Short-term Aluminum Stress Differentially Affects the Photochemical Efficiency of Photosystem II in Highbush Blueberry Genotypes

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ABSTRACT. Aluminum (Al) toxicity is a major agronomic problem in acid soils. Most studies regarding Al stress focus on phenomena occurring in the roots; however, less is known about the effects of Al stress on photosynthetic apparatus functionality. Our aim was to rank three highbush blueberry (*Vaccinium corymbosum*) cultivars according to their tolerance to acid and Al stresses. Additionally, the levels of Al toxicity for highbush blueberry were established. ‘Brigitta’, ‘Legacy’, and ‘Bluegold’ were grown in a greenhouse in hydroponic solutions containing different Al concentrations (0, 25, 50, 75, and 100 μM) for 0 to 48 h and were allowed to recover (without Al) over 24 h. In all Al-treated cultivars, root growth inhibition was found at the highest Al treatment. However, ‘Brigitta’ also showed root growth up to 75 μM Al. Photochemical parameters decreased substantially due to Al treatments in ‘Bluegold’ (up to 98% inhibition) and ‘Legacy’ (up to 80% inhibition) without total recovery. In contrast, ‘Brigitta’ showed a better photosystem II performance and root growth than the other cultivars. These results suggest that ‘Brigitta’ is the best cultivar for use in acid soils with Al toxicity, followed by ‘Legacy’. ‘Bluegold’ was highly sensitive to Al stress. In addition, Al toxicity levels for blueberries depend on the genotype studied.

In areas with high rainfall, soluble nutrients and other basic elements are solubilized from soils, with a concomitant pH decrease. Organic matter mineralization also results in a release of hydrogen ions (H^+), decreasing the pH of soils. H^+ releases Al as Al^{3+} predominantly retained by the negative charges of clay minerals in equilibrium with the Al^{3+} of the soil solution. Thus, the quantity of Al^{3+} in the soil solution is augmented with soil acidity to levels that can be toxic to plants (Hede et al., 2001). Aluminum toxicity is a major agronomic problem in acid soils. Acid soils may account for as much as 50% of the world’s potentially arable land (Dahlgren et al., 2004). This problem is exacerbated by the current extensive use of ammonium fertilizers and acid rain (von Uexkull and Mutert, 1995). Most studies regarding Al stress are focused on phenomena occurring in the roots because the effects of Al manifest themselves in the plant root system first (Barceló and Poschenrieder, 2002; Delhaize and Ryan, 1995). Many reports indicate that acidification generates an increase in Al concentration as Al^{3+} in the soil solution (Jarvis, 1987). This results in root growth reduction (Mora et al., 2004, 2005; Tamás et al., 2006) and sensitivity to Al toxicity (Kochian, 1995), reducing

crop yields (Bolan et al., 2003; Kochian, 1995; Matsumoto, 2000).

Little is known as to whether the early effects of Al stress on roots are reflected in the functionality of the photosynthetic apparatus. Restricted shoot growth also becomes evident only after root growth is limited by exposure to toxic levels of Al^{3+} in the rooting environment (Rengel, 1996). Comparatively less information exists about the effects of Al^{3+} on leaves than on roots. In leaves, Al toxicity induces chloroplast malformations, even though high amounts of Al may not be detected in this organelle, indicating indirect effects on chloroplast functioning (Moustakas et al., 1995). In effect, decreases in total chlorophyll content and photosynthetic rates accompanied by a partial inhibition of photosynthetic electron transport in photosystem II (PSII) in response to Al^{3+} have been reported in some species (Chen et al., 2005a, 2005b; Chen, 2006; Peixoto et al., 2002; Pereira et al., 2000). Alterations in the photosynthetic apparatus due to stress affect the metabolism and productivity of crops (Rao and Cramer, 2003).

