Effect of Al on the growth and nutrients uptake of blueberries 
(\textit{Vaccinium spp.})

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Abstract

Aluminum (Al) is the major factor limiting plant growth on acidic soils. Blueberry (\textit{Vaccinium spp.}) is an acidophilic plant. Highbush blueberry and rabbiteye blueberry are the main commercially cultivated species, while the response of which to Al is still unclear. Therefore, hydroponic experiments were conducted to determine the effect of Al (0 and 100 $\mu$mol L$^{-1}$) on the growth and nutrient uptake of highbush blueberry ‘Brigitta’ and rabbiteye blueberry ‘Brightwell’. The results showed that root biomass, root length per fresh weight, root activity and foliar nitrogen (N) concentration of ‘Brigitta’ were significantly decreased by Al, and root lipid peroxidation was increased by Al. In contrast, the biomass and root activity of ‘Brightwell’ were not affected by Al treatment, and root lipid peroxidation was significantly decreased, root length and surface area per fresh weight were increased compared with the control, which was beneficial for nutrients absorption. In fact, foliar N concentration of ‘Brightwell’ was increased in Al treatments. However, fewer Al was accumulated in leaves of ‘Brightwell’ compared to ‘Brigitta’. Therefore, it could be concluded that growth and nutrients uptake of ‘Brightwell’ was not negative affected by Al, which meant ‘Brightwell’ was resistant to Al, compared to ‘Brigitta’.

\textbf{Keywords:} Al toxicity; lipid peroxidation; root activity; root morphology

Introduction

Aluminum (Al) has been recognized as the major constraint to plant growth on acid soils. Al can restrict root elongation within minutes or hours, and subsequently inhibit the uptake of water and nutrients, resulting in poor growth and productivity (Ma \textit{et al.}, 2001; Kochian \textit{et al.}, 2015). However, many plant species have adapted to acid soils and grow vigorously without any toxicity symptoms, such as \textit{Camellia aleifera} Abel (Zeng \textit{et al.}, 2012), \textit{Lespedeza bicolor} (Dong \textit{et al.}, 2008), and \textit{Melastoma malabathricum} (Watanabe \textit{et al.}, 2008).

Blueberries (\textit{Vaccinium spp.}) is an acidophilic plant that thrives on soils with pH values from 4 to 5.5 (Eck, 1988), suggesting that it is relatively Al-tolerant. However, several studies have shown that one of the
major cultivated blueberry genotypes, highbush blueberry, was sensitive to Al stress (Patten et al., 1988; Yang and Goulart, 1997; Reyes-Díaz et al., 2011). It was reported that stress effects including inhibited root elongation by short-term Al stress (50 μmol L\(^{-1}\)) in hydroponic culture (Reyes-Díaz et al., 2009), and photosynthetic processes under long-term Al stress (Yang and Goulart, 1997; Reyes-Díaz et al., 2010) were observed on highbush blueberry ‘Bluegold’, ‘Legacy’ and ‘Brigitta’. Yang and Goulart (1996, 2000) demonstrated that the mechanism for Al toxicity in highbush blueberry was, in part, through the inhibition of root growth and limiting P and N uptake. Inostroza-Blancheteau et al. (2011) found root tips of highbush blueberry ‘Bluegold’ and ‘Brigitta’ were injured by 100 μmol L\(^{-1}\) Al treatment, and oxidative stress was induced in the roots by Al.

Rabbiteye blueberry is another main cultivated blueberry genotype, which can adapt to a wider soil pH range, compared to highbush blueberry (Retamales and Hancock, 2018). It was also reported that rabbit eye blueberry was sensitive to Al stress (Peterson et al., 1987; Patten et al., 1988). Peterson et al. (1987) observed a decreased growth of rabbit eye blueberry when a sandy loam was acidified with aluminum sulfate but not with elemental sulfur, possibly related to high leaf Al concentrations (up to 317 mg kg\(^{-1}\)). However, Korcak (1982, 1988) reported no visible toxicity symptoms were observed even more than 1000 mg kg\(^{-1}\) Al accumulated in leaves. Spiers (1990) found leaf Al content was increased with Al additions in sandy culture, but plant growth and leaf dry weight were unaffected and the toxicity symptoms of high Mn treatment was alleviated by Al.

