RESEARCH ARTICLE

Aluminium Accumulation Potential of *Alstonia scholaris*

Mugdha Ambatkar,* Kevin D'cruz

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Abstract

Aluminium toxicity commonly affects plants and considerably reduces crop production. However, some plants, particularly tropical trees, have adapted to high aluminium concentrations by using strategies such as aluminium accumulation. The present study is the first report of aluminium hyperaccumulation in *Alstonia scholaris*, which is a common tree in Mumbai. Aluminium was accidently detected in the inflorescence tissue of *A. scholaris*. Volumetric analysis and atomic absorption spectrometry revealed that the aluminium concentration exceeded 1000 µg/g (dry weight). Notably, aluminium appeared to be stored in the fresh inflorescence stalks but not in the flowers. The aluminium concentration in the stalks determined through volumetric analysis was 10689.7±846.8 µg/g dry weight. Furthermore, the aluminium concentration in oven-dried stalks determined through atomic absorption spectrometry was 4080.36±11.60 µg/g. Because *A. scholaris* accumulates aluminium in its aerial parts at a concentration exceeding 1000 ppm, it can be considered a hyperaccumulator of aluminium. The high concentration of organic acids in the flowers indicated the possible role of organic acids in compartmentalization or sequestration of aluminium in the inflorescence stalks. Investigating the molecular and genetic basis of the mechanism(s) underlying aluminium sequestration in *A. scholaris* can provide important information for the development of crop varieties that are minimally affected by aluminium toxicity.

Keywords: *Alstonia scholaris,* Aluminium, Hyperaccumulation, Organic acids, Toxicity.

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Introduction

The roles of various elements as macronutrients and micronutrients in plant growth are well established. However, thus far, no role in plant growth has been attributed to aluminium (Al) in its various ionic states including Al^{3+} (Bojórquez-Quintal *et al*., 2017). Although Al is one of the most abundant metals in the earth's crust, several studies have reported toxicity in plants caused by Al^{3+} at micromolar concentrations (Poschenrieder *et al*., 2008). Despite its ubiquitous occurrence, plants have not been reported to use Al for growth probably because biological systems are not designed to use free trivalent cations such as Al^{3+}; however, they can use Fe and Co, which can change their valency under conditions prevailing in biological systems (Vitorello *et al.,* 2005).

Under acidic conditions, or in acidic soils, Al is easily taken up by plants. Globally, acidic soils constitute approximately 35% (Ryan *et al*., 2011) to 40% (Vitorello *et al*., 2005) of available arable land. Acidic soils are naturally present in tropical regions, and several native tropical plant species have developed mechanisms of tolerance or resistance to Al. However, recently, high levels of air pollution and resulting acid rain have led to the development of acid soils in non-tropical regions (Brunner and Sperisen, 2013). Clearly, Al toxicity has become a common problem in agriculture, and it exerts considerable selection pressure for adaptation. Numerous studies have been conducted to determine the effects of Al on plants such as inhibition of root growth (Poschenrieder *et al*., 2008). According to a report, phytohormones have been shown to respond to high Al concentrations by inhibiting root growth (Daspute *et al*., 2017). Considering the increasing demand for food production, the development of new varieties of crops that are resistant to Al toxicity is necessary. Identifying the genes involved in Al tolerance or exclusion is crucial for developing Al-tolerant and Al-resistant crops, which are necessary for sustainable agriculture (Krill *et al*., 2010). Furthermore, suitable genes may be cloned and used for the development of new crop varieties (Kochian *et al*., 2005).

Plants can be classified into three categories, namely hyperaccumulators, excluders, and indicators, according to the strategies employed for responding to high concentrations of Al. Internal mechanisms for tolerating Al include the sequestration of Al into vacuoles and detoxification by Al chelation (Daspute *et al*., 2017). According to an accepted definition (Jansen *et al*., 2002), plants that accumulate more than 1000 ppm of a metal in their aerial parts (with respect to dry weight) are hyperaccumulators of that metal. Although the term hyperaccumulator was first defined for nickel (Ni), it has been applied to Al (Jansen *et al*., 2002).

