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Calcium sulfate ameliorates the effect of aluminum toxicity differentially in genotypes of highbush blueberry (*Vaccinium corymbosum* L.)

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Abstract

The effect of gypsum ($CaSO_4$) amendment in the reduction of Al phytotoxicity of blueberry cultivars differing in Al resistance (Legacy and Brigitta, Al-resistant and Bluegold, Al-sensitive) was studied in a Hoagland's nutrient solution under acidic conditions for 2 weeks. Treatments were: Control (Hoagland solution), 2.5 mM $CaSO_4$, 5 mM $CaSO_4$, 100 µM Al ($AlCl_3$), 100 µM Al + 2.5 mM $CaSO_4$, 100 µM Al + 5 mM $CaSO_4$. Physiological, biochemical and chemical features of leaves and roots were determined to establish the amendment efficiency in the reduction of Al toxicity in these cultivars. Results showed that under Al toxicity the three investigated cultivars accumulated high Al concentrations in leaves and roots. These concentrations decreased with $CaSO_4$ application. Statistically significant interactions among Al in leaves but not in roots (p=0.719) and cultivars (p<0.001), were found. The lowest Ca concentration was found in the most Al-sensitive cultivar (Bluegold) and the highest in the more Al-resistant cultivars (Legacy and Brigitta). Among the underlying processes affected by Al stress in these blueberry cultivars the most evident changes were exhibited by the Al-sensitive cultivar Bluegold, where the photosynthetic performance decreased showing a slight recovery in presence of gypsum amendment at the end of experiment. Instead, the more Al-resistant cultivar (Legacy) did not change its photosynthetic parameters in presence of the gypsum amendments during the treatment, whereas in Brigitta, only a slight recovery at the end of treatment was evidenced by the gypsum application. Thus, in relation to these parameters the gypsum amendment was efficient in complete recovery from the toxic Al effect in the Al-resistant cultivar Brigitta and a slight recovery of the toxic Al effect in the Al-sensitive cultivar Bluegold. Nonetheless, this amendment is a good alternative to ameliorate Al toxicity in Al-sensitive cultivars and additionally provides a good source of Ca and S.

Keywords: Acid soils, aluminium, amendments, calcium, gypsum.

1. Introduction

Soils derived from volcanic materials (Andisols) are frequent in Southern Chile. They are characterized principally by high organic matter (OM), low phosphorus (P) availability, and acidity (pH $\leq$ 5.5) (Mora *et al*., 1999; 2002), as well as low contents of calcium and magnesium (Meriño-Gergichevich *et al*., 2010). In these soils a main problem is the acidity, determining the presence of toxic and available forms of trivalent aluminum (Al^{3+}) in the soil solution (Borie and Rubio, 2003), which is very detrimental for plants (Poschenrieder *et al*., 2008; Ryan and Delhaize, 2010). Toxic Al^{3+} results primarily in a reduction of root growth (Mora *et al*., 2004) and eventually overall plant toxicity (Kochian, 1995) reducing crop yields (Mora *et al*., 1999; Bolan *et al*., 2003). The reduction of root growth is due to the interaction of Al^{+3} with the dividing and expanding cells of the root elongation zone, inhibiting root elongation (Sivaguru and Horst, 1998; Rangel *et al*, 2007). As consequence water and nutrient uptake are diminished (Foy *et al*., 1978; Delhaize and Ryan, 1995). Other important and common effects of Al^{3+} on plant cells are the overproduction of oxygen reactive species (ROS) (Blokhina *et al*., 2003; Ma, 2005) and lipid peroxidation (LP). Lipid peroxidation induces alterations in the plasma membrane integrity and functionality (Tamas *et al*., 2006), resulting in nutritional and metabolic disorders (Pavlovkin *et al*., 2009) not only at the root level but also in the upper organs of plants (Reich *et al*., 1994; Peixoto *et al*., 2002). Enzymatic and non-enzymatic antioxidant systems can regulate the scavenging of ROS in plants (Shao *et al*., 2008). A greater leaf antioxidant capacity was found in leaves of *Citrus reshni* under Al toxicity coinciding with an augmented requirement for scavenging reactive species (Chen *et al*., 2005.). The photosynthetic performance is also altered under Al stress in some plants such as sorghum cultivars (Peixoto *et al*., 2002) and citrus rootstocks (Pereira *et al*., 2000), among others.

To reduce Al toxicity in acid soils and to overcome Al phytotoxicity a frequent practice is the employment of Ca amendments (Toma and Saigusa, 1997; Mora *et al*., 2002) such as lime, gypsum or phosphogypsum (PG) (Campbell *et al*., 2006; Takahashi *et al*., 2006a,b). The beneficial Ca effects in ameliorating Al toxicity in different crops growing in acid soils are reported by Illera *et al*. (2004) and Mora *et al*. (2002). Meriño-Gergichevich *et al*. (2010) reviewed the Al^{3+}-Ca^{2+} interaction in plants growing in acid soils in comparison to the Al-phytotoxicity response to calcareous amendments and pointed out the importance of gypsum amendments in the reduction of toxic Al without altering pH conditions (Franzen *et al*., 2006). This occurs due to the replacement of exchangeable Al^{3+} by Ca^{2+} particularly in the subsoil and the formation of Al-hydroxyl-sulfate or aluminum sulfate complexes (Mora *et al*., 2002), which are less toxic to plants.

In current years, highbush blueberry (*Vaccinium corymbosum* L.) has been positioned as an important crop in southern Chile due to its high antioxidant capacity, which is believed to be good for human consumption and for economic return. The crop is well established on Andisols between 34º and 44º S (INE, 1998; Besoain, 1999) especially in the "La Araucanía" region and with less importance in the most southern "Los Ríos" and "Los Lagos" regions of Chile. Although this species is well adapted to acid soils (Ireland and Wilk, 2006), it is very sensitive to Al^{3+} which constitutes a problem for its productivity (Korcak, 1992; Yang *et al*., 1996; Blatt and Mc Rae, 1997; Suzuki *et al*., 1999). Physiological and biochemical features of this plant species in response to Al toxicity has been poorly studied. Reyes-Díaz *et al*.