Blueberry cannot grow well in soils with low content organic matters, therefore, peat or composed pine bark are needed to apply to the mineral soil for better growth (Julian et al., 2012). These organic amendments may bind active Al forms in soil solution, except to mediate fluctuations in soil moisture and temperature (Yang and Goulart, 1997). It has been reported that rabbit eye blueberry required lower soil organic matter content compared to highbush blueberry (Retamale and Hancock, 2018). These may hint rabbit eye blueberry could be more tolerant to Al, compared to highbush blueberry. Besides, given the conflicting evidences on Al tolerance of rabbit eye blueberry, it was interesting to clarify the response of rabbit eye blueberry to Al stress and determine whether plant growth and nutrient uptake were affected as highbush blueberry.

Materials and Methods

Plant materials and growth conditions

Rabbit eye blueberry ‘Brightwell’ and highbush blueberry ‘Brigitta’ were widely cultivated in China (Li et al., 2016), and ‘Brigitta’ was reported to be relative Al tolerant compared to other highbush blueberry cultivars (Reyes-Díaz et al., 2009). Therefore, the two cultivars were used in the study. Tissue cultured saplings with uniform size (≈15 cm tall) and well-established roots were selected. All saplings were conditioned for 4 weeks in plastic boxes filled with 18 L of aerated nutrient solution. The nutrient solution (Sugiyama and Hanawa, 1992) contained: 0.25 mmol L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), 0.5 mmol L\(^{-1}\) NaNO\(_3\), 1 mmol L\(^{-1}\) KH\(_2\)PO\(_4\), 0.5 mmol L\(^{-1}\) K\(_2\)SO\(_4\), 2 mmol L\(^{-1}\) CaCl\(_2\), 1 mmol L\(^{-1}\) MgSO\(_4\), 46.3 μmol L\(^{-1}\) H\(_3\)BO\(_3\), 9.1 μmol L\(^{-1}\) MnCl\(_2\), 0.76 μmol L\(^{-1}\) ZnSO\(_4\), 0.31 μmol L\(^{-1}\) CuSO\(_4\), 0.1 μmol L\(^{-1}\) (NH\(_4\))\(_2\)MoO\(_4\), and 19 μmol L\(^{-1}\) Fe-EDTA. The pH of solution was adjusted to 4.5 daily using a 1 mmol L\(^{-1}\) H\(_2\)SO\(_4\) solution, and the nutrient solution was renewed weekly. Beginning on June 9, saplings of ‘Brightwell’ and ‘Brigitta’ were cultured in nutrient solutions containing 0 or 100 μmol L\(^{-1}\) Al (as Al\(_2\)(SO\(_4\))\(_3\)), with four replicas of each sapling per treatment. The treatments were conducted in a growth chamber with a 14 h/25 °C day 10 h/20 °C night regime, a light intensity of 360 μmol m\(^{-2}\) s\(^{-1}\) and 70% relative air humidity. The plants were harvested on Sept. 9 for analysis.
Sample analysis

Photosynthesis parameters

Photosynthesis rate, stomatal conductance, and transpiration rate of leaves were measured with an LI-6400 portable photosynthesis system (LI-COR, Lincoln, USA) at 1200 mol m\(^{-2}\) s\(^{-1}\) light intensity on August 4. Leaf temperature in the leaf chamber was set to 25 °C, with humidity in the leaf chamber set to that in the field. Leaf chamber CO\(_2\) concentration was set to 400 mol mol\(^{-1}\) with the flow rate set to 500 mol s\(^{-1}\). All measurements were taken during the periods of 10:00-11:30 a.m.

Root morphology and plant growth

Following harvest, the plants were separated into roots, stems and leaves and the tissues were rinsed with deionized water. Subsamples of roots, stems and leaves from each plant were dried at 75 °C for at least 48 h to determine dry weight. One gram of fresh fine roots was collected from each plant and scanned using a flatbed scanner (Epson Expression 1680, Long Beach, CA, USA) to measure the root diameter, length, and surface area using the WinRHIZO Pro software (Regent Instruments, Quebec, Canada).

Root activity

Root activity was measured using the triphenyl tetrazolium chloride (TTC) method (Lindström and Nyström, 1987). Briefly, TTC is reduced by dehydrogenases, root-derived enzymes that provide a proxy for root activity. Fresh fine roots from each sapling were washed with deionized water, and then the terminal 1 cm portions of the roots were excised and treated with TTC.

