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Iron plaque formed under aerobic conditions efficiently immobilizes arsenic in *Lupinus albus* L roots☆

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ABSTRACT

Arsenic is a non-threshold carcinogenic metalloid. Thus, human exposure should be minimised, e.g. by chemically stabilizing As in soil. Since iron is a potential As immobiliser, it was investigated whether root iron plaque, formed under aerobic conditions, affects As uptake, metabolism and distribution in *Lupinus albus* plants. White lupin plants were cultivated in a continuously aerated hydroponic culture containing Fe/EDDHA or $FeSO_4$ and exposed to arsenate (5 or 20 μM). Only $FeSO_4$ induced surficial iron plaque in roots. LA-ICP-MS analysis accomplished on root sections corroborated the association of As to this surficial Fe. Additionally, As(V) was the predominant species in $FeSO_4$-treated roots, suggesting less efficient As uptake in the presence of iron plaque. Fe/EDDHA-exposed roots neither showed such surficial Fe–As co-localisation nor As(V) accumulation; in contrast As(III) was the predominant species in root tissue. Furthermore, $FeSO_4$-treated plants showed reduced shoot-to-root As ratios, which were >10-fold lower compared to Fe/EDDHA treatment. Our results highlight the role of an iron plaque formed in roots of white lupin under aerobic conditions on As immobilisation. These findings, to our knowledge, have not been addressed before for this plant and have potential implications on soil remediation (phytostabilisation) and food security (minimising As in crops).

1. Introduction

The concentration of arsenic in non-contaminated soils rarely exceeds 10 mg kg^{-1}, but several processes can result in elevated concentrations. Natural processes and, predominantly, anthropogenic activities such as the use of pesticides, mining and smelting of arsenic-containing minerals, have led to the spread of this trace element in the environment (Fitz and Wenzel, 2002). As a consequence, high concentrations of arsenic (As) have been found in groundwater of several countries, such as India and Bangladesh, exceeding the limit established by the WHO of 10 μg L^{-1} for drinking water. Arsenic is commonly found in soils as oxyanionic compounds. In oxidized soils the major species is As(V), while As(III) proportion increases in anoxic soils (Smith et al., 1998). Plants take up As from soils and accumulate it in their tissues (Zhao et al., 2009). It is a main chemical contaminant in plant-based food and therefore e.g. the European Food and Safety Authority has recommended to actively lower As in agricultural crops (EFSA, 2009). As a consequence, there is a high demand for economical remediation techniques, such as phytoremediation or *in situ* phytostabilisation. This approach is based on the immobilisation of contaminants in the soil using plants and soil amendments with the aim of decreasing the labile contaminant fraction (Vangronsveld et al., 2009; Kumpiene et al., 2012). The addition of iron amendments, such as iron oxides, iron (sulfate) salts or iron-rich by-products, has been proven to be an efficient tool for arsenic stabilization in soils. Their supply leads to the formation/accumulation of reactive oxyhydroxides in the soil, which strongly sorb As (Kumpiene et al., 2006; Komárek et al., 2013). The immobilisation process is more effective when iron oxides are formed *in situ* and seems to be stable in a long-term scale (Hartley et al., 2004).

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The re-vegetation of contaminated soils requires the use of plants that can tolerate water stress, high soil acidity and salinity as well as high pollutant concentration. Adequate plants for phytostabilisation strategies are those able to exclude metals and metalloids or to accumulate them in their roots in order to avoid transfer to the food chain (Mendez and Maier, 2008). *Lupinus albus* L. is an annual legume that has been previously proposed as a good candidate for phytoremediation strategies on As-contaminated soils. Some of its properties, such as its N_2-fixing capacity, the formation of proteoid roots to improve nutrient uptake and its tolerance to acidic soils and to As, enable this plant to survive on As-contaminated soils (Vázquez et al., 2006; Martínez-Alcalá et al., 2010). Due to its suitability for human and animal nutrition and its ability to improve soil structure (Huyghe, 1997), white lupin has also high agronomic potential.

Iron plaque formation on the roots of submerged plants has been described on several occasions (Siqueira-Silva et al., 2012; Zimmer et al., 2011). In such reducing environments, Fe(III) is reduced to Fe(II). By releasing O_2, aquatic plants create oxidising microenvironments around their roots. Fe(II) re-oxidises to Fe(III), which precipitates on the root surface, creating an iron plaque layer (Blute et al., 2004). The capacity of this iron plaque to sequester metals and metalloids from soil solution is well known, besides its possible role as a buffer and reservoir in nutrient uptake (Tripathi et al., 2014; Williams et al., 2014). This plaque has been predominantly studied in flooded rice. Rice iron plaque sequesters As mainly in amorphous and crystalline iron (oxy)hydroxides (Liu et al., 2006). It has been considered as a mechanism to restrict As uptake and translocation to shoots (Liu et al., 2004), but also as a pool that may increase As uptake and accumulation by plants or the supply of arsenic into the soil solution (Huang et al., 2012).

In this work we evaluated how different sources of iron supplied to *Lupinus albus* L. grown in hydroponic conditions influence arsenic uptake, distribution and speciation in plant tissues. Our aim was to induce iron (hydro)oxide precipitation on the root surface using continuously aerated conditions and to investigate the impact of this iron plaque on As immobilisation and transfer as well as on the As metabolism in the plant.