Differential uptake of Al^{3+} by roots among crop genotypes could account for differences in tolerance to this metal (Delhaize et al., 1993). Nevertheless, conflicting results have been reported regarding differences in Al accumulation and tolerance by different genotypes. For example, wild species that grow in acidic soils are frequently very resistant to Al stress and can accumulate high concentrations of Al in their leaves (>1% of dry weight), whereas cultivated species such as *Oryza sativa* and *Secale cereale* cannot (Kochian, 1995).

Species of commercial interest such as *Medicago sativa*, *Hordeum vulgare*, *Solanum lycopersicum*, and *Brassica napus* are sensitive to Al, whereas *Triticum aestivum* and *Zea mays* genotypes exhibit high variability in their sensitivity (Kochian, 1995; Piñeros et al., 2005). Thus, plant response to Al toxicity is highly dependent on the species or cultivar studied (Barceló

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and Poschenrieder, 2002; Giannakoula et al., 2008; Kochian, 1995).

Al toxicity seems to be significant in limiting plant growth and production in acidic soils derived from volcanic ash (Andisols) in southern Chile (Borie and Rubio, 2003; Mora et al., 2006). The preponderant current agronomic activity in this region is highbush blueberry cultivation. Although *Vaccinium corymbosum* is adapted to acidity (Ireland and Wilk, 2006), the physiological response to the interaction between acid substrate and Al and its accumulation in plant organs in different genotypes of this species remains unknown. Our aim was to rank three highbush blueberry cultivars according to their tolerance to short-term acid and Al stresses by measuring root growth, photochemical fluorescence parameters, Al, and pigment contents. This study is relevant because highbush blueberry is cultivated in Chile in acidic soils with high potential for Al toxicity.

Materials and Methods

PLANT MATERIAL. The three most frequently cultivated genotypes of *V. corymbosum* ('Brigitta', 'Legacy', and 'Blue-gold') in southern Chile were selected for this study (Guerrero, 2006). One-year-old saplings (15 cm in height) of these cultivars growing in a solid substrate (1 oat:1 shell sawdust:1 pine needles by volume) were provided by the Maquehue Station of the Universidad de La Frontera (Temuco, Chile). Saplings were conditioned in plastic boxes filled with 18 L of Hoagland's nutrient solution for 1 week (Hoagland and Arnon, 1959). The physiological status of saplings during conditioning was controlled by chlorophyll fluorescence analyses by determination of the maximum quantum yield (F_v/F_m) as described below. Due to the F_v/F_m values remaining in the range of healthy leaves (near 0.8) (Björkman and Demmig 1987), the time of conditioning was considered adequate. After conditioning, saplings were transferred to a hydroponic solution of CaCl_2 (0.5 mM) containing Al as AlCl_3 in concentrations of 0, 25, 50, 75, and 100 μM Al (treatment solution) for different times (24 and 48 h) in a greenhouse. The solutions were prepared with sterile deionized water and were filter sterilized through 0.2- μm diameter pore filters. The pH of the control (without Al) and Al treatment solutions was monitored and was adjusted daily to 4.5 using 0.1 M HCl with a high accuracy portable pH meter (model pH-0.13; Hi-Tech-Instruments, Shanghai, China). Environmental conditions in the greenhouse were 25/20 °C (day/night), 16/8 h (light/dark) light periods, 70% relative air humidity, and photosynthetic photon flux (PPF) densities of 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This light intensity was similar to that used for maintenance in Maquehue Station, where plants were previously cultivated. Solutions were aerated with an aquarium pump and were changed every 2 d. After 48 h of treatment, the cultivars were transferred to CaCl_2 solution without Al for 24 h (recovery treatment). The recovery treatment permits discrimination between Al-tolerant, -sensitive, and -intermediate genotypes (Raman et al., 2002). There were nine saplings per Al treatment in a split-plot design. The experiment was run twice. At the start of the experiment (control without Al) and after each treatment, physiological, chemical, and morphological analyses of roots and leaves were performed as indicated below.

