Journal of Inorganic Biochemistry 128 (2013) 183–187

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Short communication

Amelioration of iron toxicity: A mechanism for aluminum-induced growth stimulation in tea plants

Roghieh Hajiboland [b], Juan Barceló [a], Charlotte Poschenrieder [a,*], Roser Tolrà [a]

[a] Plant Physiology Laboratory, Bioscience Faculty, Universidad Autónoma de Barcelona, Spain
[b] Plant Science Department, University Tabriz, 51666 Tabriz, Iran

ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 21 June 2013
Accepted 1 July 2013
Available online 13 July 2013

Keywords:
Acid soil
Growth stimulation
Hematoxylin
Morin

ABSTRACT

Tea plants (*Camellia sinensis*) are well adapted to acid soils with high Al availability. These plants not only accumulate high leaf Al concentrations, but also respond to Al with growth stimulation. Decreased oxidative stress has been associated with this effect. Why tea plants not exposed to Al suffer from oxidative stress has not been clarified. In this study, hydroponically grown tea plants treated with 0 to 300 μM Al were analyzed for growth, Al and Fe accumulation, and Al distribution by means of morin and hematoxylin staining. Roots of control plants stained black with hematoxylin. This indicates the formation of a Fe–hematoxylin complex. Young leaves of controls accumulated more than 1000 mg Fe kg^{-1} dry weight. This concentration is above the Fe-toxicity threshold in most species. Supply of Al stimulated growth and reduced Fe uptake and transport. These results indicate that Al-induced growth stimulation might be due to alleviation of a latent Fe toxicity occurring in tea plants without Al supply.

Aluminum toxicity is a severe problem for crop production in acid soils [1–3]. Native plants and acid-soil-adapted crops have efficient Al-tolerance mechanisms [4]. Some highly tolerant species, like *Camellia sinensis*, hyper-accumulate large Al concentrations in shoots [5–7]. Moreover, growth stimulation by Al has frequently been observed in Al-accumulator plants [8,9]. The mechanisms of this Al-induced growth stimulation are poorly investigated. There is no experimental evidence for an essential role of Al in any organism. Amelioration of some latent stress is the main reason for growth stimulation by non-essential elements. Alleviation of proton toxicity may account for fast Al-induced enhancement of root elongation in proton sensitive plants [10,11]. Contrastingly, in tea Al-induced enhancement occurs at non-toxic pH. Several investigations attribute Al-induced growth stimulation to enhanced antioxidant defenses [9,12,13]. However, the question as to why tea is prone to oxidative stress when grown in the absence of Al remains. Iron toxicity can be an important constraint for tea development in acid soil, especially in areas affected by heavy rainfalls combined with poor soil drainage [14]. As a Fenton metal excess tissue Fe can cause oxidative stress [15]. Our working hypothesis is that in the absence of Al supply, tea can suffer from iron toxicity which is alleviated by Al leading to growth stimulation through a decrease of Fe uptake.

One month-old tea seedlings (*C. sinensis* (L.) O. Kuntze) were grown in a controlled-environment (T: 25/18 °C day/night, 14/10 h light/dark period, RH: 70/80%; photon flux density: 400 μmol m^{-2} s^{-1}) in quarter strength nutrient solution pH 4.0 [12] (2 L/plant; renewed every 3 days). After 1 week in the control solution, Al was supplied at concentrations between 0 (control) and 300 μM (125 μM Al^{3+} activity). Plants were grown for a further 4 weeks, harvested and analyzed for growth and Fe and Al concentrations [16]. For staining, 4 to 6 week-old control plants were exposed to different Al concentrations. Free-hand sections of lateral root apex, taken after 4 and 24 h exposure, were stained with morin or hematoxylin and viewed under light, fluorescence, or confocal fluorescence microscope, as previously reported [16].

Plants grew best with 100 μM Al, but even in the solution with 300 μM Al shoot biomass was not affected by Al and root growth was even higher than in controls (Table 1). This is in line with observations by Li et al. [17] who found an Al-toxicity threshold for tea between 320 and 530 μM. Aluminum accumulated to considerable amounts in both roots and leaves (Fig. 1). This suggests that the high tolerance is based on efficient chelation and compartmentation of Al in the plant tissues rather than on restriction of its uptake [18]. Extremely high Fe concentrations in the roots and leaves of control plants were observed (Fig. 1). Aluminum exposure substantially decreased Fe uptake and transport. This reduction was especially relevant in the lateral roots and the young leaves that had developed during the Al treatment period. Here Fe decreased from close to 1000 μg g^{-1} in controls to around 300 mg kg^{-1} in Al-treated plants. Morin staining of roots showed a preferential localization of Al in root tip border cells and root hairs were abundant close to the root tips (Fig. 2a,b,c). Morin revealed accumulation of Al mainly in cell walls. At a 1 mm distance to the apex, the walls of all developing cells revealed Al by morin staining (Fig. 2d). At a greater distance from the tip where vascular bundles had differentiated, morin staining was mainly found in the walls of the outermost cortex cells (not shown). This was also seen in longitudinal root sections

* Corresponding author at: Lab. Fisiología Vegetal, Facultad Biociencias, Universidad Autónoma de Barcelona, 08193 Bellaterra, Spain. Tel.: +34 935812163.
E-mail address: Charlotte.poschenrieder@uab.es (C. Poschenrieder).

0162-0134/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jinorgbio.2013.07.007

Table 1
Growth parameters of shoot and root in tea (*Camellia sinensis* L.) plants grown for 8 weeks in nutrient solution with different Al concentrations. Data of each column indicated by the same letter are not significantly different ($p < 0.05$).