(2009; 2010) reported that short and long term Al toxicity affected differentially the effective photochemical efficiency of photosystem II (PSII) in highbush blueberry genotypes.

The possibility to employ amendments such as gypsum ($CaSO_4$) which reduce Al phytotoxicity without significantly changing the pH resulting in increased crop yield on Andisols (Mora *et al*., 1999) is a very important agronomic practice for crops, which demand acidity for their growth and development, but are sensitive to toxic Al as in the case of *V. corymbosum* (see above). However, little is known about the effects of $CaSO_4$ on the physiological and biochemical traits in this plant species. Therefore, the aim of this work was to study whether $CaSO_4$ is able to ameliorate the negative effects of Al toxicity on genotypes of highbush blueberry through determining some functional and chemical characteristics of root and leaves.

2. Materials and methods

2.1. Plant Material

One-year-old saplings of three highbush blueberry cultivars (cv), with different Al resistance, (Al-resistant Legacy and Brigitta and Al-sensitive Bluegold), frequently cultivated in southern Chile (Guerrero, 2006; Reyes-Díaz *et al*.,2009; 2010) were used for this study. Plants of these genotypes were *in vitro* produced and provided by Berries San Luis, Lautaro, Chile. Two groups of saplings were conditioned in plastic boxes filled with 18 L of Hoagland's nutrient solution (Hoagland and Arnon, 1959) for 2 weeks. One group of saplings was used for physiological and biochemical analyses and the other group was used for growth determinations (see below).

After conditioning, all saplings were subsequently transferred to a Hoagland hydroponic solution with increased $CaSO_4$ concentrations (2.5 and 5 mM) without and with Al ($AlCl_3$) in a toxic concentration (100 µM) during 15 days and continuous aeration in a greenhouse. The individual treatments were: Control (Hoagland solution), 2.5 mM $CaSO_4$, 5 mM $CaSO_4$, 100 µM Al, 100 µM Al + 2.5 mM $CaSO_4$, 100 µM Al + 5 mM $CaSO_4$.

The solutions were prepared with sterile deionized water and were filter-sterilized through 0.2-µm pore diameter filters as described by Reyes-Díaz *et al*. (2009). The pH of the control (without Al) and all treatments solutions was monitored with a portable pH meter (model pH-0.13; Hi-Tech-Instruments, Shanghai, China) and adjusted daily to a pH of 4.5 using 0.1 M HCl. Greenhouse growth conditions were 25/20 ºC (day/night), a 16/8-h (light/ dark) photoperiod, 300 µmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF), and 70% relative air humidity. After treatments, roots were washed three times with double distilled water as described in Yamamoto *et al*. (2001). During the experiment, *in vivo* measurements of chlorophyll fluorescence and CO_2 assimilation were made at different times. At the end of the experiment, growth was determined and biochemical measurements were performed in roots and leaves from five individual plants per treatment as described below. For biochemical analyses, samples were harvested and stored at –80 ºC until use.

2.2. Chemical analysis

Mineral element concentrations in leaves and roots

Calcium and Al concentrations were determined according Sadzawka *et al*. (2007), using a simultaneous multi-element atomic absorption spectrophotometer (model UNICAM 969 Atomic absorption Spectrometer, England). Harvested leaves and roots were dried separately in a forced-air oven at 70ºC for 72 h and immediately ground. Samples were ashed at 500 ºC for 8 h, treated with 2 M hydrochloric acid and Ca

and Al was quantified using atomic absorption spectrophotometry

For sulfur (S) determination, the turbidimetric method described by Sadzawka *et al*. (2007) was followed. The samples (leaves and roots) dried for 48h were treated with 95% magnesium nitrate ($MgNO_3$ x $6H_2O$) and ashed at 500ºC for 8 h. Then the ashed samples were digested for 60 min in 10 mL of 2M HCl at 150 ºC and after addition of barium chloride ($BaCl_2$) and Tween-80 solution measured in an UV-VIS spectrophotometer (UNICO® 2800 UV/VIS, Spain) at 440 nm.

2.3. Physiological and biochemical analysis

Plant growth analysis

Growth was analyzed by determining the change in fresh weight of 5 plants from the beginning (W1) to the end of the treatment (15 days) (W2). Growth was expressed as the mean relative growth rate (MRGR) from the mean natural logarithm-transformed weight plants: MRGR= (lnW2)–(lnW1)/(t2– t1) (Hoffmann and Poorter, 2002), where t1 and t2 represent 0 and 15 days, respectively.

CO_2 assimilation

CO_2 assimilation was determined *in vivo* on attached leaves from the second to fourth node of shoots using a portable CO_2 infrared gas analyzer (Licor LR6400, LI-COR Bioscience, Inc., Lincoln, Nebraska, U.S. & Canada) equipped with a cuvette which controlled the light source (300 µmol $m^{-2}s^{-1}$), temperature (20ºC), humidity and CO_2. External CO_2 from air was applied to obtain a reference concentration of 360 ppm, with a flow rate of 200 mL min^{-1} and 80% external relative humidity.

Chlorophyll a fluorescence parameters of PSII

Leaf chlorophyll-a fluorescence from the second to fourth node of shoots was measured using a portable pulse-amplitude modulated fluorimeter (FMS 2; Hansatech Instruments, King's Lynn, UK) to determine the photochemical efficiency of PSII according to Reyes-Díaz *et al*. (2009). The fluorescence parameters maximum quantum yield (Fv/Fm), effective quantum yield (ΦPSII) and electron transport rate (ETR) were estimated as described by Maxwell and Johnson (2000).

Lipid peroxidation

In fresh material of leaves and roots, the thiobarbituric acid-reactive substances (TBARS) were measured using Heath and Packer's method (1968) modified by Du and Bramlage (1992). In this modified procedure, the absorbance was measured at 532, 600, and 440 nm to correct for the interference produced by TBARS sugar complexes.

