Aluminum stress differentially affects physiological performance and metabolic compounds in cultivars of highbush blueberry

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Aluminum (Al) toxicity is one of the major factors that limit the growth and production of crops in acid soils. Highbush blueberry (Vaccinium corymbosum L.) cultivars differing in resistance to Al toxicity regarding root growth and photosynthetic performance were used. In this study, we compared the physiological and metabolic strategies to cope with Al toxicity among the highbush blueberry cultivars [two new ones (Camellia and Cargo) and three established ones (Brigitta (Al-resistant), Star and Duke)]. Aluminum concentration in roots and leaves increased in all cultivars after 24 and 48 h of exposure to Al, but less so in roots of cultivar Camellia and leaves of cultivar Cargo. These two cultivars displayed minor effects of Al exposure in terms of photosynthetic activity in comparison with the established cultivars. Furthermore, Cargo did not vary fluorescence parameters, whereas Camellia exhibited a decrease in effective quantum yield (ΦPSII) and electron transport rate (ETR) and a change in non-photochemical quenching (NPQ) and maximum quantum yield (Fv/Fm) under Al after 48 h. The Al treatment increased total phenols in leaves of Brigitta, Cargo, and Camellia, whereas antioxidant activity increased in Star and Cargo after 48 h. Aluminum exposure decreased malate concentration in roots of all cultivars, but no change was noted in fumarate concentration. The antioxidant activity correlated with photosynthetic performance and the total phenol concentration in the leaves of new cultivars exposed to Al, suggesting enhanced resistance in the short-term experiment. The principal component analysis separated the new from the established cultivars. In conclusion, the new cultivars appear to be more Al-resistant than the established ones, with Star being most Al-sensitive. Regarding the Al-resistance mechanisms of the new cultivars, it is suggested that Camellia could have a root Al-exclusion mechanism under Al toxicity. This mechanism could be explained by low Al concentration in roots, suggesting that this cultivar could exude organic acid, allowing to chelate Al in the rhizosphere. Nonetheless, further researches are needed to confirm this assumption.

Acid soils are characterized by nutrient deficiency and toxicity of metals such as manganese (Mn), iron (Fe) and aluminum (Al), with Al toxicity being the main limiting factor for plant growth in acid soils6. Aluminum is incorporated into aluminosilicates and other insoluble forms, which are harmless to plants at neutral or near-neutral pH values7,8. Aluminum in acidic soils (pH$_{watt}$ < 5.0) is solubilized, being available to plants as Al$^{3+}$ and Al(OH)$_2$$^{+}$ forms4,5. Acid soils comprise around 50% of the world’s arable lands9. Aluminum toxicity to plants includes two
categories of responses: (i) short-term responses that can be observed within a few minutes to an hour after Al exposure, and (ii) long-term responses that require hours or days to occur13–15. However, the Al toxicity effects on plant growth depend on Al concentration, plant species, genotypes, plant age, and growth conditions16.

In roots, Al accumulates predominantly in the apical elongation zone, inhibiting cell elongation within a few minutes of Al exposure16. The Al-related inhibition of growth and injury to root apex cells has been observed in many plants species17–19, including highbush blueberry Vaccinium corymbosum14. The Al exposure responses are associated with changes in physiological and biochemical processes, including increase in reactive oxygen species (ROS) and damage to biological membranes, as well as negative effects on photosynthetic activity, such as decreases in photosynthetic pigments and fluorescence parameters, reduced enzymatic activity in carbohydrate metabolism, decreased stomatal conductance, and ultimately the programmed cell death15–18. In Citrus, the CO2 assimilation, non-photochemical quenching (NPQ), photochemical quenching (qP), the effective quantum yield of PSII and maximum quantum yield of PSII (Fv/Fm) were decreased by Al toxicity16. In V. corymbosum, a decrease in photosynthetic performance under Al toxicity was noted in the Al-sensitive but not Al-resistant cultivars19. Al exposure affected carbohydrate storage, translocation, and metabolism20. An increase in carbohydrate concentration in the presence of Al was correlated positively with Al resistance in Quercus serrata21. In contrast, in highbush blueberry, carbohydrate concentration decreased under Al stress compared with the control21. In the Al-sensitive Citrus grandis, a decrease in total soluble protein in leaves was reported under Al toxicity, whereas no change occurred in the Al-tolerant species C. sinensis22.

An important mechanism underpinning avoidance of Al stress is the chelation of Al (internally or externally), usually by organic acid anions (OAA) such as citrate, oxalate and/or malate and fumarate (in the order of binding strength OAA:Al)19,23–26. In Populus trichocarpa and P. tremuloides, Al-induced exudation of citrate, malate, and oxalate from roots was observed23. In addition to OAA, antioxidant compounds such as phenolics also have the capacity to chelate toxic metal ions due to their functional groups [hydroxyl (-OH) and carboxylic (-COOH)]28, reducing the harmful effects on plants29.

Highbush blueberry grows well in acid soils, with pHvalues between 4.4 and 5.530. In Chile, this species is usually cultivated in volcanic ash-derived soils31, in areas characterized by soil acidity and high availability of Al31, low concentration of exchangeable bases (Ca++, Mg++, K+, Na+), high rainfall, and severe phytotoxicity of Al32. Studies performed in the established highbush blueberry cultivars indicated that short-term Al exposure differentially affects the photochemical features, with Brigitta cultivar showing Al resistance and Bluegold cultivar being Al-sensitive33. Besides, in the long-term, cultivar Legacy had higher Al resistance than Bluegold, suggesting different strategies to cope with Al toxicity among these established cultivars19. Recently, new cultivars of blueberry such as Camellia and Cargo have been introduced to southern Chile. These cultivars are characterized by early production and high yield during the season, suggesting these new blueberry cultivars are more productive and could be more Al resistant than the established cultivars. Despite the importance of these new highbush blueberry cultivars, there is no knowledge of their Al sensitivity/resistance under acidic conditions and Al toxicity. Thus, this study aimed to compare the physiological and metabolic strategies of coping with Al toxicity between the new and established highbush blueberry cultivars.