Al supply (μM)	Shoot DW (g plant^{-1})	Leaf numbers (plant^{-1})	Root DW (mg plant^{-1})	Taproot length (cm plant^{-1})	Total root length (cm plant^{-1})
0	1.39 ± 0.12^{b}	7.2 ± 0.66^{b}	420 ± 72^{c}	17.7 ± 1.74^{c}	628 ± 81^{c}
100	1.97 ± 0.41^{a}	8.35 ± 0.21^{a}	750 ± 25^{a}	28.3 ± 1.12^{a}	1916 ± 110^{a}
300	1.68 ± 0.20^{ab}	7.99 ± 0.42^{ab}	580 ± 23^{b}	25.1 ± 1.81^{b}	1582 ± 98^{b}

where morin staining in the vascular bundles was clearly visible (Fig. 2e). Morin staining was even observed in the root tips of laterals that were just emerging from the pericycle through the cortex (Fig. 2e). In any case, control roots exhibited green fluorescence when stained with morin. In these plants only reddish or bright yellow fluorescence was observed (Fig. 2a,b). Results for hematoxylin staining were unexpected. Not only roots from Al treated plants but also those from controls heavily stained with this dye (Fig. 3). The color development of the hematoxylin complex found in controls compared to that in Al-treated roots revealed differences in tonality. While a typical violet color indicative of the Al–hematoxylin complex was observed in Al-exposed roots, in controls the color was darker, almost black. These visual differences in tonality were confirmed by digital color analysis (Fig. 3) using 31×31 pixel samples taken in the transition zone of root tips. In controls the hematoxylin stain yielded colors with a high percentage of black (K) and relatively low proportions of red (R) and blue (B) tonalities (Fig. 4). With increasing Al-supply, the percentage of black substantially decreased from 71 to 1%, while red and blue increased. Overall staining intensity decreased with increasing Al supply (Fig. 3). In control roots the black staining was observed on different structures: mucilage deposits, root cap border cells, and root hairs (Fig. 4). Abundant root hairs close to the tip were observed in the main roots. In lateral roots of control plants some recently formed tips remained free of stain while others stained down to the tip. The abundant root hairs stained black in controls, but violet in Al-treated plants. Morin staining of roots shows the presence of Al in root hairs close to the root tip (Fig. 2). A similar distribution of morin-stainable Al has also been observed in *Brachiaria decumbens*, a tropical grass that is a hyper-resistant Al excluder [19]. This distribution of morin-stainable Al contrasts with a preferential uptake of Al by root tips reported in other species [20,21]. Aluminum in tea seems to move mainly apoplastically following the transpiration stream and accumulates in the cell wall of the epidermal cells [18]. The distribution of Al revealed by morin staining may reflect Al movement through the roots because morin apparently stains Al bound to organic acids, i.e. soluble Al complexes, but not the Al tightly bound to cell walls [21]. The green fluorescence of the Al–morin complex is quite specific. Iron can also strongly bind to morin, but Fe quenches the fluorescence [22]. Competition between Al and Fe in the roots of tea plant is supported by the unusual hematoxylin staining pattern of the roots (Figs. 3 and 4). Roots without Al supply usually do not stain, while roots from Al treated plants exhibit a typically violet coloration due to the formation of the Al–hematoxylin complex [23,24]. It is well known that iron forms black complexes with hematoxylin, while the Al–hematoxylin complex is violet [25]. Less staining in Al-exposed

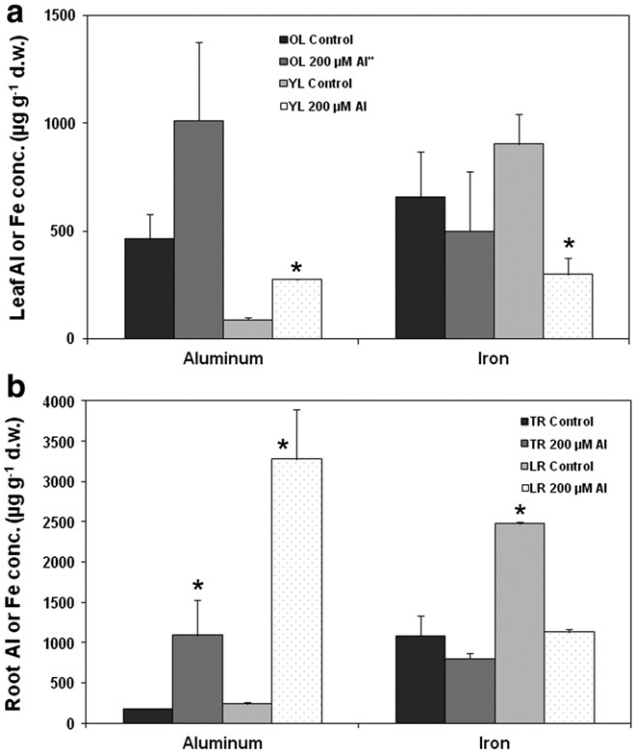

Fig. 1. Concentrations of Al and Fe in leaves (a) and roots (b) of tea plants exposed to control or Al supplemented nutrient solutions. OL, old leaves; YL, young leaves; TR, taproot; LR, lateral root. Values are means ± SD ($n = 3$). For each organ, columns marked with an asterisk represent significant differences between Al-treated and control plants (ANOVA; LSD, $p < 0.05$).