Radical scavenging activity

The radical scavenging activity (RSA) of roots and leaves was performed using the method of free radical 2.1-diphenyl-1-picrylhydrazyl (DPPH) scavenging as described by Chinnici *et al*. (2004), with some modifications. The absorbance was measured at 515 nm using Trolox as standard.

Superoxide dismutase activity

The frozen plant material was homogenized in 50 mM pH 7.0 potassium phosphate buffer (K_2HPO_4–KH_2PO_4). The homogenate was centrifuged at 4°C

for 15 min at 11,000 g, and the supernatant was used as enzyme extract. Superoxide dismutase (SOD; EC. 1.15.1.1) activity was assayed by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) and measured using an UV-VIS spectrophotometer (UNICO® 2800 UV/VIS, Spain) at 560 nm. One SOD unit was defined as the amount of enzyme corresponding to 50% inhibition of the NBT reduction (Donahue *et al*. 1997). SOD activity was calculated on a protein basis. The protein concentration in the enzyme extracts was measured spectrophotometrically using the method described by Bradford (1976).

2.4. Experimental design and statistical analysis

The experiment was arranged as a split-plot design with 3 genotypes x 6 treatments x 5 replicates each. We used three boxes for treatments and their position with 5 plants each was changed every day to minimize positional effects. *In vivo* measurements of photochemical parameters of PSII and of photosynthesis were taken at 0-1-3-6-10-15 days. Growth, Ca, S and Al concentrations, lipid peroxidation, radical scavenging capacity and SOD activity were determined at the end of the experiment after 15 days of treatment. For statistical analysis, reported values correspond to the mean of five individual replicates for each cultivar, treatment and time. All data were subjected to the normality and equal variance tests according to Kolmogorov-Smirnov. Data were then subjected to a two-way analysis of variance (ANOVA) (where the factors were genotypes and treatments) for chemical and biochemical analyses and to a three-way ANOVA (where the factors were genotypes, treatments and times) for *in vivo* measurements. A Tukey test was used to identify means with significant differences. Both analyses were performed with Sigma Stat 2.0 software (SPSS, Chicago, IL). Differences between the means were considered significant at $p \leq 0.05$.

3. Results

3.1. Plant chemical analysis

Al concentrations

Aluminum treatment alone significantly increased the Al concentrations of roots and leaves of all cultivars with cultivars Brigitta > Legacy > Bluegold in the leaves (Figure 1). $CaSO_4$ treatment reduced Al concentrations in leaves and roots. Roots showed higher Al concentrations than leaves under Al treatment (100 µM) without cultivar differences. Root Al concentrations were also greatly reduced by $CaSO_4$ supply with respect to Al treatment alone but not to the control level even at the highest $CaSO_4$ supply (Figure 1). ANOVA revealed a significant interaction between cultivars and treatments for leaves ($p \leq 0.01$) but not for roots ($p = 0.719$).

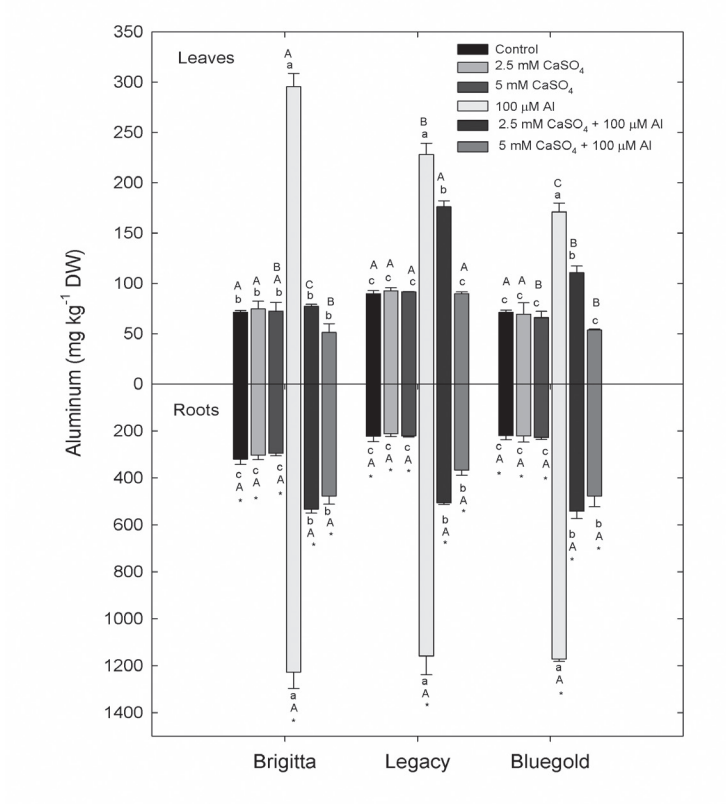

Figure 1. Aluminum concentrations in leaves and roots of three cultivars of highbush blueberry under Al and $CaSO_4$ treatments. Values represent means ± SE (n=5). Different lower case letters indicate significant differences ($p \leq 0.05$) between treatments within cultivars and plant tissue. Different upper case letters show differences ($p \leq 0.05$) between cultivars within treatments and plant tissues. Asterisk (*) indicates statistically significant differences between tissues (leaves and roots) for the same cultivar and treatments.

Calcium concentrations

The most striking differences in Ca concentrations were between the cultivars with cv Brigitta having the by far highest Ca concentrations in the roots (Figure 2). Cultivar Legacy had the lowest Ca concentrations in the leaves which did show differences with the two other cultivars. Al treatment reduced the leaf Ca concentrations in cv. Bluegold more than in cv. Brigitta, but not in cv Legacy. In the roots, Al supply decreased Ca concentrations particularly in cv. Brigitta. Likewise, in all cultivars, under Al supply $CaSO_4$ application restored the Ca concentrations in the leaves and the roots almost to the control levels (Figure 2). ANOVA revealed a significant interaction between cultivars and treatments for Ca concentrations in leaves and roots ($p = 0.001$).

