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Distribution and mobility of aluminium in an Al-accumulating plant, *Fagopyrum esculentum* Moench.

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Abstract

Buckwheat (*Fagopyrum esculentum* Moench. cv. Jianxi) accumulates high concentrations of Al in the leaves without showing any toxicity. To understand the accumulation mechanism of Al in buckwheat, the distribution and mobility of Al in buckwheat were investigated. Relatively long-term treatment (28 d) with Al led to a decrease in Al concentration from old to young leaves, while a short-term (1 d) exposure to Al resulted in a uniform distribution of Al in the leaves. When the fourth leaf was wrapped inside a transparent plastic bag to suppress transpiration, the Al concentration of this leaf was only one-quarter of that in the corresponding leaf without wrappng. Within a leaf, the Al concentration at the margins was much higher than that in the centre. These results indicate that Al distribution in the leaves is controlled by both rate and duration of transpiration. The mobility of Al between old and new leaves was studied by first growing plants in a solution with Al, followed by culture in a solution without Al. The Al content in the two new leaves appeared after removal of external Al was very low, whereas that in the old leaves did not decrease but continued to increase. The increased Al content was found to be translocated from Al remaining in the roots. It is concluded that Al is not mobile once it is accumulated in the leaf.

Key words: Accumulation, aluminium, buckwheat, distribution, mobility, transpiration.

Introduction

Ionic aluminium (Al^{3+}) in acid soil is toxic to most plant species at micromolar concentrations. However, some plant species, known as Al-accumulating plants, can tolerate high concentrations of Al^{3+} and accumulate Al to high concentrations in the shoot without showing any signs of toxicity. Tea and hydrangea are typical Al accumulators. Tea can accumulate Al to concentrations up to 30 000 mg kg^{-1} on a dry weight basis in old leaves and up to 600 mg kg^{-1} in young leaves (Matsumoto *et al*., 1976). The Al concentration in the leaves of hydrangea plants with blue sepals reaches 3000 mg kg^{-1} during a growth period of several months (Ma *et al*., 1997*a*). Some trees in tropical cloud forests (such as *Richeria grandis*) also accumulate high levels of Al (more than 1000 mg kg^{-1}; Cuenca *et al*., 1990). *Melastoma malabathricum* and *Vaccinium macrocarpon*, which are adapted to low-pH soils, accumulate large amounts of Al in either roots or leaves (Osaki *et al*., 1997; Watanabe *et al*., 1998).

Al in some accumulating plants is localized in the cells at millimolar concentrations (Ma *et al*., 1997*a*, 1998). Although free Al at cytosolic pH (7–7.5) is reduced to nanomolar orders of concentration (Martin, 1988), these are still potentially phytotoxic (Taylor, 1991). Internal detoxification of Al in some Al-accumulating plants is achieved by complexation with organic compounds. In tea leaves, Al is chelated with catechin (Nagata *et al*., 1992). A complex of dephindin 3-glucoside-Al-3-caffeoylquinic has been identified in blue-coloured hydrangea sepals (Takeda *et al*., 1985*a*, *b*), whereas Al in the leaves is present in the form of a 1 : 1 Al-citrate complex (Ma *et al*., 1997*a*).

Recent studies reported that buckwheat also accumulates high concentrations of Al in the leaves. A 10 d intermittent exposure to 50 μM Al results in an accumulation of 450 mg Al kg^{-1} in leaves (Ma *et al*., 1997*b*), with about 90% of the leaf Al being present in the cell sap at a concentration higher than 2 mM. Specific processes for uptake, translocation and accumulation of Al exist in the buckwheat plants, in conjunction with a series of changes

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in the chemical form of Al (Ma and Hiradate, 2000). The roots take up Al in the form of ionic Al (Al^{3+}). Following uptake, Al is chelated with internal oxalate in the root cells, forming a stable, non-phytotoxic complex of Al-oxalate at a 1 : 3 ratio. When Al is translocated from the roots to the leaves, Al-oxalate (1 : 3) is converted to Al-citrate (1 : 1) in the xylem. When Al-citrate moves from the xylem to the leaf cells, re-conversion from Al-citrate to Al-oxalate (1 : 3) occurs (Ma and Hiradate, 2000). However, information about the distribution of Al in the shoot organs is limited. This is because most plant species are unable to translocate Al to the shoot, and most known Al-accumulating plants are woody species, in which it would be difficult to study Al distribution during a short time. To understand accumulating mechanisms of Al in buckwheat further, the distribution and mobility of Al was examined.