ROOT GROWTH. Before treatment, the initial sizes of the longest primary roots of nine saplings in the control and Al

treatment groups of the three cultivars were measured and recorded. Thereafter, the root length after 24 and 48 h of treatment and at recovery was monitored.

ALUMINUM CONTENT IN PLANTS. Leaves and roots of the samples were separated and dried by placing them in a 70 °C forced-air oven for 48 h. After weighing, samples were ashed at 500 °C for 8 h and were treated with 2 M hydrochloric acid. Al was quantified using a simultaneous multielement atomic absorption spectrophotometer (model 969; UNICAM, Cambridge, UK) as described by Sadzawka et al. (2004).

PIGMENT DETERMINATIONS. Total chlorophylls and total carotenoids were measured in extracts of completely expanded nonshaded leaves by a spectrophotometer (Genesys 5; Spectronic New York) at 663, 646, and 470 nm. Pigments were extracted with ethanol 96% and concentrations were calculated according to Lichtenthaler and Wellburn (1983).

CHLOROPHYLL FLUORESCENCE PARAMETERS. Chlorophyll fluorescence parameters were used to determine the photochemical efficiency of PSII in leaves. The basic protocol of Reyes-Díaz et al. (2005) was followed. Fluorescence of attached nonshaded leaves was measured using a portable pulse-amplitude modulated fluorimeter (FMS 2; Hansatech Instruments, Norfolk, UK). Completely expanded leaves of the three cultivars were dark-adapted for 30 min (to obtain open centers) with leaf-clips with a mobile shutter plate. The fiber optic and its fiber optic adapter were then attached to a ring located 10 mm above the leaf sample. Different light pulses (see below) were applied following standard routines programmed into the machine. Signal recording and calculation were performed on a personal computer using data analyses and control software (Hansatech Instruments). Minimal fluorescence (F_0) was determined by applying a weak-modulated light (0.4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and maximal fluorescence (F_m) was induced by a short pulse (0.8 s) of saturating light (9000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). After 10 s, actinic light (120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was turned on to obtain fluorescence parameters during steady-state photosynthesis. Saturating pulses were applied after steady-state photosynthesis had been reached to determine maximal fluorescence in light-adapted leaves (F_m') and steady-state fluorescence (F_s). Finally, the actinic light was turned off and a 5-s far-red (FR) pulse was applied immediately to obtain minimal fluorescence in light-adapted leaves (F_0'). In this article, we used the definitions of fluorescence parameters [maximum quantum yield of the PSII (F_v/F_m), effective quantum yield of the PSII (ϕPSII), electron transport rate (ETR), and nonphotochemical quenching (NPQ)] as described by Maxwell and Johnson (2000). ϕPSII was calculated as $(F_m' - F_s)/F_m'$. ETR was calculated as $\text{PPF} \times 0.5 \times \phi\text{PSII} \times 0.84$. NPQ was calculated as $(F_m - F_m')/F_m'$ (Maxwell and Johnson 2000).

STATISTICAL ANALYSES. Reported values of chlorophyll fluorescence parameters correspond to the mean of four to six individual replicates for each cultivar and experiment, six individual replicates for root growth, and three individual replicates for Al and pigment contents. Data were subjected to a two-way analysis of variance (ANOVA; where the factors were Al treatments and time), with repeated measurements for one factor (cultivars) (Quinn and Keough 2006). A Tukey test was used to identify those values with significant differences. Both analyses were performed with Sigma Stat software (version 2.0; SPSS, Chicago). Differences between the values were considered significant at $P \leq 0.05$.

Results

No root elongation was observed in the *V. corymbosum* cultivars investigated under the effect of the highest Al treatment (Fig. 1). Root growth was observed in the 75 μM of Al treatment in 'Brigitta' and 'Legacy' (around 6%; $P \leq 0.05$), but not in 'Bluegold'. The largest elongation without Al was observed in 'Brigitta' roots (23% and 32%) at both times and after the recovery treatment, respectively, when compared with their initial values ($P \leq 0.05$; Fig. 1).