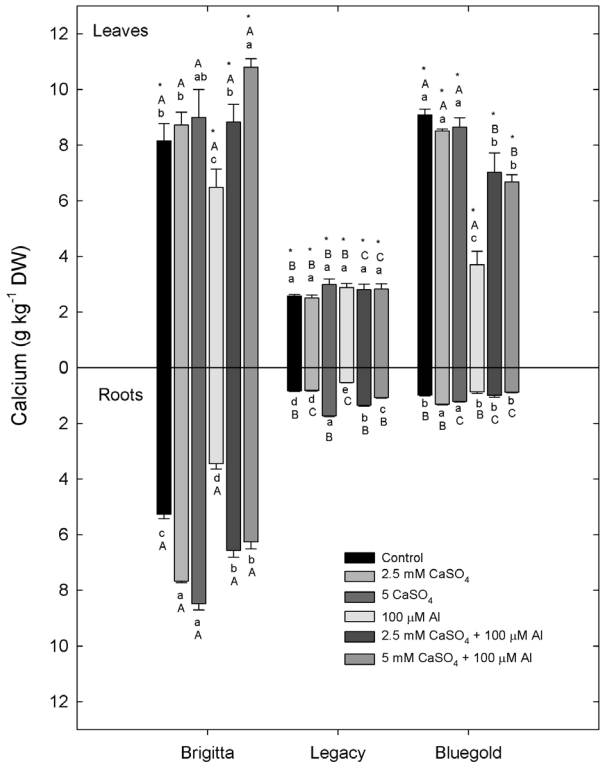

Figure 2. Calcium concentrations in leaves and roots of three cultivars of highbush blueberry under different treatments. Values represent means ± SE (n=5). Different lower case letters indicate statistically significant differences ($p \leq 0.05$) between treatments for the same cultivar and tissue. Different upper case letters show differences ($p \leq 0.05$) between cultivars for the same treatments and tissue. Asterisk (*) indicates statistically significant differences between tissues (leaves and roots) for the same cultivar and treatments.

Sulfur concentrations

Application of $CaSO_4$ enhanced S concentrations in the leaves in all cultivars; in roots only cv. Bluegold responded with increased S concentrations (Figure 3). Al supply did not affect the S concentrations neither in leaves nor in roots with the exception of cv. Bluegold, where Al prevented an increase in S concentrations in leaves at both $CaSO_4$ supplies (Figure 3). Roots and leaves S content showed a statistically significant interaction between cultivar and treatments ($p<0.001$).

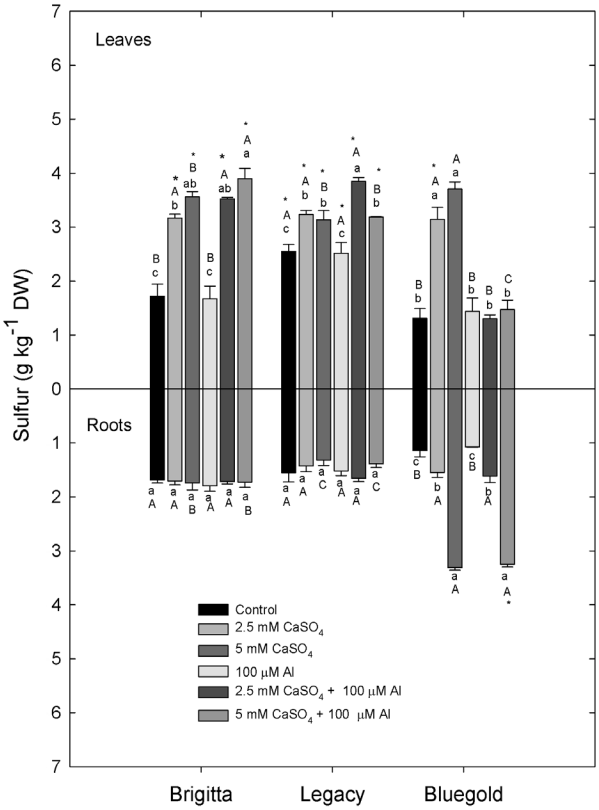

Figure 3. Sulfur concentrations in leaves and roots of three cultivars of highbush blueberry under different treatments. Values represent the average of five replicates ± SE (n=5). Different lower case letters indicate statistically significant differences ($p \leq 0.05$) between treatments for the same cultivar and tissue. Different upper case letters show differences ($p \leq 0.05$) between cultivars for the same treatments and tissue. Asterisk (*) indicates statistically significant differ ences between tissues (leaves and roots) for the same cultivar and treatments.

3.2. Physiological and biochemical analysis

Mean relative growth rate

In cv. Legacy, mean relative growth rate (MRGR) was not affected neither by Al nor $CaSO_4$ treatments (Figure 4). In contrast, Al treatment significantly reduced MRGR in cvs Brigitta and Bluegold, whereas $CaSO_4$ supply restored the Al-reduced MRGR in these cultivars. Surprisingly, in Bluegold the highest $CaSO_4$ rate decreased significantly the MRGR in absence of Al compared to the control (Figure 4). For this physiological parameter statistically significant interaction between cultivars and treatments were found (p = 0.001).

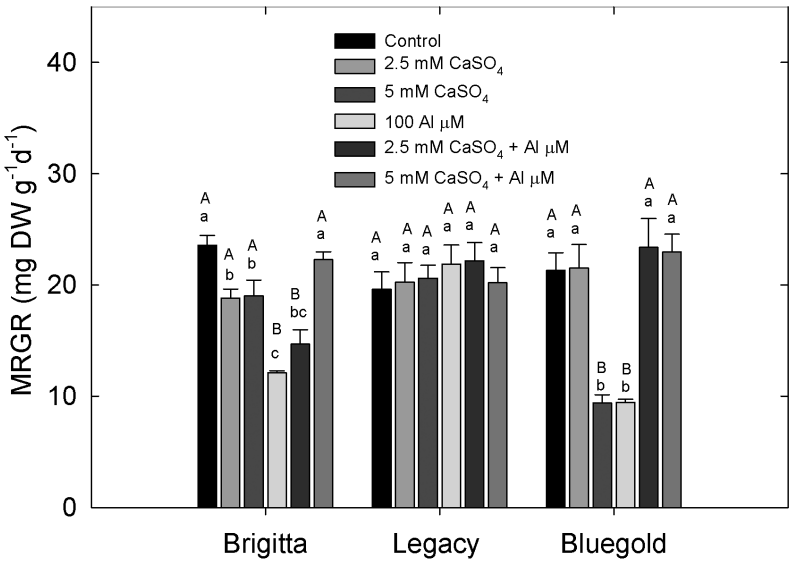

Figure 4. Mean relative growth rate (MRGR) of three cultivars of highbush blueberry grown for 15 days under different treatments. Values represent the means ± SE (n=5). Different lower case letters indicate statistically significant differences ($p \leq 0.05$) between treatments within cultivars. Different upper case letters show differences ($p \leq 0.05$) between cultivars within treatments.