A phylogenetic study investigating the trait of Al hyperaccumulation among various plant families revealed that some members of family Apocynacea are hyperaccumulators (Jansen *et al*., 2002); however, the listed genera (*Bonafousia*, *Carpodinus* and *Willughbeia*) did not include *Alstonia*. Furthermore, the status of *Alstonia scholaris*, a tropical tree species, with regard to Al accumulation is unknown. Moreover, the findings of the aforementioned phylogenetic study indicated that Al accumulation is a primitive trait from an

Department of Botany, St. Xavier's College, 5, Mahapalika Marg, Mumbai-400001, Maharashtra, INDIA

***Corresponding author:** Dr. Mugdha Ambatkar, Department of Botany, St. Xavier's College, 5, Mahapalika Marg, Mumbai-400001, Maharashtra, INDIA; Mobile: +91-9324385275; Email: mugdhaambatkar@gmail.com

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evolutionary view point.

The uptake and storage of large amounts of Al in aerial tissues have been reported in numerous tropical tree species because coping with high concentrations of Al^{3+} is critical for survival (Brunner and Sperisen, 2013). In the present study, the Al concentration in the inflorescence stocks of *A. scholaris* was estimated. Initially, the presence of Al was detected using qualitative cation analysis. The concentration of Al was initially estimated using a volumetric method. However, atomic absorption spectrometry (AAS) was used for specifically and conclusively determining the concentration of Al.

The present study is part of a larger study on the flowers of *A. scholaris*. The inflorescences, particularly the flowers, produce a yellow flavonoid-based natural dye that is extractable in water. During the optimisation of the pH of the extraction medium all specimens collected from different locations showed identical behaviour; the inflorescence stocks but not the flowers exhibited the formation of a white gelatinous precipitate at and above pH 8.0. The cobalt nitrate test was used to confirm that the precipitate contained Al. The confinement of Al to the inflorescence stalks and the prevention of migration of Al into the floral or reproductive tissue indicate the possible presence of a mechanism for excluding Al ions. The findings of the present study can provide a starting point for studies on genes and mechanisms of Al exclusion in *A. scholaris*, which have applications in improvement of crop production by reducing Al toxicity in crop plants.

Material and Methods

Collection of *A. scholaris* inflorescences

Shoots with inflorescences of *A. scholaris* were collected from trees growing in three locations, namely Dhobi Talao, Powai and Parel, in Mumbai. The inflorescences were immediately separated from the shoots by cutting. The flowers and inflorescence stalks were segregated, washed, dried and stored in separate containers at room temperature until further use. All the chemicals used in the study were analytical grade chemicals purchased from Merck, Germany, unless specified.

Qualitative detection of Al^{3+}

Aqueous extracts of the fresh flowers and inflorescence stalks from each location were prepared by gently heating 1 g each of the stalks and flowers in 20 ml of distilled water [material to liquor ratio (MLR) 1:20] in lidded beakers for 15 min by using a water bath. Each liquor was filtered through a coarse filter paper, and the filtrate was centrifuged at 4000 rpm (1789×g) for 5 min. The supernatant was used for qualitative detection of Al^{3+} by using a cobalt nitrate solution.

Estimation of Al^{3+} by using complexometric titration

The method followed for estimation was based on a previously reported method (Malati, 1999). A 5 mL aliquot each of a solution of 10 mmol/L $Al_2(SO_4)_3$ and a 10% w/v aqueous extract (MLR 1:10) of fresh inflorescence stalks was used for titration against a standard solution of 10 mmol/L $ZnSO_4$ in the presence of 10 mmol/L EDTA under alkaline conditions. A mixture of solochrome black T and KNO_3 (9:1 w/w) was used as the indicator. The endpoint of the titration was Prussian blue to wine red. The titration was repeated three times. The concentration of Al^{3+} was calculated per unit of fresh and dry weight of inflorescence tissue.