Roots showed higher Al content (63%–92%) than leaves in all the cultivars (Fig. 2), with the highest percentages frequently being found after 48 h of treatment. Al content decreased after 24 h of recovery treatment, with the exception of 'Brigitta' (Fig. 2). In all cultivars, a significant increase in Al content was found at each time in Al-treated roots, with respect to the controls and the recovery period ($P \leq 0.05$). Root Al content in 'Brigitta' was similar in all Al treatments (Fig. 2), whereas in 'Bluegold', it increased with Al concentration. For 'Legacy', Al content significantly decreased after the recovery treatment

(Fig. 2). A statistically significant interaction between Al treatments and time was found in roots in 'Legacy' ($P < 0.001$) and 'Bluegold' ($P = 0.021$).

In leaves, all cultivars increased their Al content at the higher Al treatments with respect to the control (50%–75%; $P < 0.05$). The highest content in leaves was found in 'Brigitta' in all Al treatments at 24 h (75%) and 48 h (67%) with respect to the controls (Fig. 2). After the recovery period, 'Brigitta' (55%), and to a lesser degree 'Legacy' (40%), showed higher Al content than the controls. By contrast, 'Bluegold' had similar Al content than its respective control (Fig. 2). Statistically significant interactions were found between Al treatments and times in all cultivars ($P \leq 0.001$).

Differences between the concentrations of total chlorophylls (Chl *a* + *b*) in different Al treatments compared with their controls were found at each time and by recovery in all cultivars (Fig. 3). At the end of Al treatments (48 h) and at the highest Al treatment (100 μM Al), a statistically significant decrease in Chl *a* + *b* was found in 'Legacy' (33%) and 'Bluegold' (56%; $P \leq 0.001$). Nevertheless, after the recovery treatment, only

Fig. 1. Root growth of highbush blueberry cultivars at different Al treatments. Values represent the average of six replicates $\pm$ SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between aluminum treatments for the same cultivar and exposure time. Different upper case letters show differences ($P \leq 0.05$) between exposure times for the same cultivar and treatments.

Fig. 2. Aluminum content in leaves and roots of three cultivars of highbush blueberry at different times and Al treatments. Values represent the average of three replicates $\pm$ SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between aluminum treatments for the same cultivar and exposure time. Different upper case letters show differences ($P \leq 0.05$) between exposure times for the same cultivar and treatments.

Fig. 3. Changes in chlorophyll pigments of three cultivars of highbush blueberry under aluminum stress. The panels on the left show total chlorophylls (Chl *a* + *b*) and the panels on the right show chlorophyll ratio (Chl *a*/*b*). Al exposure times were: 24 h, 48 h, and recovery for 24 h without Al. Values represent the average of six replicates $\pm$ SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between aluminum treatments for the same cultivar and exposure time. Different upper case letters show differences ($P \leq 0.05$) between exposure times for the same cultivar and treatments.

'Bluegold' reached and slightly surpassed the Chl *a* + *b* content of the controls ($P < 0.05$; Fig. 3). Chl *a* + *b* content of each cultivar and Al content of leaves were negatively correlated. Moreover, a higher negative correlation between these parameters was found in 'Brigitta' ($r = -0.7$; $P = 0.001$) and 'Legacy' ($r = -0.6$; $P = 0.001$) than in 'Bluegold' ($r = -0.4$; $P = 0.02$). A statistically significant interaction between time and Al treatments was detected for Chl *a* + *b* content in 'Legacy' and 'Bluegold' ($P = 0.038$ and 0.001 , respectively).

