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Aluminium detoxification in facultative (*Passovia ovata* (Pohl ex DC.) Kuijt and *Struthanthus polyanthus* Mart. - Loranthaceae) and dependent (*Psittacanthus robustus* (Mart.) Marloth - Loranthaceae) Al-accumulating mistletoe species from the Brazilian savanna

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ABSTRACT

Mechanisms to detoxify aluminium (Al) is a hot topic for cultivated plants. However, little information is known about the mechanisms used by native plants to deal with Al-toxicity. In Cerrado, some generalist mistletoe species, such as *Passovia ovata* (Pohl ex DC.) Kuijt and *Struthanthus polyanthus* Mart. can parasitize Al-accumulating and Al-excluding plant species without any clear symptoms of toxicity and mineral deficiency, while *Psittacanthus robustus* (Mart.) Marloth, a more specialist mistletoe, seems to be an Al-dependent species, parasitizing only Al-accumulating hosts. Here we (i) characterized the forms and compartmentalization of Al in leaves of *P. robustus*; (ii) compared Ca and Al leaf concentration, and leaf concentration of organic acids and polyphenols between facultative Al-accumulating (*P. ovata* and *S. polyanthus*) and Al-dependent (*P. robustus*) mistletoe species infecting *Miconia albicans* (Sw.) Steud. (Al-accumulating species). *P. robustus* chelated Al^{3+} with oxalate and stored it in the phloematic and epidermic leaf tissues. Leaf Ca and Al concentration did not differ among species. Leaf oxalate concentration was higher in the Al-dependent species. Concentrations of citrate and phenolic compounds were higher in the leaves of the facultative Al-accumulating species. These results show that facultative Al-accumulating and Al-dependent species use different mechanisms to detoxify Al. Moreover, this is the first report on a mistletoes species (*P. robustus*) with a potential calcifuge behaviour in Cerrado.

1. Introduction

Aluminium trivalent (Al^{3+}) is a toxic element for many cultivated and native plants in acidic soils with pH < 4.5 (Kochian et al., 2015). Even at concentrations lower than micromolar, Al^{3+} disrupts the root system, reduces water and nutrient uptake and compromises the plant development (Barceló and Poschenrieder, 2002; Brunner and Sperisen, 2013). Nevertheless, some native and cultivated plants are tolerant to Al-toxicity due to efficient mechanisms for detoxifying Al externally (Al-excluders) or internally (Al-accumulating).

The Al-excluders release small organic acids and phenolic compounds into the soil through their roots avoiding Al uptake (Barceló and Poschenrieder, 2002), while Al-accumulating species uptakes and accumulate high concentrations of Al in leaves and roots (> 1000 mg Al kg^{-1} leaf dry mass) (Haridasan, 2008) without symptoms of toxicity. For some Al-accumulating species (*Miconia albicans* (Sw.) Steud - Metastomataceae, *Vochysia thyrsoidea* Pohl and *V. tucanorum* Mart. - Vochysiaceae) from the Brazilian savanna (Cerrado) with a calcifuge behaviour (i.e., sensitive species to high Ca availability in the soil), Al may be considered a beneficial element reducing the toxic effects of high Ca concentration (Haridasan, 1988, 2008; Souza et al., 2017).

During the last thirty years a massive investment in research was devoted to understand the mechanisms used by Al-excluders (mostly crops) to deal with Al-toxicity (Barceló and Poschenrieder, 2002; Brunner and Sperisen, 2013; Kochian et al., 2015). However, a reduced number of studies was conducted to understand the mechanisms used