CO_2 assimilation

In all cultivars the CO_2 assimilation rate of the controls remained constant over the treatment time at about 8 μmol CO_2 $m^{-2}s^{-1}$ (Figure 5). Al treatment reduced the assimilation rate after one day but recovered to the control level in cvs Brigitta and Legacy. However, in cv. Bluegold the assimilation rate continued to decrease up to 10 days of Al treatment and remained at this low level until treatment end. Both $CaSO_4$ treatments partly restored the Al-induced decrease in assimilation rate in Bluegold, but not at the same degree as in the other cultivars. ANOVA revealed a significant interaction between cultivars and treatments for all times ($p<0.01$) with exception for 1 day ($p=0.139$). Furthermore, ANOVA also revealed a statistically significant interaction between times and treatments in Bluegold and Legacy ($p<0.01$), but not for Brigitta ($p=0.988$).

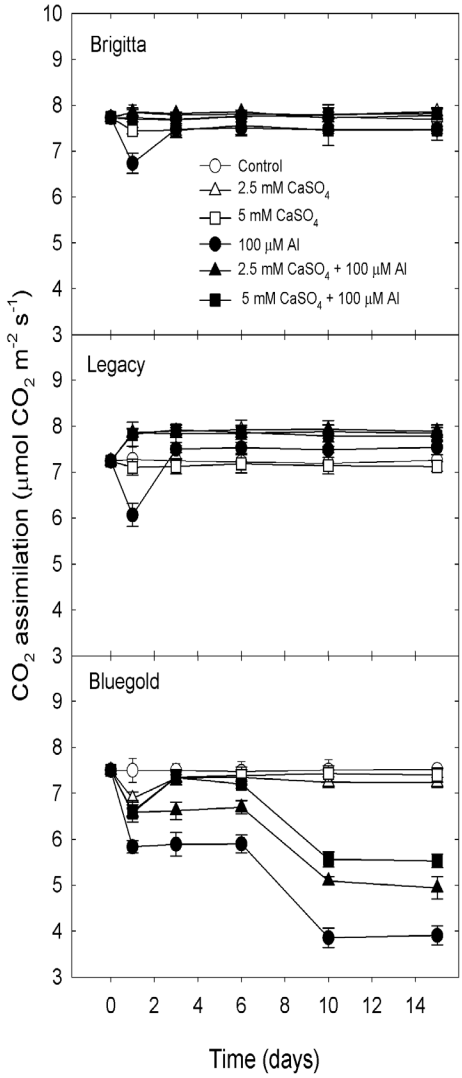

Figure 5. Effect of Al and $CaSO_4$ treatment on CO_2 assimilation rate of leaves of three cultivars of highbush blueberry during the treatment period. Values represent means ± SE (n=5).

Photochemical efficiency of PSII

The maximum quantum yield (Fv/Fm) of the three cultivars did not vary under the different treatments and times remaining at around 0.8 (data not shown) a value which is indicative of healthy plants. Nonetheless, the effective quantum yield (ΦPSII) and the electron transport rate (ETR) responded to the $CaSO_4$ and Al treatments in a cultivar-specific way (Figure 6). In cvs Legacy and Brigitta, $CaSO_4$ and their respective combination with Al reduced ΦPSII and ETR after one day of treatment, recovering to the control level

up to 15 days. However, in Bluegold $CaSO_4$ reduced ΦPSII and ETR at the first day with recovery only at both $CaSO_4$ levels. Aluminum supply equally reduced both photochemical parameters; however, no recovery occurred at the end of experiment in cvs Brigitta and Bluegold. Increasing the $CaSO_4$ supply counteracted the negative Al impact completely in cv Legacy, partly in Brigitta, but not in cv Bluegold (Figure 6).

For the photochemical parameters (ΦPSII and ETR) a statistically significant interaction between cultivar and treatment was found (p <0.01). For ΦPSII of the three cultivars the effect of different levels of time depends on what level of treatment is present (p<0.01). Similarly, for ETR this interaction occurs only in Brigitta and Bluegold (p<0.01), but not in Legacy (p=0.374).

