Aluminum-activated Oxalate Secretion does not Associate with Internal Content among Some Oxalate Accumulators

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Abstract

Although aluminum (Al)-activated secretion of oxalate has been considered to be an important Al-exclusion mechanism, whether it is a general response in oxalate accumulators and related to oxalate content in roots are still not clear. Here, we examined the oxalate secretion and oxalate content in some oxalate accumulators, and investigated the role of oxalate secretion in Al resistance. When oxalate content in amaranth roots was decreased by about 50% with the increased ratio of NH₄⁺-N to NO₃⁻-N in nutrient solution, the amount of Al-activated oxalate secretion still remained constant. There was no relationship between the content of the water soluble oxalate in four species of oxalate accumulators and the amount of the Al-activated oxalate secretion in roots. Furthermore, oxalate secretion is poorly associated with Al resistance among these species. Based on the above results, we concluded that although all of the oxalate accumulators tested could secrete oxalate rapidly, the density of anion channels in plasma membrane may play a more important role in Al-activated oxalate secretion.

Key words: aluminum toxicity; Amaranthus; anion channel; oxalate accumulator; oxalate secretion.


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Aluminum (Al) toxicity is a major constraint for crop production in acid soils, which occupy approximately 50% of potentially arable lands worldwide (von Uexküll and Mutert 1995). On the other hand, there is a wide variation in the resistance to Al toxicity among plant species or cultivars within the same species, and the breakthrough has been made in understanding the physiology of resistance exhibited by some species (Kochian et al. 2004). Among the resistant mechanisms, organic acid anions secretion is the most widely documented (Ma et al. 2001; Ryan et al. 2001; Kochian et al. 2004), because their secretion tends to be localized to the root apices and organic acid anions can chelate Al effectively, thus making it less toxic.

Ma et al. (1997) first reported that buckwheat roots (Fagopyrum esculentum Moench cv. Jiangxi) exude oxalate rapidly in response to Al stress. Zheng et al. (1998) further suggested that de novo oxalate synthesis is not involved in the Al-activated oxalate secretion because oxalate is abundant in the cytosol of buckwheat roots. Another oxalate accumulator, spinach (Spinacia oleracea L. cv. Quanneng), also exhibits Al-dependent rapid oxalate secretion in roots, but seems to be very sensitive to Al stress due to its poor resistance to acid stress (Yang et al. 2005a). Recently, we screened nine cultivars of buckwheat for differential Al resistance and found large genotypic variation among them in response to Al stress (Yang et al. 2005b). However, the Al-sensitive buckwheat cultivar also has the same Al-dependent oxalate secretion pattern, indicating that oxalate secretion can not fully explain the genotypic differences among buckwheat cultivars (Zheng et al. 2005). We further demonstrated that cycloheximide, a broad protein-synthesis inhibitor, fails to inhibit Al-activated oxalate secretion in buckwheat but could effectively inhibit citrate secretion in Cassia tora L. plants,
further confirmed that de novo synthesis of protein is not involved in the process of oxalate secretion in buckwheat (Yang et al. 2006). These reports lead to the hypothesis that oxalate secretion under Al stress in the oxalate accumulators might be a common feature.

To test this hypothesis, some oxalate accumulator species or cultivars were chosen to investigate the Al-dependent oxalate secretion. We also tested whether the alteration of oxalate content affects the amount of Al-activated oxalate secretion. Here, we demonstrated that all oxalate accumulators tested can exude oxalate rapidly when exposed to Al stress, and the amount of Al-activated secretion was not related to oxalate contents in roots.

**Results**

**Comparison of oxalate secretion among oxalate accumulators**

Al-activated oxalate secretion from all of the oxalate accumulators was tested (eight cultivars from four plant species). The water soluble oxalate was the highest in amaranth roots and lowest in tomato. However, the Al-activated secretion of oxalate was the lowest in amaranth roots, and the largest in spinach roots. Overall, there was no correlation between oxalate content and Al-activated secretion in these oxalate accumulators (Figure 1).

**Manipulation of oxalate content and Al-activated oxalate secretion**

As shown in Figure 2, oxalate content in amaranth roots was decreased with the increasing ratio of $\text{NH}_4^+:\text{NO}_3^-$ application. The water soluble oxalate in roots was decreased by 18%, 34%, and 52%, respectively, when the ratio of $\text{NO}_3^--\text{N}:\text{NH}_4^+:\text{N}$ declined from 15:1 to 3:1, 1:1, and 1:3. However, the amount of Al-activated oxalate secretion maintained at the same level.

**Correlation between Al resistance and oxalate secretion among oxalate accumulators**

Among the tested four plant species, buckwheat showed higher Al resistance than spinach, amaranth, and tomato. Root elongation of spinach, amaranth, and tomato were 6.1, 5.3, and 6.3 mm respectively after 24 h growth in 0.5 mmol/L $\text{CaCl}_2$ solution (pH 4.5), and that of five buckwheat cultivars were 17.9, 21.0, 28.3, 26.7, and 17.2 mm as shown in Figure 3. The root growth of amaranth and tomato plants almost ceased when exposed to 50 $\mu$mol/L Al, the relative root elongation being less than 10% as compared with the no Al control. However, the amount of oxalate secretion showed no correlation with their Al resistance.
Seeds for oxalate; effectively and make synthesis of oxalate is not required. Another relationship between Al resistance and Al-activated oxalate de novo solution (pH 4.5) containing 0 or 50 mM can chelate Al for relative root elongation).

n secretion, thus maintaining ion balance and apoplastic secretion in eight cultivars from four plant species.