Materials and methods

Plant materials and growth conditions

Seeds of buckwheat (*Fagopyrum esculentum* Moench. cv. Jianxi) were soaked in distilled water overnight and then germinated on a net tray in the dark at 25 °C. On day 2 the net was floated on a plastic container filled with 0.5 mM $CaCl_2$ solution (pH 4.5). The solution was renewed daily. On days 4 or 5, seedlings were transplanted to 1.0 l plastic pots (4 seedlings per pot) containing aerated one-fifth strength Hoagland solution. This nutrient solution contained the following macronutrients (mM): KNO_3 (1.0), $Ca(NO_3)_2$ (1.0), $MgSO_4$ (0.4), and $(NH_4)H_2PO_4$ (0.2); and the micronutrients (μM): Fe-EDTA (20), H_3BO_3 (3), $MnCl_2$ (0.5), $CuSO_4$ (0.2), $ZnSO_4$ (0.4), and $(NH_4)_6Mo_7O_{24}$ (1). The solution was adjusted to pH 4.5 with 0.4 M HCl and renewed every other day. Plants were grown in a temperature-controlled greenhouse (20 °C) from August to October 2000, with natural lighting. Each experiment was repeated at least twice with three replicates each.

Distribution of Al in buckwheat

To estimate the Al distribution in buckwheat, 10-d-old seedlings were exposed to 50 μM $AlCl_3$ in 0.5 mM $CaCl_2$ (pH 4.5) every other day. The seedlings were grown in nutrient solution on alternate days. The reason for this intermittent Al treatment was to avoid interaction between Al and other nutrients such as P. Plants were harvested after 28 d. Leaves were separated into cotyledons and the first to the eighth leaf (numbered from oldest to youngest).

To examine Al distribution with short-term exposure to Al, 22-d-old seedlings were subjected to 100 μM $AlCl_3$ in 0.5 mM $CaCl_2$ (pH 4.5) for 24 h. The five fully developed leaves and the cotyledons were then harvested separately as described above.

The effect of transpiration on Al distribution was investigated by wrapping the fourth leaf of 22-d-old seedlings with a transparent plastic bag. After 24 h of exposure to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 100 μM Al, each leaf was harvested.

To study the distribution of Al in a single leaf, the second and fifth leaves were harvested after exposure to 50 μM $AlCl_3$ in 0.5 mM $CaCl_2$ (pH 4.5) and one-fifth Hoagland nutrient solution on alternate days for 20 d. Each leaf was cut into three parts with scissors as illustrated in Fig. 3.

Mobility of Al in buckwheat

Ten-day-old seedlings were exposed to 50 μM $AlCl_3$ as described above until the appearance of the fifth leaf (12 d). Half of the seedlings were harvested, while the remaining half were transferred to nutrient solution without Al. After two new leaves (sixth and seventh leaves) appeared, the plants were harvested and separated into roots, stems and individual leaves. After drying in an oven at 70 °C for 2 d, the weight of each sample was recorded.

To examine Al translocation from the root to the above-ground parts following removal of external Al, the stem was severed 3 cm above the roots and xylem sap was collected for 1 h with a micropipette at 0, 24 and 72 h from the cessation of Al treatment. The Al concentration in the xylem sap was determined as described below.

Al determination

Plant samples were dried in an oven for 2 d (70 °C), and then ground to a fine powder. The samples were digested with concentrated HNO_3 (heavy-metal grade). Al concentration in the digested solutions and fresh xylem sap was determined by flameless Atomic Absorption Spectrometry (Hitachi Z-5000, Tokyo, Japan), after an appropriate dilution with 0.1 N HNO_3.

Results

With 28 d of intermittent Al treatment, buckwheat plants grew normally and no symptoms of Al toxicity were observed. The Al concentrations in the roots and stem were 4200 and 80 mg Al kg^{-1} dry weight, respectively. The Al concentration in leaves varied with leaf position; the highest concentration was found in the oldest leaf (first leaf, 1680 mg Al kg^{-1}), and the lowest in the youngest leaf (eighth leaf, 200 mg Al kg^{-1}) (Fig. 1). A decrease in Al concentration was observed from the oldest to youngest leaves.