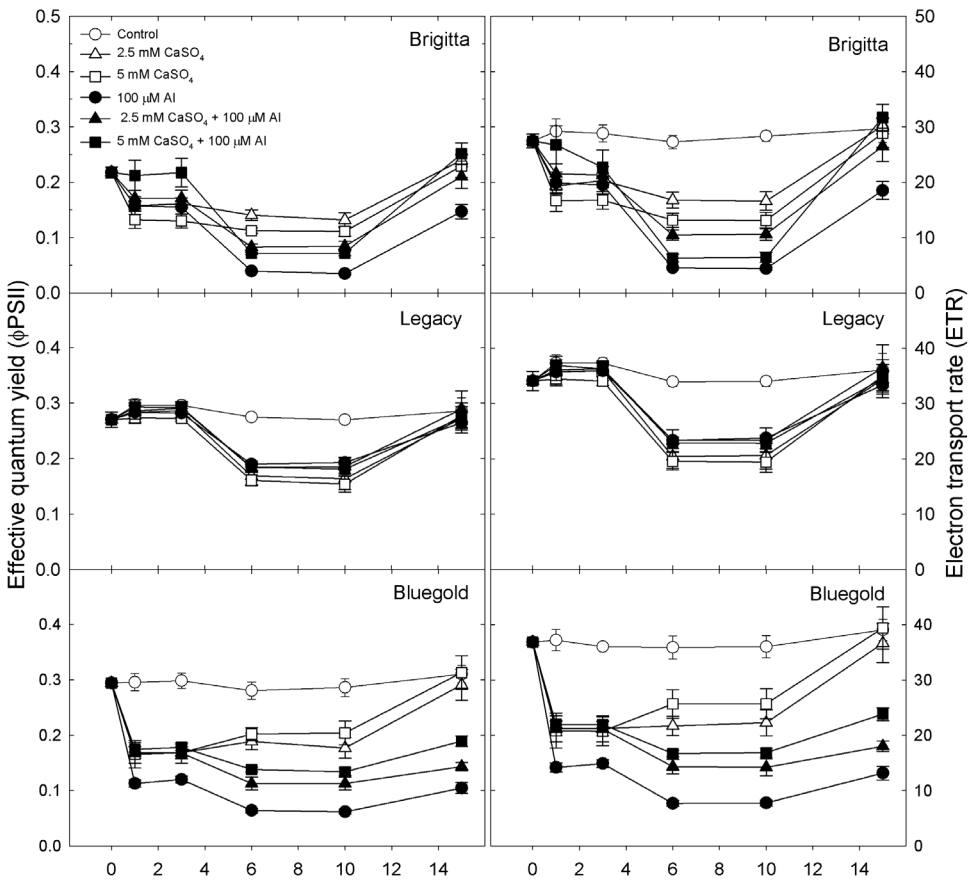

Figure 6. Changes in the effective quantum yield (ΦPSII) and electron transport rate (ETR) of three cultivars of highbush blueberry at different times under different treatments. Values represent the average of five replicates ± SE.

Lipid peroxidation

Leaves showed higher lipid peroxidation than roots in all cultivars (Figure 7). Aluminum-treated plants presented the highest lipid peroxidation in the investigated cultivars. Both $CaSO_4$ amendments improved significantly the lipid peroxidation of leaves in cvs Brigitta and Legacy, whereas in Bluegold this occurred only by the highest $CaSO_4$ level (Figure 7). Aluminum treatment significantly enhanced lipid peroxidation in the roots of all cultivars, but several fold more in cvs Brigitta and Bluegold than in cv. Legacy, which showed generally very low levels of lipid peroxidation (Figure 7). Application of $CaSO_4$ alleviated the lipid peroxidation induced by the Al treatment in cv. Legacy but only partly in cvs Brigitta and Bluegold. For shoots and roots there was statistically significant interaction between cultivars and treatments for lipid peroxidation ($p<0.01$). Likewise, in the three cultivars the effect of different levels of treatment depends on the plant organ ($p<0.01$).

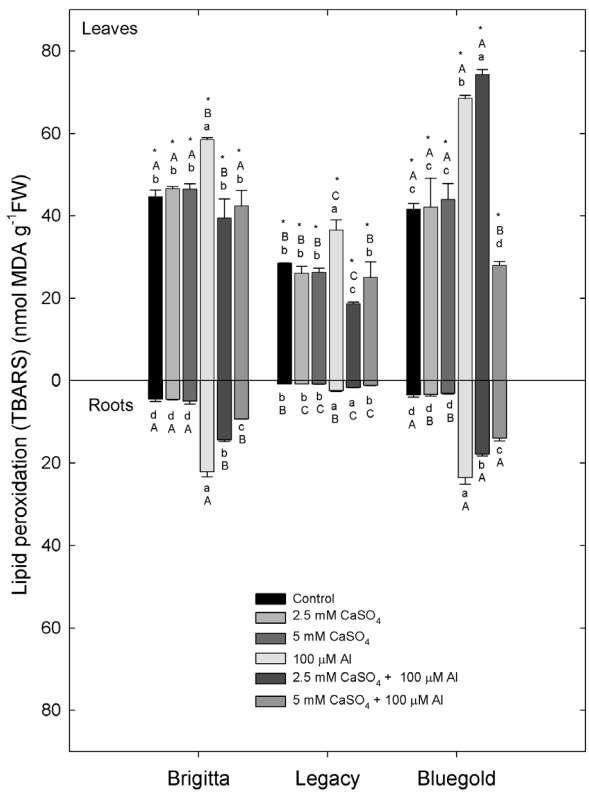

Figure 7. Lipid peroxidation measured as malondialdehyde (MDA) concentration of roots of highbush blueberry cultivars grown for 15 days under different treatments. Values are means ± SE (n=5). Different lower case letters indicate statistically significant differences ($p\leq0.05$) between treatments within cultivars. Different upper case letters show differences ($p\leq0.05$) between cultivars within treatments. Asterisk (*) indicates statistically significant differences between tissues (leaves and roots) for the same cultivar and treatments.

Radical scavenging capacity

Leaves of all three cultivars showed a higher radical scavenging activity (RSA) than roots independent on the treatment (Figure 8). In cv. Brigitta, Al treatment increased RSA in shoots and roots significantly, an effect which was further enhanced by the application of $CaSO_4$. Cultivars Legacy and Bluegold did not respond to Al supply, but the response to $CaSO_4$ was similar but weaker than in cv. Brigitta. Particularly, in cv Bluegold RSA was least responsive to the treatments (Figure 8). A statistically significant interaction among cultivars and treatments were found for RSA in roots and leaves ($p \leq 0.001$).