Figure 3. Relationship between Al resistance and Al-activated oxalate secretion in eight cultivars from four plant species.

Seedlings of amaranth (triangle), buckwheat (circles), spinach (diamond), and tomato (square) roots (4–5 cm of primary root) were exposed to 0.5 mM CaCl₂ solution (pH 4.5) containing 0 or 50 μM Al. Root length was measured before and after 24 h treatment. The numbers refer to the different buckwheat cultivars: 9279-21 (no. 1), Xiaosanliang (no. 2), Longyou (no. 3), Jiangxi (no. 4), and Shanxi (no. 5). Vertical bars represent ± SE (n = 3 for oxalate; n = 10 for relative root elongation). FW, fresh weight.

among these plant species, and even among five buckwheat cultivars (Figure 3).

Discussion

Organic acid anions, mainly malate, citrate and oxalate, secretion plays an important role in excluding Al from the plant roots (Ma et al. 2001; Ryan et al. 2001; Kochian et al. 2004). Although the underlying basis of Al-activated malate and citrate secretion is emerging (Kochian et al. 2004; Delhaize et al. 2007), the processes leading to secretion of oxalate still remain unclear. There is evidence that Al can affect tricarboxylic acid (TCA) cycle and glycolytic pathway. Therefore, some studies have related Al-activated secretion of organic acid anions to their metabolism. Genetic manipulation of organic acid biosynthesis (malate or citrate) through overexpressing genes of enzymes involved in the TCA cycle (de la Fuente et al. 1997; Delhaize et al. 2001; Tesfaye et al. 2001; Anoop et al. 2003) has been reported. However, there is no report on the manipulation of oxalate biosynthesis by transgenic technology so far. Oxalic acid, the simplest dicarboxylic acid, is often considered as an end product of metabolism. There are several possible pathways responsible for the oxalate biosynthesis with L-ascorbic acid possibly the major pathway (Franceschi and Nakata 2005).

However, the key enzymes involved in the pathway are still poorly understood. On the other hand, it has been reported that the nitrogen source can influence oxalate biosynthesis, and the oxalate content decreases with increasing ammonium supply (Palaniswamy et al. 2004; Ji and Peng 2005). In the present study, we also found that the water soluble oxalate in roots decreased significantly with the increasing ratio of ammonium to nitrate. However, the secretion of oxalate under Al stress was not affected (Figure 2). One of the possible explanations for this result is that there is still a sufficient amount of oxalate in roots (about 28 μmol/L per g fresh weight (FW) at the least) and de novo synthesis of oxalate is not required. Another possibility is that the amount of oxalate secretion is mediated by the amounts of anion channels activated by Al stress in the plasma membrane. In order to further analyze the relationship between oxalate content and its secretion, eight cultivars from four plant species that accumulate high oxalate were used to investigate the oxalate secretion in response to Al stress. Apparently, the higher content of oxalate was not associated with higher secretion (Figure 1), further demonstrating that it is anion channels rather than oxalate content that play a more important role in regulating oxalate secretion.

There is no doubt that Al resistance is related to organic acid anions secretion, because these anions, especially citrate³⁻, oxalate²⁻, and malate²⁻ can chelate Al³⁺ effectively and make it less or non-phytotoxic. However, the theory is, in some cases, problematic. For example, Piñeros et al. (2005) and Zheng et al. (2005) demonstrated that citrate and oxalate secretion cannot fully explain genotypic Al resistance in maize and buckwheat cultivars, respectively. Here, we found that Al-activated oxalate secretion showed no correlation with Al resistance among plant species and cultivars (Figure 3), also arguing against the theory. We think that there are two possibilities responsible for the present results. One is that the high sensitivity of amaranth, tomato, and spinach plants to Al toxicity may be due to their high sensitivity to acid stress according to our previous report (Yang et al. 2005a). However, this explanation is certainly not suitable for the genotypic differences among buckwheat cultivars. The other is that the mode of action of oxalate secretion in Al resistance remains unknown (Kinraide et al. 2005). Although Al-dependent malate secretion in wheat (Ryan et al. 1995) and citrate secretion in soybean (Shen et al. 2004) is accompanied by K⁺ secretion, thus maintaining ion balance and apoplastic pH, it is still unknown in Al-regulated oxalate secretion and there is evidence that oxalate secretion can acidify the surrounding medium (Godoy et al. 1990).