Materials and Methods

Plant materials and growth conditions. In this study, we used three established cultivars (Brigitta, Star, and Duke), and two new cultivars (recently introduced from USA) (Camellia and Cargo) of highbush blueberry (Vaccinium corymbosum L.). One-year-old plants with 40 cm in height were conditioned in plastic pots containing 18 L of Hoagland solution34 for two weeks. The composition of this nutrient solution was 3.0 mM KNO3, 2.0 mM Ca(NO3)2, 1.0 mM MgSO4, 1.0 mM KH2PO4, 1.0 mM NH4NO3, 0.4 µM CuSO4, 2.0 µM ZnSO4, and 0.07 µM (NH₄)₆Mo7O24; it was renewed every 3 days. The growth chamber conditions were 16/8 h light/dark photoperiod, 22 ± 2 °C temperature, 70% relative air humidity and light intensity around 300 µmol photons m⁻² s⁻¹. The treatments were no Al (control treatment) and 200 µM AlCl3 at pH 4.5 adjusted daily; this is a toxic concentration for highbush blueberry as observed in previous studies19,33. The physiological parameters were evaluated after 24 and 48 h of Al, the times considered short-term exposure to Al35 for woody plant species1,20,33. At these times, fully-expanded leaves and root tissues were harvested for metabolic analyses at the mid-point of the light period. The samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

Determination of Al concentration. Aluminum concentration was analyzed as described previously35. For this, 1.0 to 3.0 g of dried tissues were ground, dry-ashed in a muffle furnace at 500 °C for 8 h and digested with 2 M HCl. The concentration of Al was determined using a multi-element atomic absorption spectrophotometer (EAA, Model 969, Unicam, Cambridge, UK).

Gas-exchange and chlorophyll a fluorescence parameters. Photosynthesis-related parameters were determined in fully-expanded leaves as described previously36. Shortly, the measurements were performed in the morning using a portable infrared CO2 analyzer (Licor LI6400, Lincoln, NE, USA), equipped with a measurement cuvette with its light source (300 µmol photons m⁻² s⁻¹), and control of temperature (20 °C) and CO2 (400 µL/L) according to Reyes-Díaz et al.37. Chlorophyll a fluorescence parameters measured in leaves at the second to fourth shoot node were used to determine the effective quantum yield of PSII using a portable pulse-amplitude-modulated fluorimeter (FMS 2; Hansatech Instruments, King’s Lynn, UK) according to Reyes-Díaz et al.37. The fluorescence parameters of effective quantum yield (ΦPSII), electron transport rate (ETR), and non-photochemical quenching (NPQ) were estimated as described previously37.
Determination of photosynthetic pigments. Chlorophyll a and b and carotenoids were extracted with 100% acetone at 4 °C under safe green light and centrifuged at 10,000 rpm at 4 °C according to Lichtenthaler and Wellburn38. Pigments were quantified according to García-Plazaola and Becerril 39 using phase-reversed solvent-gradient high-performance liquid chromatography (HPLC, Agilent Technologies Inc., San Jose, California, USA).

Antioxidants assays. The antioxidant activity (AA) in roots and shoots was determined based on the method described previously 40 using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Plant samples were ground in liquid nitrogen and soaked in 1 mL of 80:20 (v/v) methanol:water. The absorbance was measured at 515 nm by a spectrophotometer (UNICOR 2800 UV/VIS, Spain) using Trolox as the standard. The values were expressed in μg Trolox equivalents g−1 fresh weight (FW).

Total phenols. The total phenols (TP) were determined by the Folin-Ciocalteau method, as described by Slinkard and Singleton41. Absorbance was measured at 765 nm and expressed in chlorogenic acid equivalents (CAE) g−1 FW.

Metabolite analyses. Approximately 15 mg of dry ground material was used for metabolite analyses. Samples were subjected to methanol extraction without Ribitol, according to Medeiros et al.42. The methanol soluble phase was transferred to a 1.5 mL tube for the quantification of sugars, organic acids, and amino acids. The resulting pellet was subjected to three washes with the same extracting solution. Starch and total protein concentrations were quantified in the pellet obtained43,44. The supernatants and pellets were stored at −20 °C until further analyses.

The starch and soluble sugars (glucose, fructose, and sucrose) were analyzed as described by Daloso et al.45 and Stitt et al.46, with minor modification. The concentrations of total proteins and amino acids were quantified as described by Cross et al.44. The concentrations of malate and fumarate were determined as described by Nunes-Nesi et al.47. All measurements were performed in a VersaMaxTM Microplate Reader (Molecular Devices®).

Experimental design and statistical analyses. The experiment was performed in a split-plot design with five cultivars, three durations of Al exposure, and three replicates. When the data passed the normality and equality of variances after the Kolmogorov-Smirnov test, we performed a two-way analysis of variance (cultivars x duration of Al treatment) and the Tukey test. If data did not pass the Kolmogorov-Smirnov test, the Dunn test and Bonferroni transformations were performed. The Pearson correlation analysis was conducted with a significance level of P ≤ 0.05 to examine the relationships among variables. In order to identify the variables that explained the differences between the new and established cultivars, a multivariate analysis by principal components analysis (PCA) was made. All analyses were performed by XLSTAT-base v.2018.5.

Results

Aluminum concentration. The statistically significant interaction between cultivars and duration of Al exposure was noted for Al concentration in roots and leaves (p < 0.001) (Fig. 1). The higher Al concentration was observed after 48 h compared with 24 h in roots and leaves of all cultivars. In new cultivar Cargo, roots exhibited the highest Al concentration at 48 h (39-fold), followed by established cultivars Brigitta (13-fold) and Star (13-fold), whilst in Duke and Camellia an increase in Al concentration was smaller (4- and 1.4-fold, respectively) in relation to their controls (Fig. 1a). In leaves, Brigitta showed the highest Al concentration at 48 h (4.5-fold), followed by Duke (4.4-fold), Star (3.2-fold), Camellia (2.8-fold), and Cargo (1.8-fold) cultivars compared to the respective controls (Fig. 1b).

Figure 1. Aluminum concentration in highbush blueberry cultivars under Al toxicity. Aluminum concentration in (a) roots and (b) leaves after 0, 24 and 48 h of exposure to Al (200 μM Al) in Brigitta, Star, Duke, Camellia, and Cargo cultivars. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Uppercase letters indicate significant differences (p ≤ 0.05) among cultivars, and lowercase letters indicate significant differences (p ≤ 0.05) among exposure times, according to Tukey test.
Photosynthetic parameters.

The significant interaction between cultivars and duration of Al exposure was observed for CO₂ assimilation (p < 0.001) (Fig. 2a) and stomatal conductance (p ≤ 0.05) (Fig. 2b). The CO₂ assimilation rate in the established cultivars (Brigitta, Star, and Duke) decreased (by 48, 37 and 32%, respectively) under Al treatment at 24 h, whereas cultivars Star and Duke restored their photosynthesis after 48 h to similar values as the control (Fig. 2a). In new cultivars (Camellia and Cargo), the photosynthesis remained unaltered with respect to the control treatment (Fig. 2a). Stomatal conductance was reduced in Star and Duke (42 and 23%, respectively) after 24 h of Al treatment compared to the control, followed by an enhancement at 48 h (Fig. 2b). In Brigitta, a decrease in stomatal conductance (32%) was noted after 48 h of Al exposure. New cultivars (Camellia and Cargo) did not change stomatal conductance during Al exposure (Fig. 2b).