A short-term (1 d) exposure to 100 μM Al resulted in a uniform distribution of Al in different leaves (Fig. 2A).

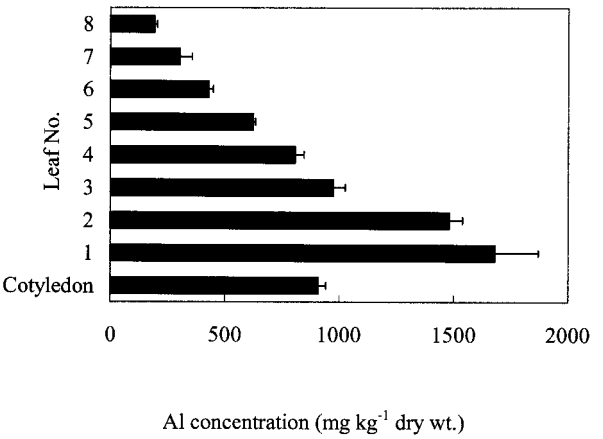

Fig. 1. Distribution of Al in different leaves of buckwheat plants. Ten-day-old seedlings were exposed to 50 μM $AlCl_3$ in 0.5 mM $CaCl_2$ (pH 4.5) and nutrient solution on alternate days for 28 d. Thin bars represent the standard deviation of the mean ($n = 3$).

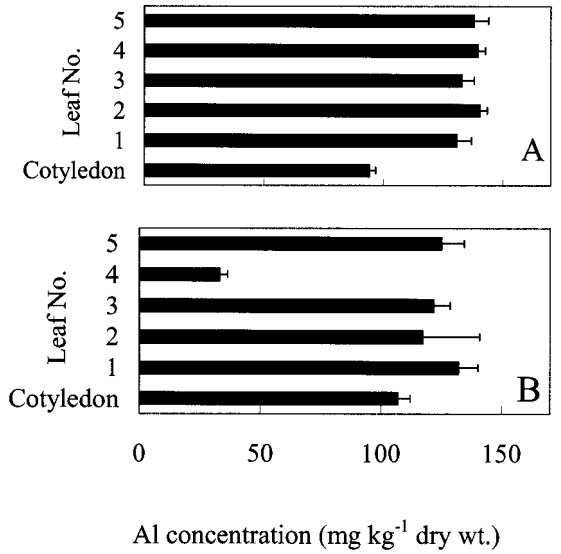

Fig. 2. Al distribution in different leaves of buckwheat following a short exposure to Al, and the effect of transpiration on Al distribution. The fourth leaf was allowed to transpire normally (A), or was wrapped in a transparent plastic bag (B). After 1 d of exposure to 100 μM $AlCl_3$, the cotyledons and each expanded leaf were harvested and Al concentrations were determined. Thin bars represent the standard deviation of the mean ($n = 3$).

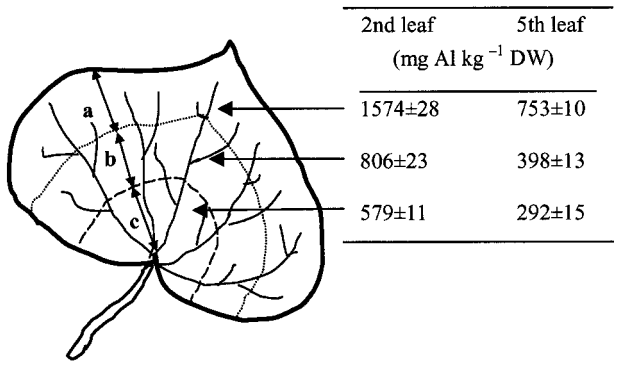

Fig. 3. Distribution of Al in different parts of a leaf. Ten-day-old seedlings were exposed to 50 μM $AlCl_3$ in 0.5 mM $CaCl_2$ and nutrient solution on alternate days for 20 d. The second and fifth leaves were harvested. The leaf tissues were cut with scissors by hand along the dotted lines. The dimensions of a, b, and c are similar. Data represent means ± SD ($n = 3$).