Lipid peroxidation

Lipid peroxidation in roots was estimated by measuring their malondialdehyde content (de Azevedo Neto et al., 2006). For each sample, 200 mg of fresh roots were homogenized in a solution of 0.3% (w/v) thiobarbituric acid in 10% trichloroacetic acid. The homogenate was heated at 95 °C for 30 min and then quickly cooled in an ice bath and centrifuged for 10 min at 10,000 g. The concentration of malondialdehyde (MDA) was calculated as the difference of the absorbance at 532 and 600 nm using an extinction coefficient of 155 mmol L\(^{-1}\) cm\(^{-1}\).

Plant nutrients

Subsamples of dried roots, stems and leaves from each plant were ground and passed through a 2-mm sieve. A portion of these samples was digested in concentrated H\(_2\)SO\(_4\) and H\(_2\)O\(_2\). The digested solution was analyzed for N concentration by the micro-Kjeldahl method, P concentration by the molybdate-blue method, and K concentration by flame photometry (Lu, 1999). Another portion of the ground sample was digested in concentrated HNO\(_3\) and HClO\(_4\) (83:17, v v). The concentrations of Al and micronutrient minerals (Fe, Mn, Cu, Zn, B, Mg, and Ca) in the digested solution were determined by inductively coupled plasma–atomic emission spectroscopy (ICP-AES) (IRIS-Advantage, Thermo Elemental, Boston, MA, USA).

Statistical analysis

Data were analyzed with a paired t test using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance was used to test for significant differences at a 0.05 level.
Results

Effect of Al on plant growth

No differences in biomass (dry weight) were found in Al-treated 'Brightwell' plants compared with no-Al plants (Figure 1). In contrast, roots of Al-treated 'Brigitta' had lower biomass than no-Al plants, which was decreased by 36.8% by Al stress. However, leaf and stem biomass were not significantly affected (Figure 1).

Root morphology of both cultivars has been affected by Al stress (Table 1). Average root diameter of 'Brigitta' was 0.238 mm, which was similar to 'Brightwell' and average root diameter of both cultivars was not affected by Al stress. Root length per fresh weight of 'Brightwell' was increased by 23% in Al treatment, while it was significantly decreased on 'Brigitta' with Al. Root surface area per fresh weight of 'Brightwell' was increased by 22% in Al treatment, whereas it was unaffected by Al on 'Brigitta'.

Root activity of 'Brightwell' was unaffected by Al application, whereas it was decreased by 34% for 'Brigitta' with Al (Figure 2). Lipid peroxidation of 'Brigitta' was significantly increased by Al treatment, which was decreased by 35% with Al treatment for 'Brightwell' (Figure 3).

Table 1. Root morphological structure of 'Brightwell' and 'Brigitta' grown in solution with no Al (CK) or 100 μmol Al L⁻¹. Values are the mean ± SD, n=4. Different letters indicate significant differences between treatments (p<0.05).

|                | Brigitta | Brightwell |
|----------------|----------|------------|
|                | CK Al    | CK Al      |
| Average diameter (mm) | 0.238±0.0110a | 0.246±0.0117a |
| Root length (mg g⁻¹ FW) | 30.0±2.32a | 26.6±1.51b |
| Surface area (cm² g⁻¹ FW) | 224±11.7a | 207±16.8a |

Figure 1. Biomass of 'Brightwell' and 'Brigitta' grown in solution with no Al (CK) or 100 μmol Al L⁻¹ (Al). Asterisks (**) indicate significant differences between treatments (p<0.05).

Effect of Al on nutrient uptake

The N, P, and K concentrations in 'Brightwell' plant tissues were largely unaffected by Al application, except for an increase in the N concentration of leaves and a decrease in the P concentration of stems (Figure 4). Al also not significantly impacted N, P, and K concentrations in 'Brigitta', except a decrease of N concentration in leaves.

Al concentration in the tissues of 'Brigitta' and 'Brightwell' were significantly increased in Al treatment compared to the control. In addition, foliar Al concentrations of 'Brigitta' were 2.6 times higher than 'Brightwell' (Table 2). For 'Brigitta', Fe, Mn, Cu, Zn, B, and Ca concentrations of roots, Fe, Mn, Cu, and Zn
concentrations of stems, Mn and Cu concentrations of leaves were decreased by Al treatment. While the concentrations of Fe, Mn, Cu, Zn, B, Ca, and Mg in 'Brightwell' were unaffected by Al treatment, except that Cu, B, and Ca concentrations in roots were decreased.

