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Short communication

Subcellular distribution and chemical forms of lithium in Li-accumulator *Apocynum venetum*

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ABSTRACT

Apocynum venetum is a promising species to remediate an emerging environmental contaminant lithium (Li). However, no research has been conducted so far relating Li tolerance mechanism. In order to improve the understanding of Li transportation and detoxification, subcellular accumulation and distribution of different chemical forms of Li was studied in *Apocynum venetum*. Subcellular Li compartmentalization analysis showed that majority of Li was located in vacuole (45.52–72.65%) and cell wall (14.84–29.02%) under Li treatment. Furthermore, water soluble and ethonal extracted Li (inorganic Li) are the main chemical forms of Li taken up by *A. venetum*. With the increase of Li concentration in the medium, Li content in all subcellular fractions and proportion of F-ethanol form with high mobility increased. The greatest amount of Li was found in soluble fraction in leaves at 25 mg L^{-1} Li treatment, followed by soluble fraction in leaves at 2.5 mg L^{-1}. These results suggest that Li compartmentation in leaf vacuoles is important in Li detoxification and Li accumulation of *A. venetum*.

1. Introduction

Lithium (Li), the lightest alkali metal, occurs naturally in trace amounts in soil and water (Aral and Vecchio-Sadus, 2008). However, as a consequence of frequent industrial activities and extensive use of Li-ion batteries, Li pollution is becoming a serious environmental problem (Shahzad et al., 2017). Due to the toxicity of Li, excess Li decreases plant growth and interrupts several physiological metabolisms such as carbon assimilation, uptake of other mineral elements, and antioxidant system (Franzaring et al., 2016; Kalinowska et al., 2013; Shahzad et al., 2016).

In heavy metal polluted soils, plants exhibited various defense mechanisms including metal exclusion from roots, restricted accumulation and transportation in heavy metal sensitive plant tissues, metal ligands in cell wall and compartmentalization in vacuoles (Li et al., 2017; Volkmar et al., 1998). For example, salt glands of *Tamarix smyrnensis* can accumulate and excrete cadmium from leaves (Manousaki et al., 2008). Plants vary greatly in their capacity for Li tolerance and several species are recognized as Li-accumulator (Kalinowska et al., 2013; Robinson et al., 2018). However, the mechanisms and strategies of plant Li resistance have not been well elucidated.

Specific capacity of plants to accumulate different amount of Li in different organs appears to play an important role in Li adaptation. Previously, it was showed that Li was accumulated mainly in roots to avoid disruption of key metabolic and biochemical processes (Kalinowska et al., 2013). However, Li-accumulator *Apocynum venetum* displays high potential for Li uptake and translocation in the above ground parts (Jiang et al., 2014). Other mechanisms are also involved in plant resistance to Li such as Li excretion from roots (An et al., 2007; Hagemeyer and Waisel, 1988; Li et al., 2009).

Subcellular accumulation and distribution of different chemical forms of metal are highly correlated with metal tolerance in plants (Hao et al., 2018; Xu et al., 2018). Vacuolar compartmentalization, one type of subcellular distribution, is the main strategy for euhalophytes to response Na^+ stress (Manousaki and Kalogerakis, 2011). Chemical forms of metals in plants determine their mobility and toxicity (Zhang et al., 2014). Soluble metal forms own high migration ability and bioavailability (Fu et al., 2016; Jabeen et al., 2014). The low bioavailable forms integrated with compounds of undissolved phosphate or oxalate, contribute to the metal tolerance and detoxification (Liu et al., 2014). Information is limited about Li distribution patterns and chemical forms in Li-accumulator plants.

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Apocynum venetum, known as luobuma and used as traditional Chinese and Uygur medicine, distributes in saline-alkali soil in northern China where Li content in soils is relatively high (Xie et al., 2012). It was noted that, *A. venetum* accumulates high Li content in leaves (Jiang et al., 2014). Therefore, this species is an ideal material to study Li tolerance, accumulation and distribution. The aim of this study was to investigate Li tolerance mechanism in *A. venetum* by examining the distribution of different chemical forms of Li in different subcellular fraction. Based on the similar chemical traits of Na^+ and Li^+, we hypothesized that main portion of Li is in vacuolar in soluble forms. Shortly, we addressed the following questions in this study: What is the pattern of Li subcellular distribution in *A. venetum*? Which form is the main chemical form of Li for different organs of *A. venetum*?

2. Materials and methods

2.1. Plant growth and Li treatment

Apocynum venetum seeds (collected from Linhai Park of Karamay, XinJiang; N 45°28′,E 84°59′) were surface sterilized with sodium hypochlorite (10%) for 15 min, and then rinsed with deionized water. Seed were germinated in darkness at 28 °C for 3 days on water-moistened sponges. Thereafter, plant cultivation was carried out in a glasshouse (natural light; 18–31 °C; 60–71% relative humidity). Seedlings were transplanted in plastic containers (10 L) and filled with full-strength Hoagland's nutrient solution. There were ten seedlings per container and three replicates per treatment. The nutrient solution was maintained at pH 6.0. The nutrient solution was completely replaced each week. After 28 days growth, plants were subjected to different Li treatments. LiCl (Fuchen Chemical Reagents, TianJin, China) was added into solutions at the concentration of 0, 2.5, and 25 mg L^{-1}. After 28 days exposure to Li, plants were harvested and separated into leaves, stems and roots. The roots were soaked in deionized water (4 °C) for 20 min to remove Li adhering to the root surface (Jackman, 1965; Zhang et al., 2014). Different parts were washed with running deionized water and absorbed by tissue paper. They were then stored at −20 °C until use within 28 days.