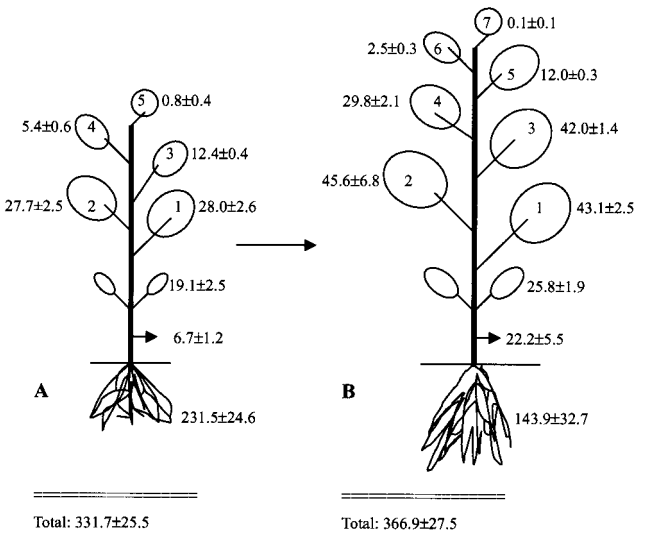

Fig. 4. The mobility of Al in buckwheat. Al content (μg) within each tissue is shown. (A) Plants were harvested after growing in Al solution intermittently for 12 d. (B) Plants were harvested after a further 12 d of growth in a nutrient solution without Al. Data represent means ± SD ($n = 3$).

The Al concentration in these leaves ranged between 130 and 140 mg Al kg^{-1}.

When the fourth leaf was wrapped in a transparent plastic bag for 1 d, the Al concentration of this leaf decreased to one-quarter of that in the corresponding leaf without wrapping (Fig. 3B). This treatment did not affect the distribution of Al in other leaves (Fig. 2A, B).

The second and fifth leaves, which had different Al concentrations (Fig. 1), were chosen for an examination of the Al distribution within a leaf. In both leaves, an increase in Al concentration from the central part to the marginal part was observed (Fig. 3). The Al concentration of the leaf margins was 1.7-fold and 1.6-fold higher than in the centre of second and fifth leaves, respectively (Fig. 3).

Al concentrations in the two youngest leaves (sixth and seventh leaves), which had appeared after the cessation of Al treatment, were extremely low, at only 50 and 4 mg Al kg^{-1} in the sixth and seventh leaves, respectively. As older leaves also continued to expand after the plants were transferred to −Al solution, the Al content, not the Al concentration of tissues was compared between plants harvested immediately after termination of Al treatment and those harvested following the appearance of two new leaves in the −Al solution. The Al content of individual older leaves increased. For example, the Al contents of the first and fifth leaves increased from 28 to 43.1 μg, and from 0.8 to 12 μg, respectively. The Al content of the roots decreased from 231.5 to 143.9 μg Al, but levels of Al in the whole plant did not change (Fig. 4). Removal of Al did not change the distribution pattern of Al as seen in Fig. 1 (data not shown).

The Al concentration in the xylem sap was about 130 μM immediately after transfer of plants from +Al solution to −Al solution. Xylem sap Al decreased to 73 μM at 24 h and 8 μM at 72 h after removal of the Al treatment (Fig. 5).

Discussion

The translocation of an element from root to shoot (mainly leaves) via xylem is driven by the gradient in hydrostatic pressure (root pressure) and by the gradient in the water potential (transpiration). The distribution of

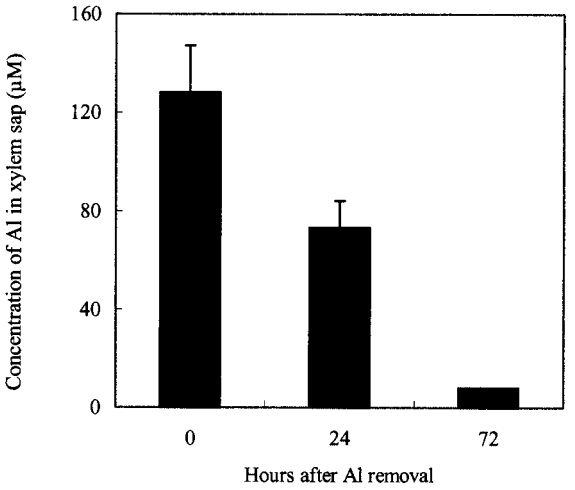

Fig. 5. The Al concentration in the xylem sap of buckwheat plants at different times after removal of Al. Ten-day-old seedlings were cultured in 0.5 mM $CaCl_2$ with 50 μM $AlCl_3$ (pH 4.5) and nutrient solution on alternate days for 12 d. After transferring the plants to nutrient solution without Al, the stem was severed 3 cm above the roots and the xylem sap was collected for 1 h with a micropipette at 0, 24, and 72 h. Thin bars represent the standard deviation of the mean ($n = 3$).

