UNIVERSIDAD DE LA FRONTERA
Facultad de Ingeniería, Ciencias y Administración
Programa de Doctorado en Ciencias de Recursos Naturales

EFFECT OF Ca/Al RATIO ON PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE ASSOCIATED WITH OXIDATIVE STRESS IN HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.) GROWING IN AN ANDISOL

TESIS PARA OPTAR AL GRADO ACADÉMICO DE DOCTOR EN CIENCIAS DE RECURSOS NATURALES

CRISTIAN JORGE MERIÑO GERGICHEVICH

TEMUCO – CHILE
2012

UNIVERSIDAD DE LA FRONTERA
Facultad de Ingeniería, Ciencias y Administración
Programa de Doctorado en Ciencias de Recursos Naturales

EFFECT OF Ca/Al RATIO ON PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE ASSOCIATED WITH OXIDATIVE STRESS IN HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.) GROWING IN AN ANDISOL

Doctoral Thesis
In Fulfillment of the
Requirements for the Degree
Doctor of Sciences in Natural Resources
By

CRISTIAN JORGE MERIÑO GERGICHEVICH

TEMUCO – CHILE
2012

Advisor: DRA. MIREN ALBERDI LAG
Departamento de Ciencias Químicas y Recursos Naturales
Center of Plant, Soil Interaction and Natural Resources Biotechnology,
Scientific and Technological Bioresource Nucleus (BIOREN-UFRO)
Facultad de Ingeniería, Ciencias y Administración
Universidad de La Frontera

Co advisor: DRA. MARJORIE REYES DÍAZ
Departamento de Ciencias Químicas y Recursos Naturales
Center of Plant, Soil Interaction and Natural Resources Biotechnology,
Scientific and Technological Bioresource Nucleus (BIOREN-UFRO)
Facultad de Ingeniería, Ciencias y Administración
Universidad de La Frontera

Reviewers: DRA. MARÍA DE LA LUZ MORA GIL
Departamento de Ciencias Químicas y Recursos Naturales
Center of Plant, Soil Interaction and Natural Resources Biotechnology,
Scientific and Technological Bioresource Nucleus (BIOREN-UFRO)
Facultad de Ingeniería, Ciencias y Administración
Universidad de La Frontera

DRA. PAULA CARTES INDO
Departamento de Ciencias Químicas y Recursos Naturales
Center of Plant, Soil Interaction and Natural Resources Biotechnology,
Scientific and Technological Bioresource Nucleus (BIOREN-UFRO)
Facultad de Ingeniería, Ciencias y Administración
Universidad de La Frontera

DR. LEÓN BRAVO RAMÍREZ
Departamento Ciencias Agronómicas y Recursos Naturales
Facultad de Ciencias Agropecuarias y Forestales
Center of Plant, Soil Interaction and Natural Resources Biotechnology,
Scientific and Technological Bioresource Nucleus (BIOREN-UFRO)
Universidad de La Frontera

DR. GUSTAVO ZÚÑIGA NAVARRO
Departamento de Biología
Facultad de Química y Biología
Universidad de Santiago de Chile

DR. ANTONIO VIOLANTE
Dipartamento di Scienze del Suolo, della Pianta, dell´Ambiente e delle Produzioni Animali
Università degli Studi di Napoli Federico II
Portici, Napoli, Italy

EFFECT OF Ca/Al RATIO ON PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE ASSOCIATED WITH OXIDATIVE STRESS IN HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.) GROWING IN AN ANDISOL

Tesis realizada bajo la supervisión de la directora de Tesis Dra. MIREN RITA ALBERDI LAG, perteneciente al Departamento de Ciencias Química y Recursos Naturales de la Universidad de La Frontera y es presentada para su revisión por los miembros de la comisión examinadora.

CRISTIAN JORGE MERIÑO GERGICHEVICH

.....	.....
DIRECTOR PROGRAMA DOCTORADO EN CIENCIAS DE RECURSOS NATURALES	DRA. MIREN ALBERDI LAG
	.....
	DRA. MARJORIE REYES DÍAZ
.....	.....
DIRECTOR PROGRAMA UNIVERSIDAD DE LA FRONTERA	DRA. MARÍA DE LA LUZ MORA GIL
	.....
	DRA. PAULA CARTES INDO
	.....
	DR. LEÓN BRAVO RAMÍREZ
	.....
	DR. GUSTAVO ZÚÑIGA NAVARRO
	.....
	DR. ANTONIO VIOLANTE

“El futuro pondrá las cosas en claro,
y cada quien en su sitio según sus méritos.
El presente les pertenece.
El futuro, que es en realidad
para lo que yo trabajo, será mío”

Nikola Tesla (1856-1943)

Agradecimientos

Al final de esta etapa quiero agradecer el apoyo brindado por mi familia, en especial mi madre y Paula, quienes siempre confiaron en mí desde el instante en que decidí tomar este camino. En momentos difíciles, me aconsejaron y respaldaron para continuar adelante. Gracias.

Agradecer también a las Doctoras Miren Alberdi Lag y Marjorie Reyes Díaz, de la Universidad de La Frontera, por su dedicación no sólo con la realización de esta tesis, sino con todo mi proceso de formación en este programa de doctorado. Les deseo mucho éxito en el futuro, especialmente a Marjorie.

Dedico un reconocimiento para la Dra. María de la Luz Mora, por sus consejos y opiniones, sugerencias y críticas, oportunidades y respaldo. Todos con el fin de hacer de uno un mejor profesional e investigador. Además agradezco las posibilidades que me brindó para participar en diferentes actividades llevadas a cabo por el programa.

Por su extraordinaria ayuda para realizar mi pasantía doctoral agradezco al Dr. Gabrijel Ondrašek, así como también a Mg. Monika Zovko, Mg. Helena Bakić, y al Departamento de Mejoramiento de Suelos de la Facultad de Agronomía, Universidad de Zagreb en Croacia. También la ayuda entregada por la Dra. Snježana Mihajlević y la doctorante Dunja Šamec, del Departamento de Biología Molecular perteneciente al Instituto Ruđer Bošković en Zagreb, Croacia. En estas mismas circunstancias también a Milan Mešić del Departamento de Cooperación Internacional de la universidad anteriormente mencionada, Diana Lahowsky de Hellea d.o.o. Plantaža Borovnica de Zagreb por la extraordinaria ayuda con el material vegetal, y a Germán Ibarra, Embajador de la República de Chile en Croacia.