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by native Al-accumulating plants to deal with Al-toxicity. In Al-accumulating plants, the contribution of low-molecular organic acids (e.g. citrate, malate and oxalate) and phenolic compounds (e.g. catechin) to detoxify Al were basically investigated in cultivated plants such as tea (*Camellia sinensis* (L.) Kuntze - Theaceae), buckwheat (*Fagopyrum esculentum* Moench - Polygonaceae) and *Hydrangea macrophylla* (Thunb.) Ser. (Hydrangeaceae) (Barceló and Poschenrieder, 2002; Brunner and Sperisen, 2013). An exception are investigations on some Al-accumulating members of the Myrtales and Santalales (Maejima et al., 2014, 2017; Souza et al. 2017, 2018a). The first study investigating the mechanisms of Al-detoxification in Cerrado species was recently published. The authors reported that, while the mistletoes *Passovia ovata* (DC.) Kuijt and *Struthanthus polyanthus* Mart. (Loranthaceae) chelate Al with citrate, the host *M. albicans* chelate Al with oxalate (Souza et al., 2018b). They also observed significant variation on leaf concentration of organic acids between mistletoes infecting an Al-accumulating and Al-excluding host.

Mistletoes are parasitic plants that penetrate the bark of the hosts' branches through a modified root, the haustorium, connecting to their host xylem (Lamont and Southall, 1982). Therefore, the mistletoe-host system is a very interesting model to understand nutrient relations in plants, especially Al toxicity, since the same mistletoe species can be found parasitizing different hosts with contrasting Al and nutrient concentrations in the xylem. Tropical mistletoes from the Loranthaceae family are highly diverse, represented by 73 genera and over 1500 species (Nickrent et al., 2010; Nickrent, 2011), and varying widely in the degree of host specificity. In the Brazilian Savanna (Cerrado), some mistletoes from the Loranthaceae family can be classified as facultative Al-accumulating (*P. ovata and S. polyanthus*) and Al-dependent (*Psittacanthus robustus* Mart.) species (Scalon et al., 2013; Souza et al., 2018b), depending on the host relative specificity. Facultative Al-dependent species are found parasitizing a large number of species, including Al-accumulating and Al-excluding, while the Al-dependent species is restricted to Al-accumulating hosts (Monteiro et al., 1992; Scalon et al., 2013). Despite the report of Al-accumulating tree species from Cerrado with a calcifuge behaviour (Haridasan, 1988, 2008; Souza et al., 2017), until date there are no report of tropical mistletoe species with a calcifuge behaviour.

The aims of this study was to (1) compare the leaf concentration of Ca and Al between facultative Al-accumulating (*Passovia ovata* and *Struthanthus polyanthus*) and Al-dependent species (*Psittacanthus robustus*) infecting *Miconia albicans* (calcifuge and Al-accumulating tree species); (2) characterize the forms of Al and their compartmentalization on leaves of *P. robustus* (Al-dependent species) infecting *M. albicans*; (3) compare the leaf concentration of organic acids and phenolic compounds between facultative Al-accumulating (*P. ovata* and *S. polyanthus*) and Al-dependent species (*P. robustus*) parasiting *M. albicans*. We expect to find significant differences on the mechanisms to detoxify Al between facultative Al-accumulating and Al-dependent species, reflecting the distinct strategies to deal with high Al availability on the host xylem. Based on the mechanisms used by the Al-dependent species, we expect to propose the first case of calcifuge behaviour in tropical mistletoes.

2. Results

We observed no significant variation on the leaf concentration of Ca ($x^2 = 2.72$, $p = 0.44$) and Al accumulation ($x^2 = 4.11$, $p = 0.25$) between the facultative and dependent Al-accumulating mistletoe species (Fig. 1a and b). Putting all data together, we observed that leaf Ca ($r = 0.88$, $p < 0.001$) and Al ($r = 0.58$, $p < 0.01$) levels correlated positively between mistletoes and hots (Fig. 2).

The ^{27}Al-NMR analyses revealed that *P. robustus* detoxify Al with Al-oxalate complexes (Al-oxalate(1) - 6.4 ppm, Al-oxalate(2) - 11.6 ppm and Al-oxalate(3) - 16.1 ppm). We also observed a peak indicating the presence of free Al^{3+} (0.0 ppm) in leaves of *P. robustus* (Fig. 3). The histochemical analyses revealed that *P. robustus* stored Al in the phloematic tissues and the epidermis (Fig. 4).