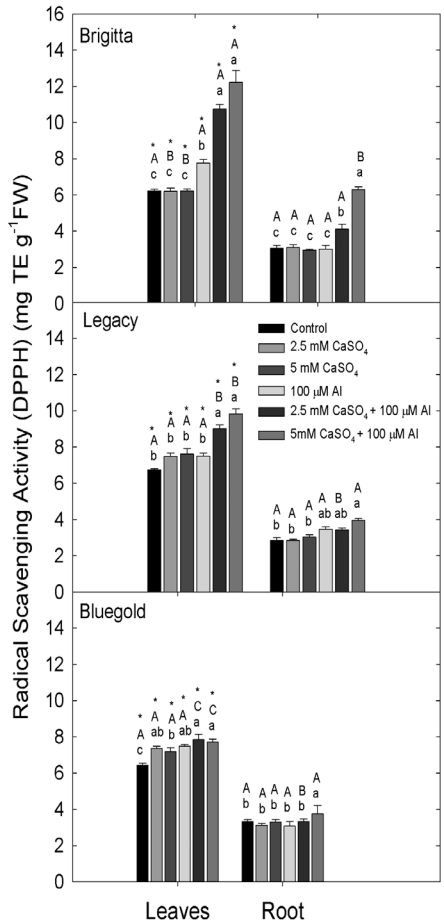

Figure 8. Radical scavenging activity of leaves and roots measured as Trolox equivalents (TE) of highbush blueberry cultivars grown for 15 days under different treatments. Values are means ± SE (n=5). Different lower case letters indicate statistically significant differences ($p \leq 0.05$) between treatments within cultivars and tissues (leaves and roots). Different upper case letters show differences ($p \leq 0.05$) between cultivars within treatments and tissues. Asterisk (*) indicates statistically significant differences between tissues for the same cultivar and treatment.

Superoxide dismutase activity

In general, leaves of three cultivars showed higher superoxide dismutase (SOD) activity than roots (Figure 9). In leaves, $CaSO_4$ supply enhanced SOD activity in all cultivars independent on Al supply (exception cv Legacy where at 5 mM $CaSO_4$ plus Al supply reduced SOD activity to the control level). SOD activity was significantly increased in response to Al treatment only in the cvs Legacy and Bluegold. In roots, the SOD activity responded less to the treatment; the highest SOD activity was found in Bluegold under Al supply, but was reduced by $CaSO_4$ treatment (Figure 9). ANOVA revealed a significant interaction between cultivar and treatment in leaves and roots for SOD activity. Likewise, statistically significant interaction between organs and treatments for all cultivars was found (p<0.01).

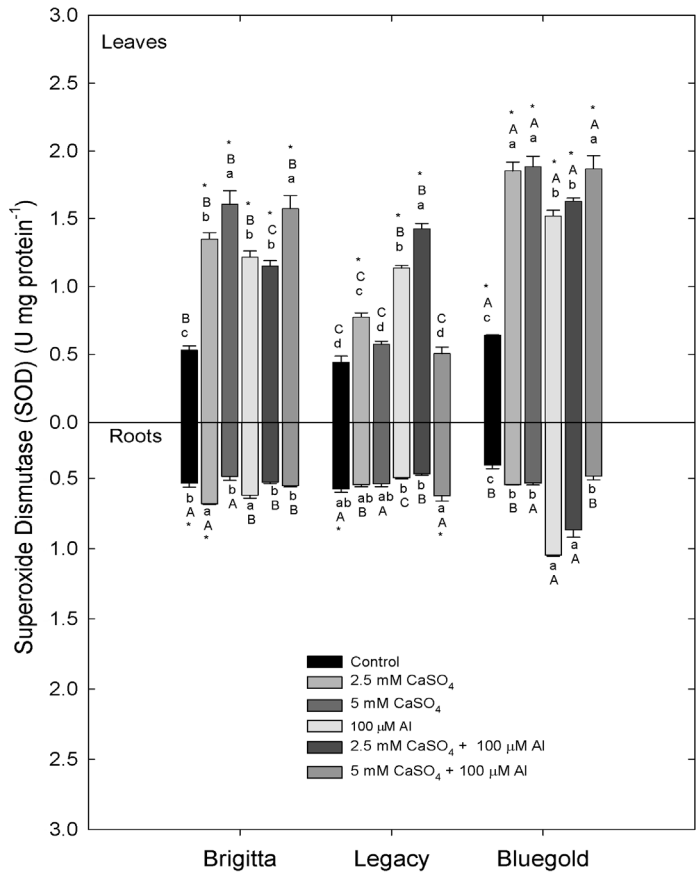

Figure 9. Superoxide dismutase (SOD) activity of leaves and roots of highbush blueberry cultivars grown for 15 days under different treatments. Values are means ± SE (n=5). Different lower case letters indicate statistically significant differences (p≤0.05) between treatments within cultivars and tissues (leaves and roots). Different upper case letters show differences (p≤0.05) between cultivars within treatments and tissues. Asterisk (*) indicates statistically significant differences between tissues for the same cultivar and treatment.

4. Discussion

Agronomical practices about use of calcareous amendment to ameliorate the negative effect on plants subjected to Al toxicity in acid soils has been reported for many crops (Caires *et al*., 2002; Ritchey and Snuffer, 2002; Caires *et al*., 2006). However, $CaSO_4$ addition on *V. corymbosum* growing in acid Andisols has been little studied. Our results about the use of $CaSO_4$ amendment in cultivars of this species under Al toxicity showed that this amendment was able to reduce the Al concentration in both roots and leaves in the three cultivars (Figure 1). Similar results were found in cultivars of barley (*Hordeum vulgare* L.) by Guo *et al*. (2006). These authors explained that Ca addition to nutrient solution reduced the accumulation of Al in tissues, due to reduction of plant Al uptake by formation of soluble aluminum sulfate ($AlSO_4^+$) complexes in nutrient solution, which are harmless to plants (Mathews and Joost, 1989; Carvalho and van Raig, 1997).

Based on the Ca concentrations of tissues, blueberry was considered as a calcifuge species by Hanson and Berkheimer (2004). These authors reported that healthy plants of this species could contain a foliar Ca level from 3.0 to 8.0 g kg^{-1} DW (0.3 to 0.8%), whereas for apple trees (*Malus domestica*) growing in an Andisol of Southern Chile, von Bennewitz *et al*. (2011) reported a foliar Ca concentration of.9.9 g kg^{-1} DW . In our study, Ca concentration of leaves was situated near to these values, depending on cultivar, showing generally higher Ca concentration than the upper limits reported by these authors in cv Brigitta. It is important to mention that these values were reached even under toxic Al in presence of the highest $CaSO_4$ concentration of nutrient solution (Figure 2). Also in roots of Brigitta, an effect of $CaSO_4$ amendment was evident in presence of toxic Al concentration. The lowest Ca concentrations of roots were found in the most Al-sensitive cultivar (Bluegold) and the highest in the Al-resistant cv Brigitta. Rout *et al*. (2001) reported that Al-sensitive cultivars of wheat (*Triticum aestivum* L.) exhibited a lower efficiency in uptake, translocation and utilization of Ca and P than the Al-tolerant cultivars. Furthermore, in our study foliar and root Ca concentrations in cultivar Bluegold and Brigitta were significantly negatively correlated with Al concentration (r = -0.7 and -0.6 for leaves and roots, respectively). These results agree with the results of Rengel and Zhang (2003), who reported that Ca addition efficiently reduced Al concentrations in different tissues by decline of plant Al uptake. The effective role of $CaSO_4$ in amelioration of Al toxicity was also confirmed by Chutichudet *et al*. (2009).