Agradezco también al Dr. Walter J. Horst, del Instituto de Nutrición Vegetal en la Universidad Leibniz-Hannover, la posibilidad de desarrollar mi pasantía en la

hermosa ciudad Hannover en Alemania. También a alumnos y profesionales de ese instituto como André Specht, Martin Duschyk, Katharina Klug, Sebastian Klein y Sascha Schulze. Y mencionar especialmente a Ingrid Dusy por su invaluable ayuda durante mi estadía en ese país.

Al proyecto MECESUP FRO 0601 y Beca de Término de Tesis CONICYT por el financimiento de mantención. Además agradezco al proyecto FONDECYT Nº 11080231 de la Dra. Marjorie Reyes Díaz, del cual nace esta tesis doctoral, así como su realización y difusión de resultados. Lo cual también fue posible gracias al financiamiento otorgado por CONICYT a través de las Becas de Apoyo a Realización de Tesis Doctoral AT-24100159 de 2010, Pasantía Doctoral en el Extranjero Becas-Chile (convocatorias 2011 y 2011-2012), Asistencia a Eventos y Cursos Cortos en el Extranjero (convocatorias 2010 y 2012) y al Programa de Apoyo para Asistencia a Congresos para Alumnos de Doctorado de la Universidad de La Frontera (convocatorias 2010, 2011 y 2012). Así como al Programa de Doctorado en Ciencias de Recursos Naturales de la Universidad de La Frontera, por el soporte económico para tal efecto.

A mis colegas Ligia, Alex, Rodrigo, Vivian, Sebastián y Daniel, de la generación 2008, y a Cecilia, Jacqueline, Gabriela y Claudio, por los grandes momentos vividos tanto académicos como de verbena, ya sea aquí o en algún otro lugar del mundo y a Edith Cantero por su amistad.

A Ximena Garrido y Jessica Yañez, quienes colaboraron estrechamente con mi trabajo experimental, y por los buenos momentos pasados.

Finalmente, agradezco a Berries San Luis de Lautaro por material vegetal fundamental donado para esta investigación. Así como a Erick Von Baer y Marcelo Labra de Semillas Baer Ltda. por facilitar el suelo requerido para el establecimiento de ensayos.

Table of contents

Chapter 3. Mitigation of Al toxicity through the regulation of Ca/Al ratios in highbush blueberry (*Vaccinium corymbosum* L.) using calcium sulfate.

Table index

Figure index

THESIS ABSTRACT

Highbush blueberry (*Vaccinium corymbosum* L.) is well adapted species to acid soils (pH≤5.5) where Al-toxicity (Al^{3+}) can be one of the most important soil constraints for plant growth and development. Plant stress is triggered by Al^{3+}, causing well documented harmful effects on physiological and biochemical features in roots. However, damages in the upper parts may also be present, decreasing normal functioning of photosynthetic parameters. On the other hand, calcium (Ca) addition plays a fundamental role in the amelioration of Al^{3+} by regulation of Ca and Al interaction at cellular level, improving processes such as water and nutrient absorption, photosynthesis and regulation of antioxidant activity associated to oxidative stress caused by toxic Al. The Ca/Al molar ratio (Ca/Al) is stoichiometric atomic ratio (molCa/molAl) to determine this interaction and used as indicator of potential stress from Al^{3+} and several studies have demonstrated that Ca/Al in foliar and root tissues is strongly correlated with the Ca/Al solution. Calcium sulfate or gypsum ($CaSO_4$) is a calcareous amendment used to ameliorate the harmful effect of Al^{3+} concomitant to an increase in Ca contents, without raise pH for crops adpted to acidity as highbush blueberry. Nonetheless, the effects of this amendment on physiological and biochemical performance by improving of Ca/Al in this species grown under Al-toxicity are remaining little known. Therefore, the objective of this work was to study the effects of Ca/Al ratio on physiological and biochemical processes in highbush blueberry cultivars grown in a calcium sulfate amended Andisol. In two separate experiments, under greenhouse conditions, one year-old plants a more Al-tolerant (Legacy) and Al-sensitive (Bluegold) highbush blueberry cultivars were disposed in a nutrient solution containing Al (100 and 200 μM) and increased $CaSO_4$ concentrations (2.5, 5, and 10 mM) for 15 days (experiment 1). Afetrward, both cultivars were grown in an Andisol with high Al-saturation amended with $CaSO_4$ at 0, 700, 1400 and 2800 mg kg^{-1} soil, for 60 days (experiment 2). In both experiment chemical, physiological and biochemical features were studied. Experiment 1 showed in both cultivars an increased, Ca content and Ca/Al up to ~100% and 180%, respectively by adding $CaSO_4$ concomitant with a reduction in

foliar Al in both Legacy and Bluegold ($r=-0.80$; $P\leq0.001$ and $r=-0.74$; $P\leq0.001$, respectively). A high Ca/Al had a positive effect on photochemical parameters in both cultivars ($P\leq0.05$) as well as in the reduction of oxidative stress and increase of total phenols and SOD, particularly in Legacy. Furthermore, highbush blueberry develops well in acid soils, where Ca/Al is typically low; $CaSO_4$ amendment, mainly at 5 and 10 mM, may represent an effective alternative to application in Chilean acid soils, as Ca source, reduction of toxic Al and Ca/Al regulation especially in Legacy. In experiment 2 calcium sulfate improved Ca/Al, plant growth, as well as photochemical parameters, carotenoids contents, and relative water content (RWC) in leaves, especially in tolerant cultivar. However, the amendment did not show clear effects on chlorophyll contents (Chl) and leaf water potential (Ψ_l). The Ca/Al molar ratio was related to a decreased lipid peroxidation (LP) in both cultivars, whereas radical scavenging activity (RSA), anthocyanins (TAN), and antioxidant enzymes were directly increased by this ratio. It was concluded that calcium sulfate can be an effective amendment to ameliorate Al^{3+} toxicity in highbush blueberry, mainly in the Al tolerant cultivar. Moreover, we observed that different Ca/Al molar ratio could be established to improve the physiological and biochemical performance in this species, depending on the cultivar. In conclusion Ca/Al was related to an improved plant performance to stress environmental conditions, showing different ranges of Ca/Al for each cultivar. This research provides valuable information about the use of calcium amendment to improve the nutrient levels and reduce the risk factors related to highbush blueberry cultivation in soil conditions such as Southern Chile.