Leaf concentrations of organic acids differed significantly between facultative and dependent Al-accumulating species. *P. robustus* (Al-dependent) had four times more oxalate leaf concentrations than *P. ovata* and *S. polyanthus* (facultative Al-accumulating) ($x^2 = 7.42$, $p = 0.02$) (Fig. 5a). On the contrary, the lowest level of citrate was observed in *P. robustus* (Al-dependent) and the highest level in *P. ovata* (facultative Al-accumulating) ($x^2 = 9.85$, $p < 0.01$) (Fig. 5b).

Leaf concentrations of phenolic compounds varied significantly between facultative and dependent Al-accumulating species ($x^2 = 7.73$, $p = 0.02$). The concentration of phenolic compounds was three-fold higher in the facultative Al-accumulating species (*P. ovata* and *S. polyanthus*) than in Al-dependent (*P. robustus*) (Fig. 6).

3. Discussion

Similar leaf Ca and Al concentrations between facultative and dependent Al-accumulating species reflected the nutrient availability in the host. This similarity was expected since hemiparasites sink nutrients from the host's xylem sap by the haustorium (Scalon et al., 2013). Correlations of leaf nutrient concentrations between mistletoes and hosts were previously reported for tropical and subtropical plants, including Al-accumulating and Al-excluding species (Glatzel and Geils, 2009; Scalon et al., 2013; Scalon and Wright, 2015; Souza et al., 2018b). Despite similar leaf concentrations of Ca and Al among the species, the ratio of Ca:Al was higher in the facultative Al-accumulating species (1.05 - *P. ovata*; 0.97 - *S. polyanthus*) than in the Al-dependent species (0.58 - *P. robustus*) suggesting that these two groups may use different mechanisms to deal with Al and Ca toxicity.

Chelation with low-molecular organic acids is one of the most important mechanisms to detoxify Al and Ca in the leaves of both Al-accumulating species and calcifuge species (Lee, 1998; Brunner and Sperisen, 2013). In the present study we observed that the Al-dependent mistletoe species (*P. robustus*) detoxify Al with oxalate, while Souza et al. (2018b) found that facultative Al-accumulating mistletoe species (*P. ovata* and *S. polyanthus*) chelate Al with citrate. The Al-citrate complex observed in the facultative Al-accumulating mistletoes (Souza et al., 2018b) is considered the generalist complex for Al detoxification being used for both internal and external Al detoxification for Al-accumulating and Al-excluding species (Ma et al., 1997; Brunner and Sperisen, 2013; Kochian et al., 2015). The broad use of citrate to detoxify Al^{3+} is attributed to the tricarboxylic anions that forms a more stable complex with Al^{3+} than oxalate and malate that are dicarboxylic anions (Kochian et al., 2015).

Furthermore, the Al-oxalate complexes observed in *P. robustus* are frequently found in Al-accumulating species with a calcifuge behaviour, such as *M. albicans* (Souza et al., 2018b), *Melastoma malabathricum* L. (Watanabe et al., 1998; Maejima et al., 2017) (Metastomataceae) and *Symplocos chinensis* (Lour.) Druce - Symplocaceae (Maejima et al., 2014). Oxalate not only detoxify Al^{3+} but also acts on the regulation of Ca concentrations by balancing soluble and insoluble forms, reducing the toxic effects of high Ca^{2+} concentrations (Lee, 1998; Nakata, 2012). This is the first report of Al-oxalate complexes to detoxify Al and Ca in Loranthaceae family. The calcifuge behaviour, combined with the dependency of high Al-concentrations in the host xylem, may influence host selection by this mistletoe species and may explain the high specificity exhibited by *P. robustus* for the Al-accumulating hosts within the Vochysiaceae and Melastomataceae families (Scalon et al., 2013; Guerra et al., 2017).

In contrast to the facultative Al-accumulating mistletoes (*P. ovata* and *S. polyanthus*) that store Al exclusively in the phloematic fibres (Souza et al., 2018b), the histochemical analyses showed that *P. robustus* (Al-dependent species) stores Al in both phloematic tissues and epidermis. The compartmentalization of Al ion in the epidermis has previously been observed in other Al-dependent species belonging to