For all the fluorescence parameters, the significant interaction between cultivars and duration of Al exposure was observed. Concerning the chlorophyll a fluorescence parameters, new cultivar Camellia exhibited a significant reduction (p ≤ 0.05) of 40% in ΦPSII and ETR at 24 h, whereas established cultivars (Brigitta and Duke) decreased by 32 and 27% after 48 h of Al treatment (Table 1). In Cargo and Star plants, ΦPSII and ETR remained unchanged. On the other hand, the NPQ and Fv/Fm values were unchanged after 24 and 48 h of Al exposure. However, the NPQ values were highest in cultivar Cargo, followed by Star, Camellia, Duke, and Brigitta. Fv/Fm in all cultivars was around 0.8, which is in the range of healthy values for plants (Table 1).

The significant interaction between cultivars and duration of Al exposure was found for chlorophyll pigments. Total chlorophyll content (Chl a + b) in Brigitta, Duke, and Camellia leaves decreased by 24, 20 and 18%, respectively, after 24 h of Al treatment compared with the control, whereas in Star no significant difference was observed after 24 h of Al exposure. New cultivar Cargo had around a 24% increase in total chlorophyll in the Al treatment (Fig. 2c). In established cultivar Brigitta at 48 h, Chl a + b recovered to the control values (Fig. 2c). There was no significant difference in the Chl a/b ratio in all cultivars in the Al treatment, with the exception of an increase in Brigitta after 48 h of Al exposure (Fig. 2d).

The significant interaction between cultivars and duration of Al exposure was found for leaf carotenoids. New cultivar Camellia had higher concentrations of carotenoids than Duke, Brigitta, Cargo, and Star under Al exposure, whereas Star and Cargo displayed increases. However, cultivars Brigitta, Duke and Camellia exhibited a decrease in carotenoid concentration under Al stress compared to the control (Fig. 3). The leaf β-carotene in new cultivar Camellia was decreased (38%) by the Al treatment, whereas in new cultivar Cargo a significant increase (41%) was observed (Fig. 3). Lutein declined significantly (44%) in established cultivar Brigitta at 24 h, increasing afterward. This metabolite increased by around 20% in Cargo and Star under Al toxicity (Fig. 3). Concerning xanthophylls, the new cultivars exhibited unchanged values, whereas the established cultivar Brigitta decreased by about 32% at 24 h, increasing afterward (Fig. 3). In cultiver Star, violaxanthin increased (46%) in the Al treatment, whereas neoxanthin rose by 30% only at 24 h (Fig. 3). New cultivar Cargo increased the violaxanthin/anteroxanthin ratio (V/A) through time, but established cultivar Duke did not vary over time, showing the lower values...
Exposure at 24 and 48 h (Fig. 4a).

Amino acids and proteins. The concentration of amino acids in roots of all cultivars remained constant under Al stress, except in new cultivar Cargo (decreased by 27% at 48 h), with respect to the control (Fig. 4a). In leaves, the interaction between cultivars and duration of Al exposure was significant regarding amino acid concentration. The amino acid concentration in leaves of established cultivar Duke decreased around 52%, and in the cultivar Camellia increased 60%, after 24 h of Al exposure. Amino acids in leaves of established cultivar Star, Duke, Camellia, and Cargo at 0, 24, and 48 h. The values are the average of three measurements per cultivar (24 and 48 h). Uppercase letters indicate significant differences (p ≤ 0.05) among exposure times, according to the Tukey test. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Uppercase letters indicate significant differences (p ≤ 0.05) among cultivars, and lowercase letters denote significant differences (p ≤ 0.05) among exposure times, according to the Tukey test.

Table 1. Fluorescence parameters in highbush blueberry cultivars under Al toxicity. Effective quantum yield of PSII (ΦPSII), electron transport rate (ETR), non-photochemical quenching (NPQ) and maximum quantum yield of PSII (Fv/Fm) in control (0 μM Al) and aluminum (200 μM Al) treatments. Cultivars tested were Brigitta, Star, Duke, Camellia, and Cargo at 0, 24, and 48 h. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Under Al toxicity than under control. In new cultivar Camellia, the V/A values were lower compared to the other cultivars, whereas in established cultivars Brigitta and Star the V/A values were higher at 24 h compared to the other times (Fig. 3).

Amino acids and proteins. The concentration of amino acids in leaves of all cultivars remained constant under Al stress, except in new cultivar Cargo (decreased by 27% at 48 h), with respect to the control (Fig. 4a). In leaves, the interaction between cultivars and duration of Al exposure was significant regarding amino acid concentration. The amino acid concentration in leaves of established cultivar Duke decreased around 52%, and in the cultivar Camellia increased 60%, after 24 h of Al exposure. Amino acids in leaves of established cultivar Star increased 50% after 48 h of Al toxicity (P ≤ 0.05), whereas Brigitta did not exhibit significant differences under Al exposure at 24 and 48 h (Fig. 4a).

Protein concentration in roots was similar in all cultivars, with the exception of Cargo at 24 h of Al exposure (Fig. 4b). In leaves, the significant interaction between cultivars and duration of Al exposure was observed for protein concentration. In new cultivar Camellia leaves, protein concentration was unchanged over time, but in established cultivar Star a significant reduction (P ≤ 0.05) was noted at 24 h followed by recovery at 48 h. Cargo and Duke had the highest protein concentration at 48 h, whereas Brigitta showed reduced protein concentration after 48 h of Al exposure (Fig. 4b).
Soluble sugars and starch. Sucrose and starch in roots showed significant interaction ($P \leq 0.001$) between cultivars and duration of Al exposure, whereas glucose and fructose were significantly affected by the cultivar factor only. Root glucose in the established cultivars did not vary over time, but increased in Camellia by 1.5-fold at 24 h, and diminished by 38% in Cargo at 48 h (Table 2). Regarding root fructose concentration, the most evident change was observed in Camellia (increased 2.4- and 2.2-fold at 24 and 48 h, respectively) ($P \leq 0.05$) related to the control. In established cultivar Star, an increase in sucrose of 1.5-fold after 24 h and 1.9-fold after 48 h was observed under Al toxicity. In roots, sucrose concentration decreased significantly (by 63 and 93% in new cultivars Camellia and Cargo, respectively) (Table 2). The concentration of starch in Brigitta, Star and Cargo roots was reduced by 33, 71 and 30%, respectively, after 48 h of Al treatment, whereas in Camellia an increase (24%) was noted.