THESIS OUTLINES

Aluminum (Al) is one of the most abundant metals in the earth's crust (comprising about 7%). Fortunately, in agicultural terms mostly of Al is primarily in the form of insoluble aluminosilicates or oxides. Only under conditions of low pH, Al is soluble as monomeric form (Al^{3+}) the most toxic for plants, which can inhibit their growth and development causing toxicity. This toxic form predominates in soils with acid pH (≤5.5), as those of the Andisol Order. The first symptom of Al^{3+} injury in plants has been identified in roots affecting the water and nutrient uptake efficiency. However, in upper parts of plants such as stem, leaves and/or fruits, Al^{3+} harmful constraint are still controversial. In this way, some reports indicate that Al^{3+} affects physiological, biochemical and metabolic pathways, because triggers an excessive generation of reactive oxygen species (ROS). These ROS induces oxidative stress in cellular organelles and organic molecules, resulting even in cell death. It has been reported that Al^{3+} stress affects photosynthesis as result of a partial inhibition of photosynthetic electron transport rate (ETR) and closure of reaction centers in photosystem II (PSII). By other hand, plants have evolved defensive mechanisms against oxidative damage, by antioxidant systems both enzymatic and non enzymatic, which act by scavenging of ROS. This performance is regulated by a network of signal transduction molecules including receptors and messengers, among others. One of the key components is calcium (Ca), which is essential for several physiological and biochemical processes, playing an important structural and functional role, related to the cell wall, biological membranes, cytoplasm, vacuoles, and other cell organelles. Although Ca is less abundant than other nutrients in the plant cell is, is essential for development and plant production.

Chapter two focuses on reviewing the interaction between Al^{3+} and Ca^{2+} and its effects on the physiological and biochemical processes in crops growing on acid soils, and then to determine the alternatives of calcareous amendments used in acid soils providing the background support for followed chapters. This report indicates that interaction between Ca^{2+} and Al^{3+} is probably the most important factor that affects Ca^{2+} and other nutrients uptake and transport in plants grown in acids soil. This relation can be described as Ca/Al molar ratio (Ca/Al) and it has been discussed

how this interaction takes place, because both are inhibitors of another one depending on the conditions under which it developed. Literature reports that the degree of Al stress in plants is correlated with the Ca/Al rather than the Al concentration in the soil solution. Thereby, an inadequated Ca/Al disrupts the role of Ca in cell function.

On the other hand, it has been recognized that Al toxicity is ameliorated by basic cations in particularly Ca. The role of Ca adding (e.g. calcareous amendments) on reduction of Al^{3+} concentration in acid soils has been studied, but differences regarding the application manure, effectiveness, and the plant species or genotype have been matter of controversy. Currently, the use of calcareous amendments have been studied by several authors, who reported results demostrating the effectiveness of amendments to reduce Al toxicity to a greater or lesser extent depending on the source of Ca used, soil condition and the treated crop.

In South Central Chile highbush blueberry (*Vaccinium corymbosum* L.) is an important cultivated plant. It is well known that this high priced small fruit crop is increasingly cultivated due to its flavor and nutritional properties. This crop is well adapted to acid soils as Andisol but is sensible to high level of Al toxicity which decreases its productivity. Nevertheless, the effect of Ca addition to reduce Al^{3+} has been little studied for this fruit crop in our Andisol conditions. Therefore, this thesis aimed to study the relation between Ca and Al that allow a better nutrient balance and Al detoxification reflected by photosynthetic and antioxidant performances in highbush blueberry cultivars amended with calcium sulfate ($CaSO_4$) or gypsum grown in an Andisol of Southern Chile.

Therefore, before to study highbush blueberry plants subjected to high Al saturation in an Andisol, it was necessary to evaluate $CaSO_4$ treatments under controlled conditions. Chapter three to ascertain the $CaSO_4$ addition on Ca/Al and its effect on chemical, physiological and biochemical features in cultivars of highbush blueberry with contrasting tolerance to Al Legacy (Al-tolerant) and Bluegold (Al-sensitive) grown in Al-toxified Hoagland`s nutrient solution containing increased $CaSO_4$ concentrations (2.5, 5 and 10mM) and Al^{3+} (100 and 200μM), in an experiment of 15 days. According with chemical determinations both cultivars exhibited increased Ca content and Ca/Al (up to 180%) by adding $CaSO_4$ in parallel

to a reduction of Al (leaves and roots). The increase in Ca/Al molar ratio had a significant effect on physiological parameters such as photochemical efficiency of PSII [effective quantum yield (ΦPSII) and ETR]. For biochemical determinations, an adequated foliar Ca/Al exhibited a reduction of oxidative stress, and an increase of antioxidant activity of phenols. In summary, $CaSO_4$ treatments at 5 and 10mM showed positive effect on Ca/Al and evaluated parameters.

Afterwards, in chapter four were evaluated above studied cultivars under a soil conditions. A second experiment in an Al-saturated (~70%) Andisol from Southern Chile was carried out in plants of Legacy and Bluegold grown for 60 days. Soils received amendment with $CaSO_4$ at equivalent doses of 0 (control), 1000, 2000, 4000 kg ha^{-1}. Determinations on chemical (nutrient content), physiological (growth, hydric relations, and photochemical performance), and biochemical (lipid peroxidation, antioxidant capacity, and antioxidant compounds) features were performed. At the end of experiment improved nutrients content, Ca/Al and growth were found, as well as photochemical parameters, carotenoid contents, and relative water content (RWC) in leaves, especially in cultivar Legacy. However, $CaSO_4$ did not show clear effects on chlorophyll contents (Chl) and leaf water potential (Ψ_{leaf}). On the other hand, $CaSO_4$ addition decreased lipid peroxidation (LP) and total flavonoids content (TFA) in both cultivars, whereas radical scavenging activity (RSA), total phenols content (TPC), total anthocyanins content (TAN), and antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were significantly increased. It is concluded that $CaSO_4$ can be an effective amendment to ameliorate Al^{3+} toxicity and improve physiological and biochemical performance in highbush blueberry. Nonetheless, we suggest, that higher doses of this amendment could be required to prevent harmful effect of Al^{3+} in Al-sensitive cultivars grown in Andisols.

In summary, we propose that an adequated Ca/Al in tissues of highbush blueberry according to genotypic features will allow a better aclimation to this species against environmental stresses as Al toxicity.