Chlorophyll *a/b* ratios (Chl *a/b*) of 'Brigitta' and 'Bluegold' varied greatly with Al treatment and exposure time (Fig. 3). At 24 h, 'Bluegold' showed a statistically significant decrease in Chl *a/b* with respect to the controls up to $50 \mu\text{M}$ Al ($P < 0.05$), whereas in 'Legacy' and 'Brigitta', Chl *a/b* ratios remained constant. No significant differences in Chl *a/b* of the cultivars at 48 h were found (Fig. 3). However, after the recovery treatment, this ratio increased significantly in 'Brigitta' subjected to the two highest Al treatments with respect to the control ($P < 0.05$). Chl *a/b* decreased in 'Bluegold' and remained unchanged in 'Legacy' (Fig. 3). There was a significant inverse correlation between Chl *a/b* ratio and Al treatments in 'Bluegold' ($r = -0.63$; $P < 0.001$). A statistically significant interaction between Al treatments and times was detected only in 'Brigitta' ($P = 0.034$).

Carotenoid content did not change in 'Bluegold' at each time and Al treatment, whereas in 'Legacy', a decrease in carotenoids was found at the highest Al treatments at 24 h (37%) and 48 h (18%).

In contrast, in 'Brigitta', carotenoids increased with treatments up to $50 \mu\text{M}$ Al at 24 h (34%; Fig. 4). A negative, but not significant, correlation between carotenoids and Al treatment was found in 'Legacy' ($r = -0.3$; $P = 0.07$). No interaction between times and Al treatment was found for carotenoid contents ($P > 0.151$).

F_v/F_m did not change in 'Brigitta' with Al treatments (Fig. 5). F_v/F_m slightly decreased in 'Legacy' after 24 h (8%), 48 h (13%), and recovery treatment (7%) compared with their controls ($P < 0.05$). In 'Bluegold', F_v/F_m decreased (12%) at both times of Al exposure and after recovery treatment (8%; $P < 0.05$; Fig. 5).

ϕPSII and ETR of the three genotypes were differently affected at each Al treatment (Fig. 6). In 'Bluegold' and 'Legacy', a statistically significant decrease ($P < 0.05$) of ϕPSII and ETR values was found at each time in Al-treated with respect to Al-untreated plants. In 'Brigitta', this was true only after 24 h of Al treatment (Fig. 6). ϕPSII and ETR were lower in 'Bluegold' than in the other cultivars ($P < 0.05$). The best recovery of ϕPSII and ETR was shown by 'Brigitta', which, at 48 h, showed an increase in these parameters with respect to the 24 h Al treatment (Fig. 6). These values were very similar to the controls. A highly negative correlation between ϕPSII , ETR, and Al content in leaves was found in all cultivars ($r = -0.7$ to -0.85 ; $P \leq 0.002$). No interaction between time and Al treatment was found for ϕPSII and ETR ($P > 0.391$). NPQ of 'Brigitta' and 'Legacy' was unaffected by the Al treatment (Fig. 5). In 'Bluegold', a tendency to reduce NPQ under Al treatment was found after 24 h ($P > 0.05$; Fig. 5).

Discussion

The present data demonstrate that short-term Al stress produced differential effects on the chemical and physiological features of *V. corymbosum* cultivars. Al accumulation was higher in roots than in leaves in the three cultivars (up to 92%). A good correlation between Al treatments and Al contents in both organs was found ($r = \text{from } 0.6 \text{ to } 0.9$; $P \leq 0.05$). It appears that the best root growth of 'Brigitta' was concomitant with the better recovery of the photochemical parameters, suggesting that 'Brigitta' was the most Al-tolerant cultivar. Also, this cultivar accumulated more Al in its roots and leaves than the other cultivars. A high Al accumulation in leaves was directly associated with Al tolerance in *Sorghum bicolor* cultivars (Gonçalves et al., 1996). Some reports indicate that Al-tolerant genotypes accumulate less Al in their roots than the Al-sensitive ones under Al exposure (Ma, 2005; Piñeros et al.,

Fig. 4. Total carotenoids and nonphotochemical quenching (NPQ) of three cultivars of highbush blueberry under aluminum stress. Al exposure times were the same as in Fig. 3. Values represent the average of six replicates $\pm$ SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between aluminum treatments for the same cultivar and exposure time. Different upper case letters show differences ($P \leq 0.05$) between exposure times for the same cultivar and treatments.