In leaves, sucrose ($P = 0.024$) and starch ($P < 0.001$) showed the significant interaction between cultivars and duration of Al exposure. Sucrose decreased in leaves of Star, Duke, and Cargo only. Leaves of Duke and Cargo showed increased, and Brigitta and Camellia decreased, starch concentration under Al exposure (Table 2). In leaves of Star, Duke, Camellia, and Cargo, the concentration of glucose did not change, whereas in Brigitta a slight increase (16%) in glucose was found at 48 h (Table 2). In leaves, fructose was unchanged in all cultivars (Table 2).

Malate and fumarate concentrations. The significant interaction between cultivars and duration of Al exposure was found for malate concentration in roots ($P < 0.001$). The concentration of malate in roots of all cultivars was reduced by up to 83% compared to the control after 48 h of Al treatment. In leaves, an increase (by 19%) in malate was observed in Brigitta at 24 h (Table 3). Regarding the fumarate concentration, established cultivar Brigitta exhibited changes in roots and especially in leaves, decreasing by 41% in roots at 24 h and by 69% in leaves at 48 h with respect to the control (Table 3).

Antioxidant activity. The lowest values of antioxidant activity in roots and leaves subjected to Al toxicity were observed in new cultivar Camellia compared to the other cultivars (Fig. 5). In roots, the interaction between cultivars and duration of Al exposure was significant ($P < 0.001$). The major differences were observed in Camellia roots at 48 h, being 2.5-fold higher than at other time points. Similarly, in the roots of established cultivar Star higher antioxidant activity (1.2-fold) was observed at 48 h compared with other times (Fig. 5a). In leaves of established cultivar Brigitta, there was a significant decrease (14%) at the end of the Al treatment, whereas new cultivar Cargo showed an increase of 12% at the same time (Fig. 5b). Conversely, antioxidant activity in Star increased (35–90%) with Al exposure (Fig. 5b).

Total phenols. In roots after 48 h of Al exposure, total phenols decreased in Brigitta (12%), Star (66%), Camellia (14%), and Cargo (10%), but not in Duke (Table 4). In contrast, leaves showed an increase in total phenols in Brigitta (67%), Camellia (28%), and Cargo (12%) at 48 h. Conversely, in shoots, significant reductions in this parameter were found in Star and Duke at 24 h under Al stress (Table 4).
In all cultivars. In the new cultivars, positive correlations were observed between total proteins and starch concentration in roots and leaves in control (0 μM Al) and aluminum (200 μM Al) treatments in five cultivars at 0, 24 and 48 h. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Uppercase letters denote significant differences (p ≤ 0.05) among exposure times, according to the Tukey test.

**Table 2.** Soluble sugars and starch in highbush blueberry cultivars under Al toxicity. Glucose, fructose, sucrose, and starch concentration in roots and leaves in control (0 μM Al) and aluminum (200 μM Al) treatments in five cultivars at 0, 24 and 48 h. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Uppercase letters denote significant differences (p ≤ 0.05) among cultivars, and lowercase letters indicate significant differences (p ≤ 0.05) among exposure times, according to the Tukey test.

Pearson correlations and principal component analysis. To evaluate the association between the evaluated features, we calculated Pearson correlation coefficients for all pairs of metabolites at 0, 24, and 48 h of Al treatment (Fig. 6). When the data sets characterizing roots of Brigitta, Star and Duke (established cultivars) were grouped, the most correlations were highly significant compared to new cultivars, Camellia and Cargo (Fig. 6a,b). On the other hand, the established cultivars showed a significant negative correlation between Al-concentration and total phenols in roots, whereas a positive correlation was found between Al concentration and antioxidant activity (Fig. 6a). In leaves of the established cultivars, we obtained 14 positive and 21 negative significant correlations, whereas the new cultivars exhibited 54 positive and 57 negative significant correlations. Chi a + b showed a positive correlation with most carotenoids in all cultivars. In the new cultivars, positive correlations were observed between total proteins and \( \delta_{pS} \) or ETR. Hence, these results clearly indicated different physiological and metabolic responses to Al exposure between the established and new highbush blueberry cultivars, as well as different responses in roots and leaves.

For PCA, the data obtained for all cultivars were averaged and normalized, as indicated in Fig. 7. For root tissues, the first principal component (PC1), which explained 41.9% of the total variance, included total phenols, starch, and proteins as the main contributing variables (Fig. 7a). The second principal component (PC2) explained 30.6% of the total variance and grouped fructose, glucose, amino acids, malate, and fumarate (Fig. 7a). When we compared the PCA score plots (Fig. 7b) for roots, we observed a clear separation between established (Brigitta, Star, and Duke) and new (Camellia and Cargo) cultivars of highbush blueberry (Fig. 7b), which is very important given that root tissues are the first targets of Al toxicity.

For all studied parameters evaluated in leaves, PC1 and PC2 explained 33.9 and 23.97% of the total variance, respectively (Fig. 7c). The first principal component (PC1) included chlorophyll b, chlorophyll a, chlorophyll \( a + b \), neoxanthin, lutein, antheraxanthin, fructose, and Al concentration (Fig. 7c). The PCA score plot (Fig. 7d) for leaves showed a clear separation among the cultivars of highbush blueberry (Fig. 7d).
Table 3. Internal organic acid anion concentrations in highbush blueberry cultivars under Al toxicity. Malate and fumarate concentration, and total phenols in roots and leaves in control (0 μM Al) and aluminum (200 μM Al) treatments in five cultivars at 0, 24, and 48 h. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Uppercase letters indicate significant differences (p ≤ 0.05) among cultivars, and lowercase letters denote significant differences (p ≤ 0.05) among exposure times, according to the Tukey test.