Chapter 1. *General Introduction*

1.1 GENERAL INTRODUCTION

In acid soils (pH ≤ 5.5), several studies have reported that aluminum (Al) can be an important constraint for plant physiological and biochemical processes, especially in young plants (Kochian, *et al*., 2005; Ryan and Delhaize, 2010). Injuries produced by the toxic species (Al^{3+}) are firstly evident in roots (Broquen *et al*., 2005; Sivaguru *et al*., 2006; Ryan and Delhaize, 2010)**,** where it accumulates altering cell division and elongation sites (Kochian, *et al*., 2005; Horst *et al*., 2010). It causes a decrease in nutrient and water uptake capacity of roots and may subsequently affect shoots functionality performance (Mossor Pietraszewska, 2001; Rout *et al*., 2001; Langer *et al*., 2009). In spite, there are a lot of reports about harmful effects of Al^{3+} on root plants (Delhaize and Ryan, 1995; Klug and Horst, 2010; Klug *et al*., 2011), while information related to its effect in shoots or leaves is scarce. In this way, Al-phytotoxicity can induce reduction of shoot growth, leaf necrosis, and delayed leaf maturity (Rout *et al*., 2001; Zhang *et al*., 2007), resulting in a decreased of photosynthetic efficiency, pigment contents, and dioxide carbon (CO_2) assimilation (Peixoto *et al*., 2002; Zhang *et al*., 2007).

On the other hand, literature report that Al^{3+} triggers an overproduction of ROS, by impairment of membrane functions, which may be related to Al-enhanced oxidative stress in cellular organelles (Yamamoto *et al*., 2002; Jones *et al*., 2006; Ma *et al*., 2007). In addition, plants have evolved some antioxidant enzymatic and non-enzymatic mechanisms against oxidative damage, to removing and scavenging of ROS (Arbona *et al*., 2003; Huang *et al*., 2005; Sharma *et al*., 2007). Enzymatic antioxidant set includes several enzymes such as superoxide dismutase (SOD, EC. 1.15.1.1), and catalase (CAT, EC. 1.11.1.6) (Blokhina *et al*., 2003; Ma *et al*., 2007). Furthermore, phenolic compounds such as flavones, isoflavones, flavonones, anthocyanins, and catechins, have been reported to play effective non-enzymatic antioxidant functions (Velioglu *et al*., 1998; Mittler *et al*., 2004; Prior *et. al*., 2005).

Antioxidant system is regulated by a network of signal transduction molecules including receptor and messengers. One of these key components in this network is calcium (Ca) that acts as an intracellular messenger in association with a wide-range

of extracellular signals to specific responses to environmental stimuli (Cheng *et al*., 2002; Lecourieux *et al*., 2006; Wang and Li, 2006). In plants, Ca is crucial for growth, development, structural support (Hepler, 2005; Schaberg *et al*., 2006), photosynthesis, as well as water splitting in photolysis, acting as an activator of manganese (Mn) cluster in PSII (Miqyass *et al*., 2007; Yocum, 2008). To a certain extent, Ca deficiency may be a problem on acid soils, repercuting in the whole plant or in a particular organ of plants, showing symptoms such as growth reduction, browning, and tissues necrosis (Seling *et al*., 2000; Hepler, 2005).

Interaction between Ca^{2+} and Al^{3+} is probably the most important factor affecting Ca uptake and transport in plants grown in acid soils (Ryan *et al.*, 1994; Pintro *et al*., 1998; Schaberg *et al*., 2006), due to competitive inhibition between these two cations for active exchangeable sites of soil and plant roots (Ryan and Kochian, 1993; Kinraide, 1998). A large proportion of total Ca resides in the cell wall (apoplast) bound to pectins, conferring both rigidity and elasticity to it (Seling *et al*., 2000). However, according to displacement hypothesis, Al^{3+} remove Ca from critical sites of apoplast, inhibiting Ca transport to symplasm, and disrupting Ca homeostasis in cell cytoplasm (Ryan *et al*., 1993; Kinraide *et al*., 1994; Ryan *et al*., 1997). In this way, it has been reported that degree of Al-stress in acid soils is strongly correlated with Ca/Al rather than the Al concentration in soil or nutrient solution, being suggested as an indicator of Al-toxicity (Cronan and Grigal, 1995; Brunner *et al*., 2002). Thereby, to overcome effects of Al^{3+}, Ca addition has been widely studied on many crops grown in acid soils as Andisols, demonstrating its effectiveness to ameliorate Al^{3+} (Mora *et al*., 1999; Toma *et al*, 2005; Takahashi *et al*., 2006).

An agronomical practice carried out on acid soils, for ameliorating Al^{3+}stress, and improve Ca, and other nutrients in plants, is the addition of calcareous amendment such as $CaSO_4$ (Ritchey and Snuffer, 2002; Toma *et al*, 2005; Takahashi *et al*., 2006). Calcium sulfate can reduce Al^{3+} without altering soil pH necessary for development of some crops. Chemical and physical properties of Andisols, have enabled blueberry development, which is well adapted to soil pH ranges from 4.5 to 5.2 and high organic matter (OM) content >5% (Sanderson *et al.*, 1995; Trehane, 2004). However, it has been reported that excessive exchangeable Al^{3+} concentration

in these soils would cause toxicity in blueberries, decreasing root and shoot growth, nutrient uptake, and photosynthesis processes, mainly in Al-sensitive cultivars (Blatt and McRae, 1997; Reyes-Díaz *et al.*, 2009, 2010, 2011).

With over 10,000 hectares (ha) and 55,000 tons (t) production, highbush blueberry (*Vaccinium corymbosum* L.) has become an economically important fruit crop in Chile (Espinoza *et al.*, 2009; ODEPA, 2011), because it is a rich source of antioxidant compounds such as phenols, anthocyanins and flavonoids, with nutritional properties for human health (Prior *et al.*, 1998; Dragović-Uzelac *et al.*, 2010, Ribera et al., 2010).

For some woody species grown in nutrient or soil solution was possible to establish the better molar relation among Ca/Al ratios (Álvarez *et al.*, 2005; St Clair *et al.*, 2005). Therefore, it would be also possible to consider the effects of this ratio in a woody fruit species as blueberry grown in an Andisol amended with calcium sulfate. Thus, the aim of this research work was to study Ca/Al molar ratio ranges that allow a better nutrient balance and Al detoxification reflected by photosynthetic and antioxidant performances in calcium sulfate amended blueberry cultivars grown in an Andisol of Southern Chile.

1.2 HYPOTHESIS

The improvement of Ca/Al ratio in highbush blueberry grown in an Andisol amended by calcium sulfate will increase the efficiency of physiological and biochemical features associated with oxidative stress.