Gypsum ($CaSO_4$) has been recognized as a nutrient source of sulfur (~17%) (Mathews and Joost, 1989). Our findings showed similar foliar ranges of S concentrations in leaves under gypsum addition compared to those reported by Sanderson *et al*. (1995) for lowbush blueberry (*Vaccinium angustifolium*) leaves (0.7 to 3.8 g kg^{-1} DW). Likewise, high S concentrations were found in the three investigated cultivars with $CaSO_4$ amendment. Similar results are reported in ryegrass by Mora *et al*. (2005). In our work, it is remarkable that Bluegold showed a higher S concentration of leaves which was positively and statistically significant correlated with the CO_2 assimilation (r=0.6, p= 0.007), whereas in the other cultivars a same tendency was found, but without statistical significance. In *Brassica* plants, the rate of photosynthesis was higher with S supply (59.2-68.3%) than without S (Mobin *et al*. 2010). These authors explained this behavior by a closure of stomata under S deficiency inhibiting the CO_2 uptake and thus restricting the carbon assimilation.

The first and most recognized effect of Al toxicity in plants is the inhibition of the division and elongation of meristematic root cells and thereby, a reduc-

tion in root growth (Panda *et al*. 2003; Mora *et al*., 2006). Reyes-Díaz *et al*. (2009; 2010) subjected the same three blueberry cultivars (Brigitta, Legacy and Bluegold) used in this work to short- and long-term Al excess in hydroponics. Cultivar Legacy showed a greater Al accumulation in leaves and roots, maintaining its growth iun contrast to the other cultivars. This feature was directly associated with the degree of Al tolerance, suggesting that Legacy is most suitable to be established in acid soils under Al toxicity.

According with our study on *V. corymbosum*, Brigitta and Bluegold showed a reduced MRGR when plants were subjected to 100 µM Al, whereas in plants additionally supplied with $CaSO_4$ MRGR was restored (Figure 4). An increased root and shoot growth was reported for soybean (*Glycine max*) by Sanzonowicz *et al*. (1998), indicating that an increase in Ca concentration (using $CaSO_4$ as Ca source) decreased the inhibitory effects of H^+ and Al in roots, because of an increased ionic strength and a reduced predicted activity of monomeric Al species.

The most evident changes in the photosynthetic performance (CO_2 assimilation and photochemical parameters) under the effect of Al were exhibited by the Al-sensitive cv Bluegold, where these parameters decreased and did not show full recovery in presence of gypsum amendment at the end of experiment (Figures 5 and 6). Akaya and Takenaka (2001) suggested that the effect of Al on reduced root function of *Quercus glauca* was not only directly associated to a decrease of photosynthesis rate, but rather to mineral element deficiency plus high Al concentration in tissues. Reich *et al*. (1994) evaluated net photosynthesis in scots pine (*Pinus sylvestris*) under excessive soil Al and deficient Ca and Mg levels in needles, which resulted in a reduced photosynthetic capacity and increased respiration. Our findings showed no effect of toxic Al on the photosynthetic performance of the Al-resistant cv Legacy, whereas in cv Brigitta, only a slight recovery of this process by the gypsum application at the end of treatment became evident. In *Citrus grandis* the toxic effect of Al showed decreased photochemical parameters of PSII such as Fv/Fm, ΦPSII and ETR, by reduction of chlorophyll contents and enhanced energy dissipation (Jiang *et al*. 2008). These authors also suggested that decreased photosynthesis performance could be related to a photoinhibition in both donor and acceptor sites of PSII.

Some stresses trigger changes in antioxidant responses suggesting an induction of these mitigation mechanisms in response to ROS generation at the cellular level (Blokhina *et al*. 2003). In our study, RSA and SOD were differentially activated in response to Al stress in the three cultivars. Statistically significant correlations between SOD and Al concentration of leaves were obtained only in Legacy ($r = 0.821$; $p<0.001$), while in Bluegold a statistically significant correlation between SOD and Al concentration and lipid peroxidation at root level was found ($r = 0.85$; $p<0.001$ and $r = 0.84$; $p<0.001$, respectively). These results suggest that in the case of Legacy, SOD would be an important defense mechanism against Al stress by helping to maintain its growth and photosynthetic capacity. On the contrary, in the Al-sensitive cultivar (Bluegold) SOD activity is only a stress response to the Al treatment. It is know that SOD constitutes the first line of defense against ROS at the cellular level, and it has a high significance as an antioxidant defense mechanism (Alscher *et al*. 2002). Notwithstanding, it is important to mention that contradictory observations about SOD activity in different plant species under stresses have been reported, which may be explained by their different stress tolerance (Blokhina *et al*. 2003).

5. Conclusions

Based on the measured parameters it is concluded that gypsum amendment was efficient in complete recovery from the toxic Al effect in the Al-resistant and medium Al-resistant cultivars Legacy and Brigitta, respectively, but was only partly efficient in the Al-sensitive cv. Bluegold. With respect to Bluegold further investigation at the long-term may be useful to obtain a better recovery. Perhaps this cultivar needs a longer time to adjust its response mechanism. However, gypsum amendment may be a good alternative liming to alleviate Al toxicity in the long-term in the Al-sensitive cultivars and may provide a good source of nutrients such as Ca and S.

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