Drying of inflorescence tissue

The inflorescence stalks from the Dhobi Talao sample were first oven dried at 40°C for 48 hours. Next, 10 g of the stalks was heated at 500°C until a constant weight was obtained. The oven dried sample was stored in airtight containers until further use. The weight of dry inflorescence stalks obtained per unit weight of fresh tissue was calculated.

Quantification of Al^{3+} in dried inflorescence stalks through AAS

AAS of the inflorescence stalk sample was performed by Ashco Analytical Services, Mumbai, by using a SOLAAR M Series GE650041 V1.21 AA spectrometer (Thermo Scientific, USA). A deuterium lamp (serial no. S12436) was used as the light source. For estimation, a sample solution was prepared using 0.0128 g of dried tissue. A calibration curve was obtained using different dilutions of standard Al^{3+} solution. The amount of Al^{3+} in the sample solution was determined in terms of µg/g of dry tissue. Finally, the percentage (by weight) of Al^{3+} in dry tissue was determined.

Determination of organic acid concentration in floral tissue

The organic acid concentration in the floral tissue was calculated in terms of oxalic acid equivalents. A diluted aqueous extract of fresh floral tissue was titrated using a NaOH solution that was standardized using 0.1 N oxalic acid. The organic acid concentration was calculated in terms of equivalents of oxalic acid per 100 g of floral tissue. The organic acids in the floral tissue were identified using paper chromatography.

Results and Discussion

Qualitative detection of Al^{3+}

When the pH of the aqueous extracts of the flowers and inflorescence stalks was made alkaline (pH>8.0) by using liquor ammonia, both extracts exhibited an increase in the intensity of their yellow colour, which is a characteristic reaction of flavonoids. However, only the extract of the inflorescence stalks showed the formation of a thick gelatinous precipitate in addition to an increase in colour intensity. The gelatinous precipitate was separated by centrifugation and treated with aqueous $Co(NO_3)_2$. The precipitate exhibited an intense blue colour, which is characteristic of Al^{3+}. Notably, Al was detected in the inflorescence stalks but not in the flowers. The confinement of Al to the inflorescence stalks and the presumable prevention of migration of Al into the floral or reproductive tissue indicates the possible presence of a mechanism for excluding Al ions.

Estimation of Al^{3+} by using complexometric titration

The concentration of the $Al_2(SO_4)_3$ solution was determined to be 5.4 mmol/L by titration against the standard $ZnSO_4$ solution. The mean concentration of Al^{3+} in the inflorescence stalk extract

Table 1: Volumetric estimation of Al^{3+} in inflorescence stalks.

Location	*Al^{3+} concentration (ppm) in fresh tissue*	*Mean (ppm)*	*Al^{3+} concentration (ppm) in dry tissue*	*Mean (ppm)*
Parel	3173.4		9885.9	
Dhobi Talao	3405.6	3431.4 ± 271.82	10609.4	10689.7 ± 846.8
Powai	3715.2		11573.8	

Table 2: Content of metals in dried inflorescence stalks.

Element	*Mean concentration in dry tissue (ppm)*
Al	4080.36±11.60
Fe	2275.30±10.44
Mn	24.75±0.05
Ni	21.89±0.60

was 0.757 mmol/L. Accordingly, the concentration of Al^{3+} in fresh inflorescence stalk tissue was found to be 3431.4±271.8 µg/g (or ppm). The water content of the fresh tissue was 67.81%. Hence, in terms of dry weight, the calculated concentration of Al^{3+} was 10689.7 µg/g (Table 1). An inherent limitation of the volumetric method is that it is not entirely specific to Al^{3+}. Consequently, the likelihood of the results of the titration being an overestimate of Al^{3+} concentration is considerably high because of the possible interference of other ions such as calcium, magnesium, manganese (Mn), iron (Fe) and Ni.