1.3 GENERAL OBJECTIVE

To study the effects of Ca/Al ratio on physiological and biochemical processes in highbush blueberry cultivars grown in a calcium sulfate amended Andisol.

1.4 SPECIFIC OBJECTIVES

1) To evaluate the effect of Ca/Al ratio on oxidative stress levels in leaves and roots of highbush blueberry under Al-toxicity.

2) To compare enzymatic and non-enzymatic antioxidant capacity of highbush blueberry under Al-toxicity at increased Ca/Al ratio.

3) To determine physiological performances in two highbush blueberry cultivars subjected to Al-toxicity and at increasing Ca/Al ratio.

Chapter 2. *Al^{3+}— Ca^{2+} Interaction in Plants Growing in Acid Soils: Al-Phytotoxicity Response to Calcareous Amendments*

Paper published in Journal Science and Plant Nutrition 10(3): 217 – 243 (2010)

Al^{3+}-Ca^{2+} INTERACTION IN PLANTS GROWING IN ACID SOILS: Al-PHYTOTOXICITY RESPONSE TO CALCAREOUS AMENDMENTS

C. Meriño-Gergichevich[1], M. Alberdi[2], A. Ivanov[3], and M. Reyes-Díaz[2]

[1]Programa de Doctorado en Ciencias de Recursos Naturales, Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera, Temuco, Chile

[2]Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Casilla 54-D, Temuco, Chile

[3]Department of Biology and the Biotron, University of Western Ontario, London, Ontario, N6A 5B7 Canada

ABSTRACT

High aluminum (Al) concentrations as Al^{3+} represent an important growth and yield limiting factor for crops in acid soils (pH $\leq$ 5.5). The most recognized effect of Al-toxicity in plants is observed in roots. However, damages in the upper parts (including stem, leaves and fruits) may also be present. In addition, Al-toxicity triggers an increase in reactive oxygen species (ROS), causing oxidative stress that can damage the roots and chloroplasts, decreasing normal functioning of photosynthetic parameters. Al-toxicity may also increase or inhibit antioxidant activities, which are responsible to scavenge ROS. As result of the negative effects of toxic Al, root metabolic processes, such as water and nutrient absorption, are disturbed with a concomitant decrease in calcium (Ca) uptake. Calcium plays a fundamental role in the amelioration of pH and Al-toxicity through Al-Ca interactions improving physiological and biochemical processes in plants. Calcium is a useful amendment for correcting these negative effects on crops growing in acid soils. This is an agronomic practice with alternatives, such as limestone or gypsum. There is little information about the interaction between amendments and Al-toxicity in physiological and biochemical processes in crops. Thus, the main objective of this

review is to understand the interactions between Al^{3+} and Ca amendments and their effects on the physiology and biochemical responses in crops growing in acid soils.

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

2.1 INTRODUCTION

Andisols are acid soils developed from volcanic ashes materials (Nanzyo *et al.*, 1993; Iamarino and Terribile, 2008), comprising a pH $\leq$ 5.5 range (Samac and Tesfaye, 2003), a high organic matter (OM) content (Mora *et al.*, 2002; Takasu *et al.*, 2006a), low phosphorus (P) availability, as well as low Ca and magnesium (Mg) contents (Kleber and Jahn, 2007; Mora *et al.*, 2007), and high levels of extractable Al and manganese (Mn) (Toma and Saigusa, 1997).Under acidity conditions, Al-toxicity is the main stress factor for cropped plants (Poschenrieder *et al.*, 2008; Ryan and Delhaize, 2010), which could limit their production by alterations in physiological and biochemical processes (Jones and Kochian, 1997; Mora *et al.*, 1999). The decrease in root growth is the most initial and evident symptoms of Al-toxicity, which inducing a reduced capacity for water and nutrient uptake (Rengel and Zhang, 2003), although functions in upper organs also may be affected (Reich *et al.*, 1994; Peixoto *et al.*, 2002). Toxic Al triggers an overproduction of oxygen reactive species (ROS) (Blokhina *et al.*, 2003; Ma, 2005), which alters the functionality of biomembranes, favoring oxidative damage in plants (Bóscolo *et al.*, 2003; Guo *et al.*, 2006). The scavenging of ROS in plants can be regulated by enzymatic and non-enzymatic antioxidant systems (Shao *et al.*, 2008).

To overcome the limitations of Al-toxicity, Ca amendments are common agronomic practices used to reduce Al-toxicity, to restore Ca and Mg availability for plants, and adjust soil acidity (Toma and Saigusa 1997; Mora *et al.* 2002), by different economically viable options, such as limes, gypsum, and phosphogypsum (PG) (Campbell *et al.*, 2006; Takahashi *et al.*, 2006a,b). There are several studies reporting the Ca ameliorative effect on Al-toxicity in crops growing in acid soils, as

soybean (*Glycine max*) (Caires *et al.*, 2006; Bachiega *et al.*, 2007), wheat (*Triticum aestivum*) (Caires *et al.*, 2002), coffee (*Coffea arabica*) (Hue, 2005), tomato (*Lycopersicon esculentum*) (Tuna *et al.*, 2007), among others. Although the effects of different Ca amendments on physical and chemical properties of acid soils are well documented, limited information is available about the effectiveness of calcareous amendments on plant physiological and biochemical processes, such as water and nutrient uptake, photosynthesis, and antioxidant systems. Therefore, the aim of this review is to summarize the effects of Al-toxicity in plants, the interactions between toxic Al and different Ca amendments and their effects on the physiological and biochemical responses in cultivated plants growing in acid soils.

2.2 GENERAL CHARACTERISTICS OF ANDISOLS

2.2.1 Andisols and their properties

Andisols are typical soils developed from volcanic materials (Takahashi *et al.*, 2007), covering from 110 to 124 million hectares (ha) at worldwide (Sparks, 2004). These soils generally possess excellent physical properties such as low bulk density (<0.90 Mg m^{-3}), high permeability, and high water-holding capacity. In addition, are unique in terms of their aggregate structure, with well-defined and stable intra- and inter-aggregate spaces (Takahashi *et al.*, 2007; Iamarino and Terribile, 2008). Chemically, Andisols are characterized by high phosphate sorption capacity, acidity, Al and Mn toxicity (mainly non-allophanic Andisols), and are rich in cations such as Ca, Mg, potassium (K) and sodium (Na) (Nanzyo *et al.*, 1993; Kameyama and Miyamoto, 2008).