| Cultivar | Roots | Leaves |
|----------|-------|--------|
|          | 0 h   | 24 h   | 48 h | 0 h    | 24 h   | 48 h |
|          |       |        |      |       |        |      |
| Malate   |       |        |      |       |        |      |
| Brigitta | 8.61 ± 0.76Aa | 4.48 ± 0.86Ab | 5.16 ± 1.09Ab | 17.00 ± 1.35Ab | 21.04 ± 0.47 Aa | 19.87 ± 1.66Ab |
| Star     | 5.34 ± 0.17Ba | 2.21 ± 0.46Bb | 1.41 ± 0.14Bc | 17.80 ± 1.03Aa | 20.70 ± 2.53 Aa | 18.44 ± 0.01Aa |
| Duke     | 2.37 ± 0.17Ca | 1.57 ± 0.33Bb | 0.40 ± 0.40Bc | 14.16 ± 1.47Aa | 14.91 ± 0.35Ba | 12.88 ± 0.57Ba |
| Camellia | 1.98 ± 0.09Ca | 2.37 ± 0.44Ba | 1.31 ± 0.22Bb | 14.81 ± 0.96Aa | 15.78 ± 1.92Ba | 14.68 ± 1.58Ba |
| Cargo    | 1.86 ± 0.10Ca | 0.98 ± 0.42Bb | 0.79 ± 0.45Bb | 15.79 ± 1.43Ab | 20.74 ± 1.56Aa | 16.86 ± 1.56AAB |

Table 4. Total phenol concentration in highbush blueberry cultivars under Al toxicity. Total phenols in root and leaves in control (0 μM Al) and aluminum (200 μM Al) treatments in five cultivars at 0, 24, and 48 h. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Uppercase letters denote significant differences (p ≤ 0.05) among cultivars, and lowercase letters indicate significant differences (p ≤ 0.05) among exposure times, according to Dunn test, Bonferroni correction and Tukey test.

| Cultivar | Roots | Leaves |
|----------|-------|--------|
|          | 0 h   | 24 h   | 48 h | 0 h    | 24 h   | 48 h |
|          |       |        |      |       |        |      |
| Total Phenols |       |        |      |       |        |      |
| Brigitta | 0.21 ± 0.01Cb | 0.25 ± 0.01Ca | 0.18 ± 0.00Cc | 0.75 ± 0.02Db | 0.77 ± 0.07Bb | 1.26 ± 0.03Ba |
| Star     | 0.26 ± 0.01Ba | 0.19 ± 0.00Db | 0.09 ± 0.01Dc | 1.32 ± 0.02Ra | 0.89 ± 0.08Bc | 1.23 ± 0.01Bb |
| Duke     | 0.19 ± 0.00Ca | 0.16 ± 0.00Da | 0.14 ± 0.00Ca | 1.59 ± 0.04Ca | 0.75 ± 0.03Bc | 0.98 ± 0.08Cb |
| Camellia | 0.37 ± 0.01Ab | 0.64 ± 0.02Aa | 0.32 ± 0.00Ab | 0.38 ± 0.01Eb | 0.34 ± 0.02Cb | 0.48 ± 0.03Da |
| Cargo    | 0.29 ± 0.01Bb | 0.35 ± 0.03Ba | 0.26 ± 0.00Bb | 2.19 ± 0.03Ab | 2.32 ± 0.05Aa | 2.45 ± 0.03Aa |

Figure 5. Antioxidant activity in highbush blueberry cultivars under Al toxicity. Antioxidant activity in (a) roots and (b) leaves in control (0 μM Al) and aluminum (200 μM Al) treatments in cultivars Brigitta, Star, Duke, Camellia, and Cargo at 24 and 48 h. The values are the average of three measurements per cultivar and treatment. Due to a lack of differences among time points in treatments without Al, we considered the values at 0 h as the average among the start of the experiment and the respective controls for each time point (24 and 48 h). Bars represent standard error among replicates. Uppercase letters denote significant differences (p ≤ 0.05) among cultivars, and lowercase letters denote significant differences (p ≤ 0.05) among exposure times, according to Dunn test, Bonferroni correction and Tukey test.
Discussion

Impairment in root growth is a primary symptom of Al toxicity and has been used to establish differences in Al sensitivity among cultivars\(^{15,20}\). In the roots apexes, Al accumulates in the cell wall due to the trivalent Al cation binding to negative wall charges\(^{14}\). Al-tolerant genotypes of wheat accumulated 3- to 8-fold less Al in the root compared to Al-sensitive ones\(^{15,20}\). The accumulation of Al in the cell wall leads to the impairment of root growth and the inhibition of photosynthesis\(^{16}\).

Figure 6. Pearson correlations matrix. Significant correlations coefficients (\(p \leq 0.05\)) are set in bold, with positive and negative correlations being distinguished by green and red, respectively. (a) Pearson correlation matrix in roots of established cultivars; (b) Pearson correlation matrix in roots of new cultivars; (c) Pearson correlation matrix in leaves of established cultivars; and (d) Pearson correlation matrix in leaves of new cultivars. Abbreviations: Maximum quantum yield of PSII (Fv/Fm), the effective quantum yield of PSII (\(\Phi_{\text{PSII}}\)), electron transport rate (ETR) and non-photochemical quenching (NPQ).
The previous report on highbush blueberry indicated that Al-concentration was twice higher in the Al-sensitive than Al-resistant cultivar in the long-term experiment. In the study presented here, the lowest Al concentration was observed in the roots of Camellia, followed by Duke, Star, Brigitta, and Cargo (Fig. 1a), suggesting that cultivar Camellia could be the most Al-resistant of the cultivars tested.

In *Citrus reshni* subjected to Al stress, a decline was reported in CO2 assimilation, non-photochemical quenching (NPQ), the effective quantum yield of PSII (ΦPSII), and maximum quantum yield of PSII (Fv/Fm). Similarly, Al inhibited ΦPSII and ETR in *Sorghum*49. Moreover, Zhang et al.50 showed a decrease in chlorophyll content and net photosynthesis in *Glycine max* plants under Al treatment. In *Eucalyptus* sp., it was reported that low pH and Al toxicity provoked a gradual decrease in chlorophyll content, photosynthesis, and transpiration51. In highbush blueberry, a significant decrease in photosynthetic performance was reported under Al stress19,33,36. Our findings showed a similar trend, with established cultivars Brigitta and Duke showing Al-related decreases in ΦPSII, ETR (Table 1), photosynthesis and chlorophyll concentration (Fig. 2), whereas new cultivar Cargo did not vary these parameters (except chlorophyll concentration, where an increase was found). Conversely, new cultivar Camellia maintained photosynthesis, but showed decreases in chlorophyll concentration, ΦPSII, and ETR, suggesting that this cultivar may have compensatory mechanisms to cope with Al stress. In addition, we found that a decrease in photosynthesis in established cultivars (Star and Duke) was concomitant with a reduction in stomatal conductance (Fig. 2a,b). Non-photochemical quenching increased significantly in cultivar Duke, whereas in new cultivar Cargo, this parameter did not change, suggesting Cargo showed Al resistance during 48 h (Table 1). Our results showed that Fv/Fm did not change at 24 and 48 h under Al toxicity in any of the investigated cultivars over the short-term, showing normal values for plants52. This is in agreement with the reports on *Quercus glauca* and *Oryza sativa*, where Fv/Fm remained in a healthy range under long- and short-term Al exposure33,34.