2005). It is well established that the different Al accumulation in organs is highly dependant on the mechanism (exclusion or inclusion) used by plants to tolerate Al toxicity (Delhaize and Ryan, 1995; Kochian, 1995). Thus, the greatest Al accumulation in leaves found in 'Brigitta' supports our suggestion that 'Brigitta' could be considered the most Al-tolerant cultivar among those investigated in the present study.

Our cultivars showed up to $400 \text{ mg} \cdot \text{kg}^{-1}$ Al content in leaves per dry weight. This Al accumulation in Al-treated leaves was statistically significantly higher than their controls (over 60%; $P \leq 0.05$), indicating translocation of Al from roots to leaves in

all the cultivars. This translocation was higher in 'Brigitta' than in the other cultivars (around 70%; $P \leq 0.05$). The Al content values exhibited by our highbush blueberry cultivars were similar to those reported in *Saccharum officinarum* leaves by Watt (2003). Sugarcane is considered more Al tolerant than other graminaceous crops (Hetherington et al., 1986). The accumulation of Al in leaves differentially affected the photochemical efficiency of PSII (ϕPSII and ETR) of the cultivars investigated (Fig. 6); 'Bluegold' was the most negatively affected by the Al treatments. Al-treated *Citrus* leaves also accumulated Al with a concomitant decrease of the photochemical efficiency of PSII with respect to the Al-untreated leaves (Chen et al., 2005b). Although the F_v/F_m was in the normal ranges for healthy plants for the three cultivars under Al treatments (0.7–0.8) (Björkman and Demmig, 1987), and a statistically significant decrease of this parameter was observed in 'Legacy' and 'Bluegold' (Fig. 2), indicating the same degree of disturbance of the photosynthetic apparatus under Al exposure. A statistically significant negative correlation between Chl $a + b$ and Al treatment in leaves was found in all cultivars, with the highest being in 'Bluegold' ($r = -0.8$; $P = 0.0003$). The major decrease in Chl $a + b$ in 'Bluegold' suggests the possibility of selective chlorophyll photobleaching, resulting in a smaller fraction of absorbed light energy for electron transport (Habash et al., 1994; Miller and Carpentier, 1991). Peixoto et al. (2002) found similar results in *S. bicolor* cultivars where Chl $a + b$ was substantially decreased after 48 h of Al exposure. Chlorophyll a/b ratio decreased markedly under Al treatment in 'Bluegold' (80%). These decreases reflect a reduction in the chlorophyll antenna size

Fig. 5. Maximum quantum yield (F_v/F_m) of three cultivars of highbush blueberry under aluminum stress. Values represent the average of four to six replicates $\pm$ SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between aluminum treatments for the same cultivar and exposure time. Different upper case letters show differences ($P \leq 0.05$) between exposure times for the same cultivar and treatments.

Fig. 6. Changes in the effective quantum yield (Φ_{PSII}) and electron transport rate (ETR) of three cultivars of highbush blueberry at different times under aluminum stress. Al exposure times were the same as in Fig. 3. Values represent the average of four to six replicates $\pm$ SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between aluminum treatments for the same cultivar and exposure time. Different upper case letters show differences ($P \leq 0.05$) between exposure times for the same cultivar and treatments.

of the photosystems and might protect the photosystems from photoinhibition by reducing energy delivery to the reaction centers (Adams et al., 2004). This change in chlorophyll antenna size is probably a strategy to reduce light absorption and avoid possible damage to the photosystems due to Al stress. Interestingly, at the beginning of the Al treatment, the chlorophyll antenna size was also reduced in 'Brigitta' (around 60%), whereas after 48 h of Al treatment, a chlorophyll antenna size similar to the controls was obtained concomitantly with the maintenance of Φ_{PSII} (Fig. 6). This suggests a fast acclimation of this cultivar's photosynthetic apparatus to Al stress supported by favorable root growth. A similar photoprotective strategy has been reported for evergreen species under other types of stress (Adams and Barker, 1998; Adams et al., 2004; Savitch et al., 2002).

