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Salinity influences arsenic resistance in the xerohalophyte *Atriplex atacamensis* Phil.

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ABSTRACT

Arsenic (As) is a highly toxic element accumulating in the environment as a result of anthropogenic activity. In Northern Chile it accumulates in salt areas and constitutes a major threat for human population. *Atriplex atacamensis* is a perennial halophyte shrub spontaneously growing in these contaminated areas. In order to determine the impact of salinity on As absorption and accumulation in *A. atacamensis*, young seedlings obtained from seeds collected on an As-polluted area were cultivated for 2 and 4 weeks in nutrient solutions containing no As and no NaCl (control), NaCl 100 mM, As(V) 1000 μM and NaCl 100 mM + As(V) 1000 μM. Speciation analysis indicates that arsenic in the solution remained in the As(V) form. Roots accumulated high concentration of As (up to 5020 $\mu g\, g^{-1}$ DW): 55% of total extractable As was in the reduced state As(III) and 45% as As(V). More than half of the total As accumulated in cell walls. The leaves accumulated lower amounts of total As, with a majority of As(V) in the extractable fraction. Addition of NaCl to the As-containing nutrient solution reduced As concentration in the roots and increased the proportion of As(V) comparatively to As(III). Salinity strongly increased As translocation from the root to the shoot but did not modify its distribution between apoplasm and symplasm compartments. Exogenous salinity reduced the As-induced senescing ethylene synthesis in As-treated plants and contributed to a more efficient antioxydation system. Salinity and As induced the synthesis of major protective molecules (proline, glycinebetaine, trigonelline, polyamines, non-protein thiols), some of them being involved in short-term response to ion toxicities while others appeared to be required for longer period. It is concluded that *Atriplex atacamensis* is an interesting tool for the phytomanagement of As-contaminated area and that salinity improves the plant resistance to this dangerous pollutant.

1. Introduction

Arsenic (As) is a highly toxic element for all living organisms and a global concern for human health. High concentrations of As in groundwater, soils and sediments in various parts of the world have been identified. This is especially the case in Northern Chile (regions Arica and Parinacota, Tarapacá and Antofagasta) where As is predominantly released from volcanic rocks, sulphide ore deposits and their weathering products at the Andean volcanic chain. Human activities, mainly copper mining, contribute to As release in the environment (Queirolo et al., 2000; Flynn et al., 2002). As a consequence, the presence of extremely high As concentration has been reported in local places (Lara et al., 2012; Herrera et al., 2009). In the main river of this area, the Loa river, high concentration of As (average: 1400 $mg\, L^{-1}$) have been recorded (Romero et al., 2003). This high As concentration constitutes a major risk for local populations using this water as drinking water or for irrigation of vegetable garden (Smith et al., 1998; Díaz et al., 2015).

Arsenic is a metalloid occurring predominantly in inorganic form as oxidized arsenate (As(V)) and reduced arsenite (As(III)). Arsenate is an analogue of phosphate, competing for the same uptake system and can thus disrupt phosphate metabolism and

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substitute phosphate in ATP (Smith et al., 2010). In plants, arsenate may be reduced by non-specific arsenate reductase to arsenite which tends to be complexed with thiol rich-peptides and stored in vacuoles (Zhao et al., 2010). Arsenite binds to sulfhydryl groups of enzymes and proteins with subsequent inhibition of cellular functions (Meharg and Hartley-Whitaker, 2002; Zhao et al., 2003; Tripathi et al., 2007). As(III) is transported across biological membranes in the neutral form through nodulin 26-like intrinsic proteins (NIPs) aquaporin channels (Tripathi et al., 2007; Zhao et al., 2010). Arsenic toxicity is also mediated by oxidative stress through generation of reactive oxygen species (ROS) and inhibition of antioxidant defences in plant tissues (Hartley-Whitaker et al., 2001). Environmental constraints induce modifications in the plant hormonal status. Stress-induced ethylene oversynthesis may hasten the leaf senescence ultimately leading to plant death (Koyama, 2014).

Some plant species may help to reduce the risk of As dispersion from contaminated sites and stabilize substrate through avoidance of erosion processes. The fern *Pteris vittata* is a well known hyperaccumulator able to accumulate more than 4000 $\mu g\, g^{-1}$ of As in its aerial part (Fayiga et al., 2008). In Chile, however, areas affected by As contamination are also extremely arid, and *P.vittata* could not be considered as a good candidate for phytostabilization purposes. In contrast, the local xerohalophyte plant species *Atriplex atacamensis* has been recommended as a promising plant species for phytomanagement of As contaminated sites (Vromman et al., 2011; Tapia et al., 2013). *Atriplex atacamensis* Phil. is native of Northern Chile (Atacama desert) and is able to cope with high As (V) concentration (up to 1000 μM As in nutrient solution). Tapia et al. (2013) recently confirmed that *A. atacamensis* is highly resistant to As contamination in soils and does not exhibit toxicity symptoms when growing on a Pre-Andean soil contaminated with more than 100 mg As kg^{-1}.