It has been documented that Al causes harmful effects in the assimilation of nitrogen and impacts nitrogen metabolism as a whole55,56. Besides, Al-tolerant plants growing in acid soils prefer NH4+ to NO3− forms, whereas those growing in neutral or calcareous soils are Al-sensitive and prefer NO3− to NH4+.57,58. Similarly, *Vaccinium angustifolium* (lowbush blueberry), adapted to strongly acidic soils, preferred NH4+ and was strongly inhibited by...
NO₃⁻ to NO₂⁻. Al toxicity in acid soils may inhibit NO₃⁻ uptake, suggesting detrimental effects on the concentration of amino acids and proteins. In this study, we observed that protein and amino acid concentrations were unaltered in highbush blueberry roots under short-term Al exposure (Fig. 5a,b). In contrast to our findings, Somers et al. found that roots of Al-tolerant wheat showed an increase in total protein content, whereas roots of Al-sensitive cultivar exhibited no changes, suggesting that these findings are dependent on the plant species studied.

Several studies have demonstrated the accumulation of soluble sugars in response to stress, with the type and concentration depending on the plant species and stress treatments. There was evidence that Al increased sugar content in woody and cultivated plants. In roots, glucose has been reported as a key energy source to promote root growth under Al toxicity. In our case, the roots of cultivars Camellia and Star significantly increased the glucose concentration at 24 and 48 h, whereas Cargo was constant until 24 h, decreasing afterward (Table 2). The increment of glucose in roots of Camellia and Star could be associated with the strategy to cope with Al toxicity. Similar to our results, studies performed on Quercus serrata roots indicated greater glucose accumulation under Al exposure.

Organic acid anions and phenolic compounds have been related to mechanisms of Al resistance due to Al chelation to non-toxic forms. Malate in roots was positively correlated with Al resistance in several Eucalyptus species. In our experiment, malate concentration in roots decreased in all cultivars at 48 h but stayed unchanged in leaves. A potential reason for internal malate decreasing in roots, mainly in Star and Duke, maybe due to exudation. In contrast, Martins et al. reported that Plantago species accumulated citrate, oxalate, malate, and fumarate, which are involved in the internal Al detoxification in plant species such as Melastoma, buckwheat, Hydrangea, and Camellia sinensis. In our results, fumarate was present in low concentration and did not change in roots and leaves of all cultivars under Al toxicity, which was in agreement with other reports, suggesting an important role for fumarate in forming metal-ligand complexes. It appears that Al exposure decreased internal malate concentration in roots of highbush blueberry cultivars, which could be one of the mechanisms related to Al exclusion.

Phenolic compounds were exuded in Al³⁺-treated Eucalyptus camaldulensis, Melaleuca leucadendra, and Melaleuca caujaputi. We observed an increase in total phenols in roots of new cultivar Camellia at 24 h, whereas there was no change in roots of new cultivar Cargo, and there was an increase in leaves of Brigitta, Cargo and Camellia after 48 h of the Al treatment. Ofei-Manu et al. reported that phenolic compounds in the roots of some woody plant species correlated positively with Al tolerance. In the study presented here, total phenols strongly declined in both roots and shoots of established cultivar Star. For the new cultivars, we suggest that phenols could chelate Al in leaves of Cargo and Camellia, contributing to the maintenance of photosynthesis. It was suggested that polyphenols detoxify Al via chelation due to the high Al affinity to phenols. Moreover, phenolic acids have the capacity to reduce oxidative stress, so they are considered antioxidant compounds. Our findings showed that the established cultivars have higher antioxidant activity in roots than the new cultivars; whereas, in leaves, one established (Brigitta) and one new cultivar (Cargo) had higher antioxidant activity than the other cultivars. Antioxidant activity was significantly and positively correlated with photosynthetic performance and total phenols in leaves of the new cultivars, suggesting resistance to Al toxicity in the short-term. In addition, the PCA analysis separated the new cultivars from the established ones. In conclusion, the new cultivars appear to be more Al-resistant than the established ones, with Star being Al-sensitive and Camellia Al-resistant followed by Cargo. Regarding the Al-resistance mechanisms of the new cultivars, it is suggested that Camellia could have a root Al-exclusion mechanism under Al toxicity due to a low Al concentration in roots, suggesting that this cultivar could be exuded organic acid allowing to chelate Al in the rhizosphere. Nonetheless, further experiments are necessary to confirm this assumption.

References
1. Bojórquez-Quintal, E., Escalante-Magaña, C., Echezarría-Machado, I. & Martínez-Estévez, M. Aluminum, a friend or foe of higher plants in acid soils. Front in Plant Science 8, 1767 (2017).
2. Bruner, I. & Sperisen, C. Aluminum exclusion and aluminum tolerance in woody plants. Frontiers in Plant Science 4, 172 (2013).
3. Kopittke, P. M. et al. Identification of the primary lesion of toxic aluminum in plant roots. Plant Physiology 167, 1402–1411 (2015).
4. Foy, C. D., Charney, R. L. & White, M. C. The physiology of metal toxicity in plants. Annual Review of Plant Biology 29, 511–566 (1978).
5. Kinrade, T. B. Reconsidering the rhizotoxicity of hydroxyl, sulphate, and fluoride complexes of aluminum. Journal of Experimental Botany 48, 1113–1124 (1997).
6. Yamanoto, Y., Kobayashi, Y., Devi, S. R., Rikishi, S. & Matsumoto, H. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive species in plant cells. Plant Physiology 128, 63–72 (2002).
7. Kochian, L. V., Pineros, M. A., Liu, J. & Magalhaes, J. V. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. Annual Review of Plant Biology 66, 571–598 (2015).
8. Kochian, L. V., Hocegkova, O. A. & Piniéros, M. A. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. Annual Review of Plant Biology 55, 459–493 (2004).
9. Simoes, C. C., Melo, J. O., Magalhaes, J. V. & Guimaraes, C. T. Genetic and molecular mechanisms of aluminum tolerance in plant. Genetics and Molecular Research 11, 1949–1957 (2012).
10. Ahn, S. I. & Matsumoto, H. The role of the plasma membrane in the response of plant roots to aluminum toxicity. Plant Signaling & Behavior 1, 37–45 (2006).
11. Grisel, N. et al. Transcription response to aluminum stress in roots of aspen (Populus tremula). BMC Plant Biology 10, 185 (2010).
12. El-Moneim, D. A. et al. Pectin methylesterase gene and aluminum tolerance in Secale cereale. Environmental and Experimental Botany 107, 125–133 (2014).
13. Awasthi, J. P. et al. Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. Plos One 12, e0176357 (2017).
14. Inostroza-Blanchetat, C. et al. Biochemical and molecular changes in response to aluminum-stress in highbush blueberry (Vaccinium corymbosum L.). Plant Physiology and Biochemistry 49, 1005–1012 (2011).
15. Kochian, L. V., Pineros, M. A. & Hocegkova, O. A. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274, 175–195 (2005).
16. Chen, L. S., Qi, Y. P. & Liu, X. H. Effects of aluminum on light energy utilization and photoprotective systems in citrus leaves. *Annals of Botany* **96**, 35–41 (2005).