an element in the shoot organs, however, depends on transpiration rate, tissue age (duration of transpiration), mobility of the element and other factors. A considerable amount of knowledge has been acquired in relation to the distribution of essential and beneficial elements in above-ground parts of plants. For example, it is well known that Ca concentrations are higher in old leaves and lower in young leaves, and this is associated with transpiration rates, age of leaves, and low Ca mobility (Behling *et al.*, 1989). The distribution of K and P is less affected by transpiration. The distribution of Si in the shoot organs is similar to that of Ca (Marschner, 1995), whereas B distribution varies with plant species. In plant species which have an ability to mobilize B from old leaves to young leaves, the B concentration follows: expanding leaf > mature leaf > old leaf (Brown and Shelp, 1997). However, in plant species that are not able to retranslocate B, B distribution is characterized by a deceasing gradient in B concentration from the old leaves to the expanding leaves. In the present study, Al distribution was investigated in buckwheat, an herbaceous crop species which accumulates Al in the shoot to high levels (Ma *et al.*, 1997*b*). A short-term (1 d) exposure to Al resulted in a uniform distribution of Al in each leaf (Fig. 2A), while plants exposed to Al treatment for 28 d showed a decrease in Al concentration from the oldest to the youngest leaf (Fig. 1). Suppression of transpiration of a leaf led to a decrease in the Al accumulated by this leaf (Fig. 2B). These results suggest that Al distribution is controlled by both transpiration rate and the age of the leaf (duration of transpiration). The acropetal decrease of Al is also the result of the immobility of Al, as discussed below.

Within a particular leaf, a steep gradient in the Al concentration (central part < middle part < marginal part) was observed regardless of leaf position (Fig. 3). This result suggests that more Al is accumulated at the end of the transpiration stream, indicating a transpiration-mediated distribution pattern of Al in the leaf. The Al distribution pattern is similar to that of B in plant species without B mobility. However, while leaf B is predominantly bound to the cell walls (Matoh and Kobayashi, 1998), most Al is localized in the cells of buckwheat leaves in a soluble form (Ma *et al.*, 1998).

The Al concentration of the oldest leaf (first) was as high as 1680 mg kg^{-1} dry weight. Taking leaf water content into consideration, the Al concentration in the cells was estimated to be about 8 mM (Ma *et al.*, 1998). However, the leaf did not show any toxicity symptoms, remaining as green as other younger leaves. The internal Al in buckwheat leaves has been demonstrated to be detoxified by the formation of a stable complex with oxalate at a 1 : 3 ratio (Ma *et al.*, 1997*b*, 1998). Buckwheat leaves contain about 50 mM oxalate (Ma *et al.*, 1998), irrespective of Al treatment, suggesting that oxalate concentrations are high enough to detoxify this level of Al.

Mobility of a nutrient, from old organs to young organs via the phloem, varies between elements. Potassium, N, and P are well-known to have high mobility, whereas Ca and Mn are low-mobility nutrients (Marschner, 1995). Radioisotopes have been used widely to study the mobility of elements and are a powerful tool; however, there is no radioisotope available for Al. In the present study, the mobility of Al in buckwheat was investigated by firstly growing plants in a solution containing Al, followed by a further growth period in a solution without Al. If Al is mobile, Al accumulated in the older leaves would be translocated to young leaves that appeared after transfer to the solution without Al. This would result in an increased Al content in the new leaves and a decreased Al content of older leaves. However, the results of this study show that the Al content was extremely low in the two newly appeared leaves and that the Al content of old leaves did not decrease but continued to increase (Fig. 4). The continued accumulation of Al in the older leaves seems to originate from Al remaining in the roots. About 60% of total Al in buckwheat roots is present in the cells as Al-oxalate (1 : 3) (Ma *et al.*, 1998). This pool of Al might still be translocated to the shoot after removal of external Al. High concentrations of Al in the xylem sap and a decrease in root Al content after Al removal support this possibility (Figs 4, 5). Considering the results of this study collectively, it is concluded that Al is not mobile in buckwheat leaves after it has been accumulated.

Acknowledgements

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