2.2.2 Aluminum forms in acid soils

According to Kochian *et al.* (2005), Al is the third most abundant element in the earth's crust, comprising about 7% of the total mass of the earth (Delhaize and Ryan, 1995; Zhang *et al.*, 2007). Yakimova *et al.* (2007) considered that Al is one of the most abundant toxic elements with ability to pollute soil, water and trophic

chains. Nonetheless, the specific biological functions of Al for animals and plants are still unknown, and so it is not regarded as an essential nutrient (Poschenrieder *et al.*, 2008). Fortunately, in agronomic terms, most of the Al is bound to insoluble forms such as aluminosilicates and/or precipitated as Al-hydroxide-sulfate, being solubilized from silicates and oxides (not toxic forms) to Al^{3+}, which is phytotoxic only under conditions of low pH (Delhaize and Ryan, 1995; Takahashi *et al.*, 2006a,b; Wang *et al.*, 2006).

In acid soils, Al has been reported in several forms, as monomer, polymer, and solid phase, which their concentration will depend on of the degree and duration of Al compounds hydrolysis (Alva *et al.*, 1991; Delhaize and Ryan, 1995) (Figure 2.1). Under high acidity ($pH \leq 5.0$) the trivalent Al species $[Al(H_2O)_6]^{3+}$ dominate and are generally referred to Al^{3+}. With increase of pH, $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ begin to appear (Lidon and Barreiro, 2002; Abreu *et al.*,2003), Langer *et al.* (2009) showed that these forms were toxic for soybean [*Glycine max* (L.) Merrill]. These two monomeric species are not generally considered toxic for many plants species. Under conditions of soil neutrality, the $Al(OH)_3$ or gibsite maintains a high presence, while aluminates $Al(OH)^{4-}$ are predominant in alkaline soil (Kochian, 1995; Mossor-Pietraszewska, 2001). These authors also noted that another polymeric complex of Al [$AlO_4\ Al_{12}\ (OH)_{24}\ (H_2O)_{12}^{7+}$ or (Al_{13})] together with Al^{3+} are the most toxic forms for plants under acidity conditions.

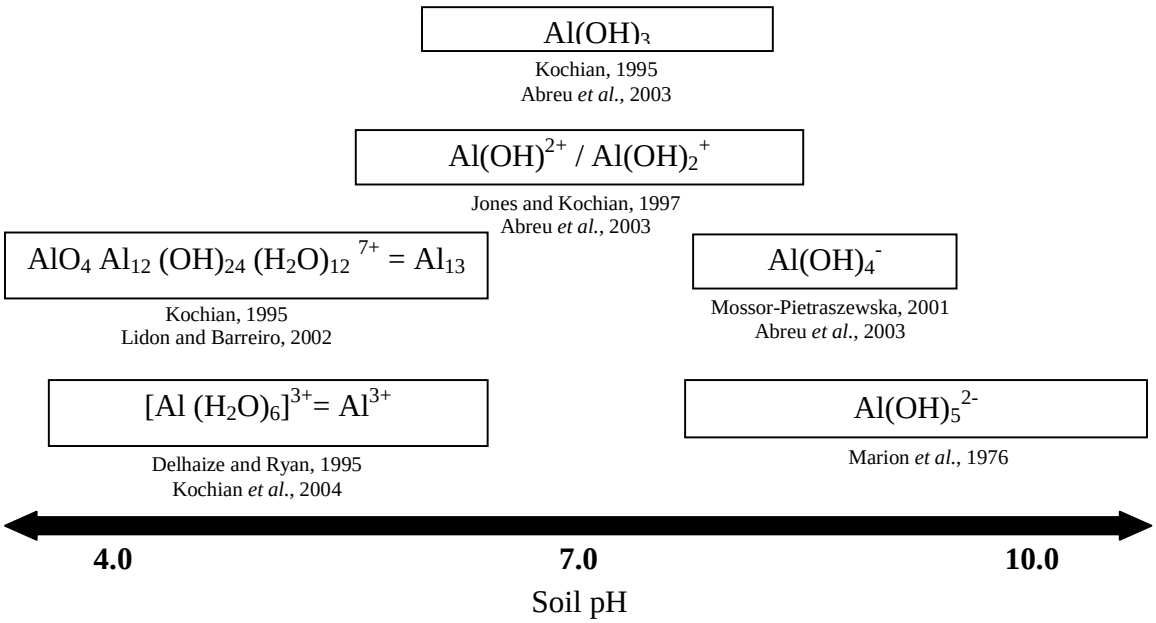

Figure 2.1. Monomeric and polymeric forms of Al and pH of soil according to various authors. When pH is lower than 5.5, soluble forms available for plants are predominant. Al^{3+} and [AlO_4 Al_{12} $(OH)_{24}$ $(H_2O)_{12}{}^{7+}$] (Al_{13}), Al species are the most toxic to plants.

Rout *et al.* (2001) found a significant correlation between low pH and high concentrations of phytotoxic Al, which is related to the reduction of exchangeable bases in the soil solution (Mora *et al.*, 2006). Soil acidification is associated with inappropriate agricultural practices (Rengel, 1996), heavy winter rainfalls that causes the loss of bases (Na^+, K^+, Ca^{2+}, Mg^{2+}) due to leaching (Mora *et al.*, 2006), use of ammoniacal fertilizer (urea) and nutrient uptake by plants (Mora *et al.*, 2006). Hede *et al.* (2001) reported that the mineralization of OM may also contribute to Al-toxicity, resulting in a hydrogen ions (H^+) release to soil environment reducing its pH, which determine an increase of Al^{3+} release.

2.2.3 Detrimental effects of aluminum on plants

Stressful conditions of Al-toxicity on plants, and hence a constraint on their agricultural production, occurs in approximately 40% of arable soil of planet (Mossor-Pietraszewska, 2001; Ma *et al.*, 2002; Ryan and Delhaize, 2010), where are

several studies demonstrating that metabolic and morphological damages caused by toxic Al in plants are expressed by a dramatic decrease in productivity (Hoshino *et al.*, 2000). Wang *et al.* (2006) rewieved that there was more than USD 600 million in estimated Al-toxicity-related losses in the agricultural sector in Australia during first years of the 21st century (with large areas of acid soils with a high content of toxic Al). Mora *et al.* (2006) reported a poor quality and forage production in a pasture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), due to high Al^{3+} concentrations in tissues, impacting in higher risk of agricultural losses, including weight gain in livestock.