**Effect of Al on photosynthesis parameters**

Stomatal conductance, Intercellular CO$_2$ Concentration and Transpiration rate of both cultivars were not affected by Al (Table 3). Photosynthesis rate of 'Brightwell' was also not affected by Al. However, photosynthesis rate of 'Brigitta' was significantly decreased by 13% under Al stress.

Table 2. Mineral concentrations (mg kg$^{-1}$) of tissues of 'Brightwell' and 'Brigitta' grown in solution with no Al (CK) or 100 μmol Al L$^{-1}$. Values are the mean ± SD, n=4. Different letters indicate significant differences between treatments ($p<0.05$)

| Mineral | Brightwell | Brigitta |
|---------|------------|----------|
|         | Leaf       | Stomata  |
| Fe      | 6.9±0.2a   | 3.9±0.2a |
| Mn      | 46.8±2.0a  | 84.6±1.7a|
| Cu      | 178±19.8a  | 780±13.9a|
| Zn      | 820±644a   | 853±205a|
| B       | 559±69.5a  | 558±83.7a|
| Ca      | 12.1±2.25a | 12.6±2.07a|
| Mg      | 2463±40.1a | 2329±17.7a|

Table 3. Photosynthesis parameters of 'Brightwell' and 'Brigitta' plants grown with no Al (CK) or 100 μmol Al L$^{-1}$. Values are the mean ± SD, n=4. Different letters indicate significant differences between treatments ($p<0.05$)

| Parameter                        | Brightwell | Brigitta |
|----------------------------------|------------|----------|
| Photosynthesis rate (μmol CO$_2$ m$^{-2}$s$^{-1}$) | 7.26±0.60a | 7.51±0.79a |
| Stomatal conductance (mmol H$_2$O m$^{-2}$s$^{-1}$) | 0.10±0.04a | 0.09±0.02a |
| Intercellular CO$_2$ Concentration (μmol CO$_2$ mol$^{-1}$) | 240±43.7a | 234±38.6a |
| Transpiration rate (mmol H$_2$O m$^{-2}$s$^{-1}$) | 1.90±0.64a | 1.75±0.48a |

Figure 2. Root activity of 'Brightwell' and 'Brigitta' grown in solution with no Al (CK) or 100 μmol Al L$^{-1}$ (Al). Asterisks (**) indicate a significant difference between treatments ($p<0.05$)
Figure 3. Lipid peroxidation of roots of 'Brightwell' and 'Brigitta' grown in solution with no Al (CK) or 100 μmol Al L⁻¹ (Al). Asterisks (**) indicate significant differences between treatments (p < 0.05).

Figure 4. N, P, K concentration of tissues of 'Brightwell' and 'Brigitta' grown in solution with no Al (CK) or 100 μmol Al L⁻¹ (Al). Asterisks (**) indicate significant differences between treatments (p < 0.05).

Discussion

Effect of Al on root growth

Root physiological responses could be detected in short time exposure of Al (Kochian et al., 2015). Lipid peroxidation was induced by Al stress, which can alter the integrity of the plasma membranes (Horst et al., 2010). Therefore, Lipid peroxidation in roots was used as a criterion for determining Al tolerance in plants (Yamamoto et al., 2001; Jones et al., 2006; Giannakoiila et al., 2008). Besides, reduction of root mitochondrial activity was also sensitive to Al exposure, and root activity was also used as an indicator of root damage under
Al stress (Ruf and Brunner, 2003; Hirano et al., 2007). In the study, Root activity of ‘Brightwell’ was unaffected, and lipid peroxidation was decreased by Al application (Figures 2 and 3), which suggested root physiological functions of ‘Brightwell’ was not inhibited by Al. Indeed, no differences in biomass (dry weight) were found in Al-treated ‘Brightwell’ plants compared with no-Al plants, while, roots biomass of ‘Brigitta’ was decreased by 36.8% by Al (Figure 1).