Quantification of Al in dried inflorescence stalks through AAS

The Dhobi Talao *A. scholaris* sample was selected for AAS because collecting soil samples for comparison of Al concentration was feasible at the Dhobi Talao location. Analysis through AAS facilitated the specific quantification of Al. The amount of Al in the undiluted tested sample, which was prepared using 0.0128 g of dry inflorescence tissue was 53.234 µg. Hence, the concentration of Al in the dry inflorescence tissue was 4080.36±11.60 µg/g or ppm. Thus, Al was found to constitute 1.27% by weight of the dry inflorescence tissue. In addition to Al, other elements, such as Fe, Mn and Ni in the tissue sample were quantified through AAS. Fe and Mn were selected for quantification because cations of both metals form gelatinous precipitates in alkaline conditions and possible interference in titration, whereas Ni was quantified mainly because of its possible interference in titration. However, among the other tested metals, only the concentration of Fe in the inflorescence was notably high (Table 2). A pooled soil sample consisting of soil from around the *A. scholaris* trees in the Dhobi Talao area showed that Al and Fe constituted 2.9% and 1.12%, respectively, of the dried soil by weight.

Determination of organic acid concentration in floral tissue

Organic acids effectively chelate Al and reduce its toxicity in plants both internally and externally. For example, organic acids secreted by roots into surrounding soil (an external mechanism) have been reported to prevent the uptake of Al^{3+} by plants. Furthermore, oxalic acid has been shown to form complexes of different molar ratios in the cell sap in leaves (an internal mechanism). The concentration of organic acids in terms of oxalic acid in the floral tissue was 5.768 equivalents/100 g. The qualitative chromatographic analysis of floral acids revealed the presence of citric, malic and oxalic acid (data not shown).

The high concentration of acids, such as oxalic acid, indicated the possibility that Al is chelated by organic acids in the inflorescence stalks and is prevented from entering the floral tissue. Oxalic acid has been shown to effectively chelate Al^{3+} ions in plants with internal and/or external Al detoxification mechanisms. Furthermore, Al binds to DNA, RNA, lipopolysaccharides and proteins (Ma, 2000). Hence, preventing Al migration into floral tissue averts possible adverse changes and genetic mutations some of which may be heritable.

The mechanism(s) preventing Al migration from inflorescence stalks to flowers, including the role of organic acids, warrant investigation. Some plants sequester Al in metabolically least sensitive parts. For example, in *Richeria grandis*, storage of Al is extracellular; it is stored in the cell walls of mature leaves. In hyperaccumulators such as *Camellia sinensis* Al is stored in the leaves in the epidermal cells and mesophyll cells. In *A. scholaris*, the inflorescences talks appear to be an Al storage site. The flowers do not appear to be storage sites probably because the flowers are metabolically active as well as reproductive in function, and Al can bind to and alter DNA, which can cause mutations. The high concentration of Al in *A. scholaris* makes the tree a potential source of natural aluminium-based mordants for dyeing fabric. Various parts of Al hyperaccumulator plants have been reported to be used as sources of alum-like natural mordants (Jansen *et al*., 2002).

The genetic and/or molecular basis of confining Al to the inflorescence stalks and preventing its entry into floral tissue in *A. scholaris* also warrants investigation. Identifying the genes involved in confining Al to the stalks can provide a foundation for genetic engineering studies for developing Al-resistant crop varieties that are likely to become a necessity for promoting sustainable agriculture in acidic soils (Samac and Tesfaye, 2003).

Conclusion

The investigation of the metal concentrations in the inflorescence stalks of *A. scholaris* has shed light on some previously unexplored aspects of the plant. The present study seems to be the first to investigate the metal concentrations in the inflorescence of *A. scholaris*. The study inferred that:

- The concentration of Al in the inflorescence stalks, which are aerial plant parts, exceeds 1000 µg/g of dry weight; hence, *A. scholaris* is an Al hyperaccumlator. The Al is accumulated or sequestered in the inflorescence stalks but is notably not detectable in the adjacent floral tissue.
- At least one of the mechanisms underlying the prevention of Al transport from the stalks to the floral tissue involves organic acids such as oxalic and malic acid.
- *A. scholaris* is a potential source of a natural aluminium-based mordant for dyeing fabric.

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