In conclusion, oxalate accumulators tested in this study secrete oxalate rapidly under Al stress, but oxalate secretion is not related to Al resistance. Furthermore, the content of oxalate in roots is also poorly associated with its secretion either in amaranth plants or in other oxalate accumulators, implying that the density of anion channels in plasma membrane may play a more important role in the Al-activated oxalate secretion.
Materials and Methods

Plant materials and growth condition

Eight cultivars from four species including amaranth (Amaranthus spp.), buckwheat (Fagopyrum esculentum Moench cvs. Jiangxi, Longyou, Xiaosanlieng, 9279-21, and Shanxi), spinach (Spinacia oleracea L. cv. Quanneng), and tomato (Lycopersicon esculentum M. cv. Zhefeng202) were used in this study. Seeds were soaked in de-ionized water overnight. Then the seeds were placed on filter paper moistened with 0.5 mmol/L CaCl$_2$ solution at pH 4.5 and kept in the dark at 26 °C. Germinated seeds were transferred to a net tray, floated on a 0.5 mmol/L CaCl$_2$ solution at pH 4.5 in a 5 l plastic container, and kept in the dark at 26 °C for one more day, and then transferred to a controlled-environment growth room with a 14 h/26 °C day at a light intensity of 250–300 μmol/L photon/m$^2$ per s and 10 h/23 °C night regime. The solution was replaced daily. At day 4, the seedlings were transplanted into 1.1-L plastic pots (12 seedlings per pot) containing aerated nutrient solution. One fifth strength Hoagland solution was used containing the following macronutrients in mmol/L: KNO$_3$, 1.0; Ca(NO$_3$)$_2$, 1.0; MgSO$_4$, 0.4; NH$_4$H$_2$PO$_4$, 0.2, and the following micronutrients in μmol/L: NaFeEDTA, 20; H$_2$BO$_3$, 3; MnCl$_2$, 0.5; CuSO$_4$, 0.2; ZnSO$_4$, 0.4; (NH$_4$)$_2$Mo$_7$O$_24$, 1. Different amounts of NH$_4$Cl were added to yield NO$_3^-$:NH$_4^+$-N ratios of 15:1, 3:1, 1:1, and 1:3 with the final total N concentration of 3.2 mmol/L. The solution was adjusted to pH 4.5 with 1 M HCl and renewed every 3 d.

Measurement of Al-activated oxalate secretion and content

For Al-activated oxalate secretion analysis, seedlings (3 weeks old) were exposed to 50 μmol/L AlCl$_3$ in 0.5 mmol/L CaCl$_2$ (pH 4.5) for 3 h. The root exudates were collected and passed through a cation-exchange resin column (16 × 140 mm) filled with 5 g Amberlite IR-102 B resin (H$^+$ form), followed by an anion-exchange resin column (16 × 140 mm) filled with 2 g Dowex 1 × 8 resin (100–200 mesh, formate form). To determine cellular oxalate concentration, seedlings (3 weeks old) were exposed to 0.5 mmol/L CaCl$_2$ (pH 4.5) for 3 h, and root tissues (approximately 0.2 g) were cut and ground into fine powder with liquid nitrogen and extracted three times with de-ionized water at 55 °C. The extracts were applied to cation- and anion-exchange resins as described above. The organic acids retained on anion-exchange resin were eluted by 1 M HCl, and the eluate was concentrated to dryness by a rotary evaporator (40 °C). After the residue was redissolved in dilute HClO$_4$ solution, pH 2.1, the concentration of oxalate was analyzed by high performance liquid chromatography (HPLC) according to Zheng et al. (2005).

Alteration of oxalate concentration in amaranth

Oxalate synthesis in amaranth plants was altered by manipulating the ratio of nitrate : ammonium in the hydroponic culture (Franceschi and Horner 1980). A part of the seedlings (10 d old) were subjected to the nutrient solution with the following macronutrients in mmol/L: KNO$_3$, 0.2; KCl, 0.8; Ca(NO$_3$)$_2$, 0.2, CaCl$_2$, 0.8; MgSO$_4$, 0.4; NH$_4$H$_2$PO$_4$, 0.2, and the following micronutrients in μmol/L: NaFeEDTA, 20; H$_2$BO$_3$, 3; MnCl$_2$, 0.5; CuSO$_4$, 0.2; ZnSO$_4$, 0.4; (NH$_4$)$_2$Mo$_7$O$_24$, 1. Different amounts of NH$_4$Cl were added to yield NO$_3^-$:NH$_4^+$-N ratios of 15:1, 3:1, 1:1, and 1:3 with the final total N concentration of 3.2 mmol/L. The solution was adjusted to pH 4.5 with 1 M HCl and renewed every 3 d. After 1 week of culture, the seedlings were exposed to 0.5 mmol/L CaCl$_2$ solution (pH 4.5) containing 0 or 50 μmol/L AlCl$_3$ for 3 h. Collection of root exudates and extraction of root tissue oxalate were conducted as described above.

Assessment of Al resistance

For assessment of Al resistance, seedlings with 4–5 cm of primary root were subjected to a compartmental hydroponic system (Yang et al. 2005b). The treatment solutions were 0.5 mmol/L CaCl$_2$ solution (pH 4.5) containing 0 or 50 μmol/L Al. The length of primary roots was measured with a ruler before and after treatments (24 h). Relative root elongation was defined as the percentage of the root elongation in 50 μmol/L Al solution to that of no-Al control.

References


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