Carotenoids play an essential role in protecting the photosynthetic apparatus against the harmful effects of light and oxygen, dissipating the excess light as heat in the antenna pigment complexes (Demmig-Adams and Adams, 1996; Niyogi et al., 1998). Our results showed similar carotenoid contents in all treatments in 'Bluegold', but a slight decrease in 'Legacy' by Al treatment was found at both times. In contrast, an increase in carotenoids was found in 'Brigitta' at the highest Al treatment with respect to the control after 24 h. This carotenoid increase was related with a decrease of photochemical parameters, suggesting that this cultivar can favor the heat dissipation pathway and thus avoid PSII photoinhibition (Demmig-Adams and Adams, 1996).

The thermal energy dissipation measured as NPQ did not present any variation between the control and Al-treated leaves, with the exception of 'Bluegold'. In this cultivar, a decrease of NPQ at all treatments and exposure times was found. Similar results were observed in the salt-acclimated halophyte *Artemisia anethifolia* (Lu et al., 2003). These authors suggested that other metabolic pathways such as the water-water cycle, Mehler reaction, and photorespiration in Al-treated leaves may be upregulated to cope with the increased excess of excitation energy. In our study, NPQ did not correspond well with changes in carotenoid content. The relationship between NPQ and carotenoid content is controversial. It has been reported that changes in NPQ correlate closely and directly with changes in carotenoid pigments (Bilger and Björkman, 1990; Demmig-Adams and Adams, 1996); however, it has also been found that carotenoids may be unrelated to NPQ (Chen et al., 2005b; Johnson et al., 1993).

It is accepted that PSII is the most vulnerable part of the photosynthetic apparatus to stress-induced damage. Therefore, the photochemical parameters of PSII are indicative, under many conditions, of the overall rate of photosynthesis (Genty et al., 1989). They give us the potential to estimate photosynthetic performance and, thereby, plant productivity under different environmental conditions (Maxwell and Johnson, 2000). Φ_{PSII} and ETR substantially decreased in 'Legacy' (around 73%) and 'Bluegold' (around 83%), without a total recovery of these parameters, especially in 'Bluegold' (Fig. 6). The photochemical parameters in 'Bluegold' were lower even under the control condition compared with the other cultivars. One possible explanation is that 'Bluegold' has already passed the fast-growing stage as evidenced by the absence of root growth even without Al treatment (Fig. 1). This was not the case in 'Brigitta' and 'Legacy' (Fig. 1). Another explanation is that 'Bluegold' may be intrinsically less efficient at managing its energy for photochemical processes than the others cultivars, which is even more noticeable with Al treatments. This cultivar-specific behavior may reflect lower productivity with respect to the other cultivars. In this respect, 'Brigitta' and, to a lesser extent, 'Legacy' managed the absorbed energy and tolerated Al exposure better than 'Bluegold' under short-term Al stress. Thus, as a first approximation, we could expect that 'Brigitta' is the best-qualified cultivar to develop in acidic soils with Al toxicity. Nevertheless, further investigation of the long-term Al response will be necessary because there exist time-dependent adjustment mechanisms. However, the current results also demonstrated that all Al treatments were toxic for 'Bluegold',

showing its high sensitivity to Al stress. In contrast, the Al toxicity level for 'Brigitta' and 'Legacy' was 100 μM . Thus, the Al toxicity levels for blueberries depend on the genotype studied. The knowledge of this work is fundamental to selecting Al-tolerant highbush blueberry cultivars, which will permit better sustainable horticultural systems in acidic soils where Al toxicity is a major problem.

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