It was reported that the alteration in root architectures can be noticeable in short time of Al exposure (Schaedle et al., 1989). Al primarily inhibits root elongation, and causes thickening and swelling of roots (Ma et al., 2001). Therefore, the maintenance of root elongation rate under Al stress has been used for Al tolerance screening purposes (Narasimhamoorthy et al., 2007). In the study, the decreased root length per fresh weight also indicated that ‘Brigitta’ was sensitive to Al, which was consistent with previous studies (Reyes-Diaz et al., 2009). While, the enhancement of root elongation of ‘Brightwell’ by Al indicated Al might be beneficial to ‘Brightwell’, like tea (Zeng et al., 2012) and Melastoma malabathricum (Watanabe et al., 2008). As root surface area per fresh weight of ‘Brightwell’ was also increased by 22% in Al treatment (Table 1). It was reported that the elongation of root and greater root surface area in Al treatment were the mechanisms of Al stimulatory effect to tea (Hajiboland et al., 2013). Baligar and Fageria (2015) reported that enhancing root surface area is good to facilitate uptake of less mobile nutrients such as P, micronutrients, and mobilization and solubilisation of unavailable organic/inorganic nutrients in soil. As limitations on acid soils are toxic levels of Al and suboptimal levels of P (Kochain et al., 2015), the higher root surface area per fresh weight in Al treatment could be beneficial for ‘Brightwell’ to absorb nutrients and adapt to acid soil.

**Effect of Al on nutrient uptake**

Due to the limited growth of roots and its disordered functions, plants treated with Al had a lower ability to absorb nutrients. Nutrients imbalances, rather than direct toxicity, were likely the other main reason for the negative effects of Al on plant growth (Ahonen-Jonnarth et al., 2003). In the study, leaf N concentration of ‘Brigitta’ was significantly decreased by Al, while, which of ‘Brightwell’ was increased by Al (Figure 4). The increased N concentration in ‘Brightwell’ may result from the higher root length and root surface area per fresh weight, which was also observed on other plants, such as Camellia aleifera, Melastoma malabathricum (Ghanati et al., 2005; Watanabe et al., 2005; Hajiboland et al., 2013). The growth and N, P, and K uptake in Melastoma malabathricum and Vaccinium macrocarpon was stimulated by Al treatment (Osaki et al., 1997). While, concentrations of Cu, B, and Ca in roots of both cultivars were decreased by Al, which may be ascribed to the competitive adsorption of Al for root apoplast exchange sites (Cronan, 1991). Besides, Mn and Cu concentrations in shoots of ‘Brigitta’ were also decreased; it suggested that ‘Brigitta’ showed a lower capability to uptake these elements under Al stress.

To tolerant Al toxicity, many mechanisms have been employed by plants (Horst et al., 2010; Kochain et al., 2015). One of the major mechanisms is the inhibited transportation of Al to shoots to protect the normal metabolisms (Poschenrieder et al., 2008). Since Al is an active element, which can interact strongly with organic molecules in the cell (Singh et al., 2017). In the study, Al concentration in the tissues of ‘Brigitta’ and ‘Brightwell’ were both significantly increased in Al treatment compared to the control. However, foliar Al concentrations of ‘Brigitta’ were 2.6 times higher than ‘Brightwell’ (Table 2). High Al level in blueberry leaves could reduce photosynthesis electron transport at PSI level, and decline photosynthesis rate, therefore, influence blueberry growth (Ulloa-Inostroza, et al., 2019). In the study, similar results were found on ‘Brigitta’ (Table 3). While, the photosynthesis parameters of ‘Brightwell’ were not affected by Al treatment. The unaffected photosynthesis rate of ‘Brightwell’ suggested it was more adapted to Al stress than ‘Brigitta’.
Conclusions

It was found that root activity and lipid peroxidation of ‘Brightwell’ were not negatively affected by Al, besides, root growth, nutrients uptake and photosynthesis rate of rabbit eye blueberry ‘Brightwell’ were also not negative affected by Al, unlike ‘Brigitta’. Meanwhile, root morphology of ‘Brightwell’ was positive affected by Al compared to ‘Brigitta’. Therefore, it can be concluded that rabbit eye blueberry ‘Brightwell’ is more resistant to Al stress than ‘Brigitta’, which was the relative Al tolerant cultivar in highbush blueberry (Reyes-Diaz et al., 2009).

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Nos.31301838; 31601709); the Natural Science Foundation of Jiangsu Province, China (Nos. BK20170615; BK20160597); Key Research and Development Plan (Modern Agriculture) of Jiangsu Province, China (No. BE2017373).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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