17. Panda, S. K., Baluška, F. & Matsumoto, H. Aluminum stress signaling in plants. *Plant Signaling & Behavior* **4**, 592–597 (2009).

18. Xu, L. M. et al. Transcriptomic responses to aluminum (Al) stress in maize. *Journal of Integrative Agriculture* **17**, 1946–1958 (2018).

19. Reyes-Díaz, M. et al. Long-term aluminum exposure effects on physiological and biochemical features of highbush blueberry cultivars. *Journal of the American Society for Horticultural Science* **135**, 212–222 (2010).

20. Silva, S. et al. Aluminum long term stress differently affects photosynthesis in ryegrass genotype. *Plant Physiology and Biochemistry* **54**, 105–112 (2012).

21. Moriyama, U., Tomioka, R., Kojima, M., Sakakibara, H. & Takenaka, C. Aluminum effect on starch, soluble sugar, and phytohormone in roots of *Quercus serrata* Thumb. seedlings. *Trees* **30**, 403–413 (2016).

22. Li, H. et al. Aluminum toxicity-induced alterations of leaf proteome in two citrus species differing in aluminum tolerance. *International Journal of Molecular Sciences* **17**, 1180 (2016).

23. Weritz, P., Chaves, A. L., Patiño, G. M., Mayer, J. E. & Rao, I. M. Aluminum stress stimulates the accumulation of organic acids in roots of *Bracharia species*. *Journal of Plant Nutrition and Soil Science* **165**, 582–588 (2002).

24. Jones, D. L. & Ryan, P. R. Aluminum toxicity: In *Encyclopedia of Applied Plant Science* (eds) Thomas, B., Murphy, D. R. & B. G. (London: Elsevier Academic Press.) pp 656–664 (2003).

25. Inostroza-Blancheme, C. et al. Molecular and physiological strategies to increase aluminum resistance in plants. *Molecular Biology Reports* **39**, 2069–2079 (2012).

26. Martins, N. et al. Physiological responses of Plantago algarbiansis and *P. almogravensis* shoots and plantlets to low pH and aluminum stress. *Acta Physiologica Plantarum* **35**, 615–625 (2013).

27. Naik, D., Smith, E. & Cumming, J. R. Rhizosphere carbon deposition, oxidative stress and nutritional changes in two poplar species exposed to aluminum. *Tree Physiology* **29**, 423–436 (2009).

28. Michalak, A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies* **15**, 523–530 (2010).

29. Winkel-Shirley, B. Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology* **5**, 218–223 (2002).

30. Vargas, O. L. & Brayla, D. R. Growth and fruit production of highbush blueberry fertilized with ammonium sulfate and urea applied by irrigation or as granular fertilizer. *Hort Science* **50**, 479–485 (2015).

31. Manquín-Cerda, K., Cruces, E., Escudey, M., Zúñiga, G. & Calderón, R. Interactive effects of aluminum and cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum L.*). plantlets cultivated in vitro. *Ecotoxicology Environmental Safety* **150**, 320–326 (2018).

32. Mora, M. L., Rossas, A., Ribera, A. & Rengel, Z. Differential tolerance to Mn toxicity in perennial ryegrass genotypes: involvement of antioxidative enzymes and root exudation of carbohydrates. *Plant Soil* **320**, 79–89 (2009).

33. Reyes-Díaz, M., Alberdi, M. & Mora, M. L. Short-term aluminum stress differentially affects the photosynthetic efficiency of photosystem II in higbush blueberry genotypes. *Journal of the American Society for Horticultural Science* **134**, 1–8 (2009).

34. Hoagland, D. R. & Arnon, D. I. The water culture method for growing plant without soil. *California Agricultural Experiment Station* **47**, 3–12 (1959).

35. Sadzawka, A., Grez, R., Carrasco, M. & Mora, M. M étodos de análisis de tejidos vegetales. Comisión de Normalización y Acreditación Sociedad Chilena de la Ciencia del Suelo. Santiago, Chile, pp 49–51 (2007).

36. Reyes-Díaz, M., Merino-Gergichevich, C., Alarcón, E., Alberdi, M. & Horst, W. J. Calcium sulfate ameliorates the effect of aluminum toxicity differentially in genotypes of highbush blueberry (*Vaccinium corymbosum L.*). *Journal of Soil Science and Plant Nutrition* **11**, 59–78 (2011).

37. Maxwell, K. & Johnson, G. Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany* **51**, 659–668 (2000).

38. Lichtenhaller, H. & Wellburn, A. R. Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochemical Society Transactions* **603**, 591–592 (1983).

39. García-Plazaola, J. I. & Becerril, J. M. A rapid high-performance liquid chromatography method to measure lipophilic antioxidants in stressed plants: simultaneous determination of carotenoids and tocopherols. *Phytochemical Analysis* **10**, 307–313 (1999).

40. Chinnici, F., Bendini, A. A., Gaiani, A. & Riponi, C. Radical scavenging activities of peels and pulps from cv. Golden delicious apples (*Malus domestica* Borkh.). *Biochemical Society Transactions* **38**, 603, 591–592 (1983).

41. García-Plazaola, J. I. & Becerril, J. M. A rapid high-performance liquid chromatography method to measure lipophilic antioxidants in stressed plants: simultaneous determination of carotenoids and tocopherols. *Phytochemical Analysis* **10**, 307–313 (1999).

42. Chinnici, F., Bendini, A. A., Gaiani, A. & Riponi, C. Radical scavenging activities of peels and pulps from cv. Golden delicious apples (*Malus domestica* Borkh.). *Biochemical Society Transactions* **38**, 603, 591–592 (1983).