2.2. Separation of subcellular fractions

Plant materials (0.5g) were homogenized in a medium containing 0.25 mmol L^{-1} sucrose, 50 mmol L^{-1} Tris-HCl (pH 7.5), and 1 mmol L^{-1} dithioerythritol at 4 °C. The homogenate was separated into five fractions at 4 °C by differential centrifugation adapted from Liu et al. (2014) and Wang et al. (2015). The homogenate was centrifuged at 300 × g for 5min and the sediment was designated as cell wall fraction (F1). The resulting supernatant solution was further centrifuged at 2000 × g for 10min and the resultant deposition considering as plastid/chloroplast (F2). The supernatant was then centrifuged at 5000 × g for 30 min and the deposition was referred as cell nucleus (F3). The supernatant of the third centrifugation step was centrifuged at 10,000 × g for 20min and the deposition was taken as mitochondrial fraction (F4). The last supernatant was considered as soluble fraction (vacuole) (F5).

2.3. Extraction of chemical forms of Li

Six chemical forms of Li in *A. venetum* were extracted by the designated solutions in the following order (Wang et al., 2008, 2015; Zeng et al., 2017): (1) 80% ethanol (ethanol-soluble protein-integrated Li and Li of amino acid salt, F-ethanol); (2) deionize water (water-soluble Li of organic acids, F-dH_2O); (3) 1 M NaCl (pectinates and protein-integrated Li, F-NaCl); (4) 2% Acetic acid (HAc) (undissolved Li of phosphate, F-HAc); (5) 0.6 M HCl (undissolved Li of oxalate, F-HCl); (6) Li in residues (F-Residue).

Plant materials (0.5g) were homogenized in extraction solution, diluted at a ratio of 1:20 (w/v), and incubated for 22 h at 25 °C. Thereafter, the homogenate was centrifuged at 5000 × g for 20 min to obtain the first supernatant solution. The precipitation was re-suspended in extraction solution, shaken for 2 h at 25 °C, and centrifuged at 5000 × g for 10 min. After collection of the former extraction solution, plant materials retained in the tube were extracted by the next extraction solution with the same procedures.

2.4. Determination of Li

Different fractions were evaporated at 65 °C to constant weight and digested with 5 ml pure HNO_3 on an electric plate at 180 °C until black brown color disappeared and the solution was transparent. After digestion, the volume of each sample was adjusted to 50 ml using deionized water. Li concentrations were measured using inductively coupled plasma mass spectrometry (8800 ICP-MS Triple Quad, Agilent Technologies).

2.5. Data analysis

All data were expressed as mean ± standard error (s.e.). Data were analyzed using one-way ANOVA to compare treatments. For comparison, least significance difference test ($P < 0.05$) was employed and SPSS was used for statistical analysis.

3. Results

3.1. Subcellular distribution of Li in A. venetum

When the stress level of Li was 2.5 and 25 mg L^{-1}, most of the Li was distributed in the soluble fraction (F5, 45.52–72.65%), some in the cell wall (F1, 14.84–29.02%), plastid (F2, 4.50–12.46%) and nucleus (F3, 4.21–9.61%), and least was noted in mitochondrion (F4, 1.66–4.48%) (Fig. 1). Li content in most subcellular fractions of different organs increased with increasing Li supply. In root, 24.84 fold and 5.43 fold higher Li were noted in soluble fraction and cell wall respectively as compared with control. In shoot and leaves, different Li treatments showed similar subcellular distribution except for cell wall (Fig. 1a and b). No change in Li concentration in cell wall was observed in shoot and leaves, suggesting a possible differential role of cell wall in Li accumulation and distribution among different plant organs.

3.2. Chemical forms of Li in A. venetum

The content of Li bound to different chemical forms was higher under high Li concentration as compared with control. The amount of Li extracted by 80% ethanol was major chemical form for all treatments and was accounted for 64.87–91.98% of the total Li in different organs (Fig. 2). The percentage of inorganic Li form (ethanol extracted) increased in the order of roots < stems < leaves, and increased in different tissues with increased Li application. Especially in leaves of *A. venetum* at 2.5 and 25 mg L^{-1} Li, the proportion of Li extracted by 80% ethanol reached 91.93% and 91.98%, respectively (Fig. 2a).

3.3. Discussion

To improve heavy metal stress tolerance in plants, it is important to understand spatial distribution of chemical forms of these heavy metals in accumulators and/or hyper-accumulators. Though the subcellular accumulation and distribution of different heavy metals in different plant tissues have been reported (Fu et al., 2011, 2016; Wang et al., 2008, 2015); however, no information is published so far regarding Li distribution at subcellular level in different plant tissues of *A. venetum*.

3.3.1. Subcellular distribution of Li

Compartmentalization of toxic ions in different subcellular fraction

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