In its natural environment, *A. atacamensis* is not only exposed to high external As doses but also frequently encounters extremely high salinities. The impact of salt on heavy metal accumulation has been extensively documented in the literature (Manousaki et al., 2008; Lefèvre et al., 2009a; Han et al., 2013). Salinity was reported to affect pollutant absorption and translocation on the one hand, and to impact the physiological strategies of the plant to cope with the accumulated heavy metals on the other hand. In contrast, data concerning salt effect on As uptake and accumulation by plants remain scarce (Guo et al., 2012). Plant tolerance to salinity may rely on the synthesis of protecting compounds such as proline (Szabados and Savouré, 2010), glycinebetaine (Ben Hassine et al., 2008), trigonelline (Rajasekaran et al., 2001) and polyamines (Lutts et al., 2013) involved in osmotic adjustment and/or protection of cellular structures. These compounds, however, are not specific to NaCl constraint and both polyamines and glycinebetaine were reported to be involved in *A. atacamensis* resistance to arsenic (Vromman et al., 2011). It may thus be hypothesized that physiological strategies triggered by NaCl on the one hand and As on the other hand may overlap to some extent. However, there is a crucial lack of information concerning the plant behaviour when both constraints are present concomitantly.

The physiological consequences of As accumulation is a direct function of As speciation within plant tissues but also As distribution between symplasm and apoplasm (Feng et al., 2015). No data are available on the putative effects of salinity on these parameters. Moreover, the plant response to abiotic stress is not only a function of stress intensity but also a direct function of the total duration of stress exposure. In the presented work, the tested hypothesis are (i) that NaCl may influence *A. atacamensis* response to As toxicity and (ii) that such an influence may vary with the duration of the treatment. For this purpose, physiological parameters (growth, water status, ethylene synthesis and osmoprotecting compounds) were quantified in plants exposed for 2 and 4 weeks to NaCl, arsenate or arsenate + NaCl in relation to As accumulation, speciation and distribution in this xerohalophyte plant species.

2. Materials and methods

2.1. Plant material and culture conditions

The seeds of *Atriplex atacamensis* Phil. were collected from female shrubs in As-contaminated site at Quillagua (UTM 19 K: 442015 E 7609153 N), which is located along the Rio Loa in the Northwest of Chile, Antofagasta region. Pooled seeds were germinated in a greenhouse on loam and sand (50/50) moistened substrate. Six-weeks old seedlings were fixed in plugged holes on polystyrene plates that were floating on 4 L plastic tanks containing an aerated modified Hoagland solution nutrient solution consisting in 1.43 mM of NH_4NO_3; 323 μM of $NaH_2PO_4{\cdot}2H_2O$; 512 μM of K_2SO_4; 750 μM of $CaCl_2{\cdot}2H_2O$; 1.64 mM of $MgSO_4{\cdot}7H_2O$; 11.4 μM of $MnSO_4{\cdot}H_2O$; 14 μM of $Na_2MoO_4{\cdot}2H_2O$; 57.8 μM of H_3BO_3; 0.96 μM of $ZnSO_4.7H_2O$; 0.4 μM of $CuSO_4{\cdot}5H_2O$, 42.7 μM of Fe-EDTA. After two weeks of acclimation, treatments were applied in order to obtain 0 (Control), 1,000 μM of As(V) (added in the form of $Na_2HAsO_4{\cdot}7H_2O$), 100 mM of NaCl and 1,000 μM of arsenate mixed with 100 mM of NaCl. The solutions were renewed weekly, and the three tanks with 15 seedlings per treatment were randomly rearranged in the greenhouse. The culture was under semi-controlled conditions. Natural lighting was supplemented during 16 h with Philips lamps (HPLR 400W) that provided a minimal photosynthetic active radiation (PAR) of 200 $\mu mol\, m^{-2}\, s^{-1}$. The temperature oscillated between 20 and 30 °C and relative humidity between 55 and 70%.

Visual MINTEQ09 software predicted that sodium in solution was at 96.8% in the form of Na^+, 2.8% as NaCl(aq) the remaining part containing minor amounts of $NaSO_4^-$, $NaNO_3$(aq) and NaH_2PO_4(aq). Arsenic was predicted to be mainly in the form of $H_2AsO_4^-$ (97.7%), while 2.1% was present as $HAsO_4^{2-}$ and only 0.1% as AsO_4^{3-}. The presence of NaCl had no impact on As speciation in nutrient solution. Nutrient solution pH was set at 5.5 and redox potential at 120 mV.

2.2. Growth parameters and plant water status

Plants were harvested after two and four weeks of treatment. Fresh and dry weights of the samples were measured for each type of organs (roots, stems and leaves) for six plants per treatment. Leaf area was measured for six leaves per plants prior to harvest with a leaf area meter (AM300 Leaf area meter; ADC BioScientific Ltd., Hoddesdon, UK). For osmotic potential (Ψ_s) determination, four leaves located at the middle portion of the stem were quickly collected on six plants per treatment. Osmolarity (C) was assessed with a vapour pressure osmometer (Wescor5500) and converted from mosmoles kg^{-1} to MPa using the formula: Ψ_s (MPa) = $-C$ (mosmoles kg^{-1}) × 2.58 × 10^{-3} according to the Van't Hoff equation (Zhu et al., 2001).

The stomatal conductance (g_s) was determined at 1:00 p.m. on three young leaves (no. 2–4 from the top of each plant) using an AP4 diffusion porometer (DELTA-T Devices Ltd., Cambridge, England) on three plants per treatment.

2.3. Ion quantification and As speciation

For total As, S, P and major cations (Ca^{2+}, K^+, Na^+, Mg^{2+}) quantification, 50 mg DW were digested in 35% HNO_3 (v/v) and evaporated to dryness on a sand bath at 80 °C. Minerals were incubated with a mix of HCl 37% – HNO_3 68% (3:1; v/v) slowly