43. Fernie, A. R., Roscher, A., Ratcliffe, R. G. & Kruger, N. J. Fructose 2,6- bisphosphate activates pyrophosphate: fructose-6-phosphate 1- phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. *Planta* **212**, 250–263 (2001).

44. Cross, J. M. et al. Variation of enzyme activities and metabolite levels in Arabidopsis accessions growing in carbon-limited conditions. *Plant Physiology* **142**, 1574–1588 (2006).

45. Dalo, D. M. et al. Tobacco guard cells fix CO2 by both Rubisco and PEPCase while sucrose acts as a substrate during light-induced stomatal opening. *Plant Cell & Environment* **38**, 2353–2371 (2015).

46. Stitt, M., Lilley, R. M., Gerhardt, R. & Heldt, H. W. Metabolite levels in specific cells and subcellular compartments of plant leaves. *Methods in Enzymology* **174**, 518–552 (1989).

47. Tanaka, H., Goto, K., Nishiyama, A. et al. Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. *Plant Journal* **50**, 1093–1106 (2007).

48. Vázquez, M. D. Aluminum exclusion mechanism in root tips of maize (*Zea mays L.*): Lysigeny of aluminum hyperaccumulator cells. *Plant Biology* **4**, 234–249 (2002).

49. Peixoto, P. H. P., Da Matta, F. M. & Cambraia, J. Response of the photosynthetic apparatus to aluminum stress in two sorghum cultivars. *Journal of Plant Nutrition* **25**, 821–823 (2002).

50. Zhang, X. B., Liu, P., Yang, F. & Xu, G. D. Effect of Al in soil on photosynthesis and related morphological and physiological characteristics of two soybean genotypes. *Botanical Studies* **48**, 435–444 (2007).

51. Yang, M. et al. Effect of low pH and aluminum toxicity on the photosynthetic characteristics of different fast-growing Eucalyptus vegetatively propagated clones. *Plos One* **10**, e0130963.

52. Björkman, O. & Demming, B. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. *Planta* **170**, 489–504 (1987).

53. Akaya, M. & Takenaka, C. Effects of aluminum stress on photosynthesis of *Quercus glauca* Thumb. *Plant Soil* **237**, 137–146 (2001).

54. Fonseca, E. M., Cambraia, J., Ribeiro, C. & Oliva, M. A. The effects of aluminum on the photosynthetic apparatus of two rice cultivars. *Experimental Agriculture* **50**, 343–352 (2014).

55. Souza, L. A., Camargos, L. S. & Aguiar, L. F. Efeito do aluminio sobre compostos nitrogenados em Urochloa spp. *Biотemas* **3**, 33–39 (2014).

56. Zhao, X. Q. & Shen, R. F. Aluminum-nitrogen interactions in the soil-plant system. *Frontiers in Plant Science* **9**, 807 (2018).

57. Maathuis, F. J. M. Physiological functions of macromineral nutrients. *Current Opinion in Plant Biology* **12**, 250–258 (2009).
58. Zhao, X. Q. et al. Aluminium tolerance in rice is antagonistic with nitrate preference and synergistic with ammonium preference. *Annals of Botany* **111**, 69–77 (2013).
59. Townsend, L. R. Effect of nitrate and ammonium nitrogen on the growth of the lowbush blueberry. *Canadian Journal of Plant Science* **66**, 209–210 (1966).
60. Somers, D. J., Briggs, K. G. & Gustafson, J. P. Aluminium stress and protein synthesis in near isogenic lines of *Triticum aestivum* differing in aluminium tolerance. *Physiologia Plantarum* **97**, 694–700 (1996).
61. Thalmann, M. & Santelia, D. Starch as a determinant of plant fitness under abiotic stress. *New Phytologist* **214**, 943–951 (2017).
62. Giannakoula, A., Moustakas, M., Mylona, P., Papadakis, I. & Yupsanis, T. Aluminium tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. *Journal Plant Physiology* **165**, 385–396 (2008).
63. Zhang, L. et al. Polyphenol-aluminium complex formation: Implications for aluminium tolerance in plants. *Journal of Agricultural and Food Chemistry* **64**, 3023–3033 (2016).
64. Silva, I. R. et al. Responses of eucalypt species to aluminium: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism. *Tree Physiology* **24**, 1267–1277 (2004).
65. Ma, J. F., Hiradate, S., Nomoto, K., Iwashita, T. & Matsumoto, H. Internal detoxification mechanism of Al in *Hydrangea* (Identification of Al form in the leaves). *Plant Physiology* **113**, 1033–1039 (1997).
66. Ma, J. F., Hiradate, S. & Matsumoto, H. High aluminum resistance in buckwheat. II. Oxalic acid detoxifies aluminum internally. *Plant Physiology* **117**, 753–759 (1998).
67. Watanabe, T., Osaki, M., Yoshihara, T. & Tadano, T. Distribution and chemical speciation of aluminum in the Al accumulator plant, *Melastoma malabathricum* L. *Plant Soil* **201**, 165–173 (1998).
68. Morita, A. et al. Chemical forms of aluminum in xylem sap of tea plants (*Camellia sinensis* L.). *Phytochemistry* **65**, 2775–2780 (2004).
69. Rangel, A. F. Short and medium term effects of aluminum toxicity and resistance in common bean (*Phaseolus vulgaris* L.). Thesis Dr. rer. hort. Von der Naturwissenschaftlichen Fakultat der Gottfried Wilhelm Leibniz Universitat Hannover. Germany, pp. 64 (2007).
70. Nguyen, N. T., Nakabayashi, K., Thompson, J. & Fujita, K. Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species. *Tree Physiology* **23**, 1041–50 (2003).
71. Ofei-Manu, P., Wagatsuma, T., Ishikawa, S. & Twaraya, K. The plasma membrane strength of the root-tip cells and root phenolic compounds are correlated with Al tolerance in several common woody plants. *Soil Science and Plant Nutrition* **47**, 359–375 (2001).
72. Tolrà, R. P., Poschenrieder, C., Lippii, B. & Barceló, J. Aluminium-induced changes in the profiles of both organic acids and phenolic substances underlie Al tolerance in Rumex acetosa. *Environmental and Experimental Botany* **54**, 231–238 (2005).

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Author Contributions
C.I.-B., M.R.-D., A.N.-N. and M.A. designed and coordinated the experiment. C.I.-B. formulated the manuscript and C.I.-B., M.P.-C., M.R.-D., M.A., R.O.-G., Z.R. and A.N.-N. revised and corrected it. M.P.-C. and M.R.-D. carried out physiological and biochemical analyses. M.P.-C. and R.O.-G. performed statistical analyses.

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