2. Materials and methods

2.1. Plant pre-cultivation

Lupinus albus (cv. Marta) seeds were sterilized in a 1% sodium hypochlorite solution and germinated on moist paper for 3 days at 28 °C. After germination, uniform seedlings were transferred to PVC pots (4 seedlings per pot) containing 3.5 L of a continuously aerated nutrient solution. The composition of the nutrient solution was (mM) $Ca(NO_3)_2$ 1.5, KNO_3 1.5, $MgSO_4$ 1.0, KH_2PO_4 0.1, K_2SO_4 0.75; (μM) NaCl 100, $MnSO_4$ 27.3, $ZnSO_4$ 1.6, $CuSO_4$ 1.6, $NiCl_2$ 1.0, $CoCl_2$ 1.0, $(NH_4)_6Mo_7O_{24}$ 1.0, H_3BO_3 20 and Fe^{3+}/EDDHA 53.8 (3 mg L^{-1} Fe). The pH of the solution was adjusted to 5.5–6 using KOH or HNO_3 and the nutrient solution was changed every 7 days. The experiment was carried out in a growth chamber under controlled conditions (night/day): T 20/25 °C, photoperiod 11/13 h, relative humidity 60/40%. The photon flux density during the light period was 520 μmol m^{-2} s^{-1}.

2.2. Iron and arsenic treatments

After four weeks of growth, the iron source was changed for half of the plants as follows: prior to the start of the iron treatment the plants were put into aerated, deionized water overnight. Then they were transferred to a nutrient solution containing 30 mg L^{-1} of ferrous iron, added as $FeSO_4 \cdot 7H_2O$ (p.a. reagent, Panreac, Barcelona, Spain). This treatment was therefore called *$FeSO_4$*. The other half of the plants were kept in the same nutrient solution in which they were previously grown. This treatment was therefore called *Fe/EDDHA*. Three days later, when a brownish coating was visible on the surface of roots treated with $FeSO_4$, plants from both iron treatments were exposed to 5 μM or 20 μM of arsenate added as $Na_2HAsO_4 \cdot 7H_2O$ by changing the entire nutrient solution, maintaining the corresponding iron treatment (in the following these treatments will be referred to as $FeSO_4$*As5 and $FeSO_4$*As20 or Fe/EDDHA*As5 and Fe/EDDHA*As20). A control (no As) was also included for each iron treatment. Four replicates were accomplished for each treatment with 4 plants in each pot, *i.e.* four pots and sixteen plants per treatment (24 pots in total). All nutrient solutions were adjusted to pH 5.5–6, continuously aerated and changed every five days. Plants were exposed to As for ten days.

2.3. Sampling and plant processing

After ten days of As exposure, all plants were harvested, washed thoroughly with tap water and distilled water and separated into shoots and roots. One plant of each pot, previously washed as explained before and separated into roots and shoots, was dried at 65 °C for 72 h, weighed and ground using a ball mill. 0.25 g of dry, ground plant material (shoots and roots) was digested in an autoclave using 3 mL of HNO_3 (65%, w/w), 1.5 mL of H_2O_2 (30%, w/w) and 4 mL of purified water (18 MΩ cm, reagent grade type I) under a pressure of 1.5 kg cm^{-2} for 30 min (Lozano-Rodríguez et al., 1995). One intact root from each pot was sampled separately and was incubated for one hour at room temperature in 40 mL 0.03 M sodium citrate and 0.125 M sodium bicarbonate, with the addition of 3 g sodium dithionite (DCB method) (modified from Taylor and Crowder, 1983). Then DCB incubating solutions were collected and roots were washed three times with distilled water, which was added to the DCB extracts and made up to 100 mL. The washed roots were dried at 65 °C for three days and digested as explained above. This iron plaque-extraction (DCB) method was applied for both iron treatments for comparing the results between $FeSO_4$- and Fe/EDDHA-treated roots. The differences in As, Fe and P concentrations between DCB-unwashed roots and DCB-washed roots were used to evaluate the distribution of these elements in the roots. For arsenic speciation analysis in plant tissues, one plant of each pot was frozen at −80 °C. Frozen shoots and roots were ground in liquid nitrogen using a mortar and pestle. Between 0.25 g and 0.5 g of the ground material were extracted with a phosphate buffer solution (PBS), containing 2 mM NaH_2PO_4 and 0.2 mM Na_2-EDTA (Sigma-Aldrich) at pH 6.0, for 1 h under sonication (Xu et al., 2007). The extracts were collected and filtered through 0.45 μm syringe filters and stored at −20 °C until their analysis.

2.4. Analytical methods

Arsenic and iron concentrations in plant digests were analysed by HG-AFS (PS Analytical, 10.055, Millennium Excalibur system, Kent, UK) and Atomic Absorption Spectrometry (Analyst 800, Perkin Elmer, Waltham MA, US), respectively. Arsenic speciation in the PBS extracts was accomplished by HPLC-HG-AFS (HPLC Agilent 1260 Infinity, Agilent, Santa Clara, USA, and HG-AFS PS Analytical 10.055, Millenium Excalibur). 100 μL aliquots of the filtered extracts were injected into the HPLC system. The isocratic separation of the arsenic species was carried out with a Hamilton PRP-X100 10 μm anion-exchange column (250 × 4.1 mm) (Hamilton, Reno, USA). An aqueous solution consisting of 20 mM Na_2HPO_4/NaH_2PO_4 adjusted to pH 6.25 was used as mobile phase at 1 mL min^{-1} flow rate. Aqueous standard solutions of As(V), As(III), DMA and MMA, prepared by dissolving appropriate amounts of $Na_2HAsO_4 \cdot 7H_2O$,