The first and most recognized effect of Al-toxicity in plants is the inhibition of division and elongation of meristematic cells and, thereby the reduction in root growth (Panda *et al.*, 2003; Mora *et al.*, 2006). In susceptible species to Al presence, symptoms of root damage have been linked to Al binding with carboxylic groups of pectins, interruption in the synthesis of cellulose and/or accumulation of callose (1,3-*β*-glucan) (Lidon and Barreiro, 2002), by interference with the enzymes involved in biosynthesis of cell wall polysaccharides which results in elevated rigidity of the cell wall (Wang *et al.*, 2006). These roots becoming thinner and darker, resulting in lower efficiency for water and nutrient absorption; being more pronounced in seedlings than in adult plants (Foy *et al.*, 1978). It has been reported that Al inhibits the absorption of nutrients as Ca and Mg, and available P (Wang *et al.*, 2006; Poschenrieder *et al.*, 2008). Despite the number of reports on various effects of Al-toxicity in roots, there is a lack of detailed information about its effects on the structural integrity and functional performance of photosynthetic apparatus (Lidon and Barreiro, 2002; Poschenrieder *et al.*, 2008) and the fact that Al may inhibit photosynthesis efficiency in some species (Reyes-Díaz *et al.*, 2009). In relation to photosynthetic efficiency (PE) of a unicellular green alga *Euglena gracilis* (strain Z, Department of Plant Physiology, University of Lund, Sweden), this process was reduced after short-term exposure (1h) at 15.0 mg Al ($AlCl_3$) L^{-1}, although in long-term (7 days) experiments, PE was partially recovered (Danilov and Ekelund, 2002). Moreover, Al-toxicity also affected the transpiration rate by reducing stomatal aperture (Kumar Roy *et al.*, 1988; Wang *et al.*, 2006).

Many plants have no more than 0.2 mg Al g^{-1} dry weight (DW) in their leaves, because the translocation of Al to the upper parts of plant is very slow, but plants species such as tea [(*Camellia sinensis* (L.) O. Kuntze] may contain up to 30 mg Al g^{-1} DW in old leaves, identified as one of the 400 accumulator species of this toxic metal (Mossor-Pietraszewska, 2001; Han *et al.*, 2007; Poschenrieder *et al.*, 2008). Among others, rye (*Secale cereal* L.), cranberries (*Vaccinium spp*.), and some members of the Proteaceae family have also been described as effective accumulators of toxic metals (Bakker *et al.*, 2000). In nutrient solution experiments, Al concentrations at micromolar range (25 - 1,600), have been sufficient to induce morphological and physiological damages in some crops (Rengel, 1996). According to Pan *et al.* (2001), Al could induce a programmed cell death in barley (*Hordeum vulgare* L.) roots after eight hours of exposure to Al treatment (0.1-50 μM) due to the presence of ROS.

2.3 CALCIUM AND ALUMINUM IN PLANTS AND THEIR INTERACTIONS

2.3.1 General role of calcium in plants

From ancient times, Ca plays a key role as essential nutrient in plants (White and Broadley, 2003), being related as a regulator of growth plant and development of root and stem (White and Brodley, 2003; Hepler, 2005), and a lot of metabolic functions/pathways (Plieth, 2005). As divalent cation, Ca^{2+} plays a structural component of cell walls and plasma membranes, forms ionic and covalent bonds with carboxylates of pectins in the polysaccharide matrix of cell wall, which is relevant for growth and texture of plants, fruits and mature vegetables (Poovaiah *et al.*, 1988; Gilroy *et al.*, 1993). Hepler (2005) reported about the antagonistic interaction between Ca and indol acetic acid (IAA), where this acid is a chelator of Ca and Mg, allowing cell division and cell elongation. However, several reports provide evidence on the inhibition of cell elongation by Ca does not prevent to IAA from stimulating cell wall synthesis. In plasma membrane, Ca interacts with phospholipids bilayer, providing stability and structural integrity, thus controlling its permeability by

interactions between phospholipids and membrane proteins (Poovaiah *et al.*, 1988; Hepler, 2005).

On the other hand, Ca participate in extra- and intracellular signaling (Yocum, 2008), by regulation of enzymatic activities (Cheng *et al.*, 2002), in both chloroplast and mitochondria, and produces electrochemical potential (Ryan *et al.*, 1997). Is involved as a secondary messenger in various signal transduction pathways in eukaryotic cells (Sanders *et al.*, 2002; Silva *et al.*, 2005), and is modulated at intracellular level in response to many signals such as hormones, light, mechanical disruption, abiotic and biotic stress (Cheng *et al.*, 2002; Sanders *et al.*, 2002). Also, Ca is involved in photosynthesis process, as activator of Mn redox chemistry, that culminates in the release of O_2 from photosystem II (PSII) during water splitting (Homann, 2002; Yocum, 2008), a process that could be inhibited by substitution of other cations such as K^+ (Ono *et al.* 2001). However, Ca deficiency in sugar beet (*Beta vulgaris* L. cv. F58-554H1) had no effect on leaf carbon dioxide (CO_2) uptake, photoreduction of ferricyanide, or ATP formation via cyclic and non-cyclic electron transport in chloroplast (Terry and Huston 1975). Whereas Homann (2002) and Yocum (2008) reported that PSII contains a set of intrinsic proteins such as Psb A, B, C, D, E, and F, that could be restored by Ca action.

2.3.2 Physiological interactions of Ca and Al in plants

It is accepted that first plant responses to Al^{3+} damage appear in roots, resulting in a decreased nutrient uptake (Wang *et al.*, 2006). Although, effects of Al^{3+} in roots have been intensively studied, on leaf structure and functions of photosynthetic machinery are little understood. In this way, some reports have indicated that Al-induced leaf necrosis (Kumar Roy *et al.*, 1988; Zhang *et al.*, 2007), leaf yellowing (Foy, 1984), stunted leaves (Wang *et al.*, 2006), and late leaf maturity (Rout *et al.*, 2001). So, these changes were accompanied by a reduction in chlorophyll contents (Wang *et al.*, 2006), photosynthesis rate (Reich *et al.*, 1994), and abnormal chloroplast structure (Akaya and Takenaka, 2001; Peixoto *et al.*, 2002). The Ca/Al molar relation is strongly associated with growth and development in a wide variety of plants (Cronan and Grigal, 1995; Schaberg *et al.*, 2006). This interaction

has been related to the toxicity that Al exerts on plants, which is principally mediated by Ca^{2+} deficiency (Álvarez *et al.*, 2005). A low Ca/Al molar ratio resulted in a reduced photosynthetic capacity and increased respiration in Scots pine (*Pinus sylvestris* L.) grown in polluted acid soils of east Europe (Reich *et al.*, 1994). By contrast, Ca addition alleviates the toxic effects caused by Al^{3+} (Rout *et al.*, 2001; Rengel and Zhang, 2003). This interaction is the factor that must affect Ca uptake and translocation in plants growing in acid soils (Mossor-Pietraszewska, 2001). It has also been shown that Al excess competes or inhibits Ca and/or Mg absorption capacity, which affects normal plants development (Watanabe and Osaki, 2002; Silva *et al.*, 2005). According to Delhaize and Ryan (1995) and Kochian (1995), three mechanisms are proposed to explain Al-Ca interactions, as follows:

2.3.2.1 Inhibition of Ca^{2+} transport via symplasm by Al^{3+}

The surface charge of plasma membranes and trans-membrane potential can be modulated by Ca^{2+}-channel activity, regulating ion transport and other processes (Rengel and Zhang, 2003). Is known that Al^{3+} affect cell membranes structure and their permeability by blocking the Ca^{2+}-channels, inhibiting influx of divalent and monovalent cations into cells, but it stimulates the anion cell influxes (Ryan and Kochian, 1993; Plieth, 2005). Binding of Al^{3+} to plasma membrane phospholipids and transport proteins, reduces the net negative membrane surface charge, permitting the movement of anions and restricting that of cations (Huang *et al.*, 1992) (Figure 2.2A). Therefore, alleviation of Al^{3+} toxicity through Ca^{2+} addition causes a reduction in the negative potential of plasma membrane, leading to a drop in the electrostatic attraction of the toxic Al^{3+} (Kinraide, 1998). Rengel (1992a), reviewed the mechanism of Al inhibition of net $^{45}Ca^{2+}$ uptake in *Amaranthys tricolor*, and concluded that Al ions affected net uptake by blocking of Ca^{2+}-channel (*i.e.* by binding to the verapamil-specific channel-receptor site), as well as by interfering with the action of the GTP proteins involved in the regulation of transmembrane Ca^{2+} fluxes. However, they not reported effects of Al on the plasma membrane Ca^{2+}-ATPase. Rengel (1992b) suggested that Al may disturb the symplasmic Ca^{2+} homeostasis by altering the Ca^{2+} flux pattern across plasma membrane, triggering an

excessive increase in cytosolic Ca^{2+}. Huang *et al.* (1992) evaluated Ca^{2+} transport mechanism in two wheat cultivars with contrasting tolerance to Al^{3+}, grown in nutrient solution (pH 4.5). In the sensitive cultivar, Al^{3+} induced an inhibition of Ca^{2+} uptake by blocking of Ca^{2+}-channels in root plasmalemma, indicating that Al effects did not involve Al-Ca interactions in the cell wall (Figure 2.2A). Lindberg and Strid (1997) reported, in two cultivars of wheat (Al-tolerant and Al-senitive), subjected to a concentration of 50 μM of Al (as $AlCl_3$), when Al was added caused reduction of cytosolic pH and free $[K^+]$ and $[Ca^{2+}]$ of root cell, and afterwards plants were removed from the Al treatment, where cytosolic pH was recovery only in Al-tolerant cultivar, caused by a hyperpolarization and depolarizations of transmembrane electrical potential of root cells.

2.3.2.2 Disruption of Ca^{2+} homeostasis in cytoplasm by Al^{3+}

Cytosolic Ca $[Ca^{2+}]_{cyt}$ in eukaryotes plant cells is approximately 100-200 nM, whereas in apoplastic fluid and some cellular organelles, $[Ca^{2+}]_{cyt}$ is 10^4 to 10^5 times higher (Hepler, 2005; Lecourieux, *et al.*, 2006). Calcium intake to cells through permeable ion channels, in the plasma membrane (White and Broadley, 2003), is gated by voltage changes, stretch and ligands such as IP3, cADP-R, glutamate, G proteins, among others (Rengel and Zhang, 2003). The $[Ca^{2+}]_{cyt}$ plays an important role as a regulator of cell expansion and division, and it is known that its changes under controlled homeostasis permit cell viability (Bush, 1995; Jones *et al.*, 1998). At very low external pH, the related control mechanisms cannot avoid a decrease in cytosolic pH; then the plant activates responses as an increase in Ca^{2+} free concentrations in cytosol (Plieth, 2005). Under acid conditions, exposure to Al^{3+} generates a disturbance of pH homeostasis and $[Ca^{2+}]_{cyt}$ (Ma *et al.*, 2002), which affects metabolic processes, such as cell division and elongation (Zhang and Rengel, 1999) (Figure 2.2B). Ma *et al.* (2002) correlated increases in cytosolic Ca^{2+} (46%) of rye root tip cells under two Al concentrations (50 and 100 μM), with a slight increase in Ca^{2+} at 50 μM and 100 μM. Aluminum 100 μM triggered an inhibition of root growth after two hours of exposure. These disruptions are generally believed to be a primary trigger of Al-toxicity, because an increase in $[Ca^{2+}]_{cyt}$ activate synthesis of

callose (1,3-β-glucan) in plant cells, which is accompanied by strong reduction of root elongation (Ma *et al.*, 2002). Other authors have indicated that this homeostasis disruption leads to an inhibition of Ca^{2+}-dependent signal transduction, affecting cell division and cell elongation (Rengel, 1992a; Kochian, 1995; Jones *et al.*, 1998).

2.3.2.3 Ca^{2+} displacement by Al^{3+} from apoplast

The importance of Ca in cell wall structure is due to its role in the interaction of Ca^{2+}-pectate, as a regulator of growth and antagonism with IAA (Hepler, 2005). Cell wall components and intercellular spaces are critical sites in the apoplast, as this is the first contact site between roots and potentially toxic Al species in a soil solution (Ryan *et al.*, 1997; Rengel and Zhang, 2003). In plants growing in acid soils, Al^{3+} reacts with these cell wall components in roots, particularly with Ca^{2+}-pectate (Blamey *et al.*, 1997), where Al binds to carboxyl groups (Rengel, 1996; Poschenrieder *et al.*, 2008). Ma *et al.* (2007) mentioned that about 85-99% of total Al, corresponds to apoplastic content, and Ca^{2+} is displaced from negative binding sites by Al^{3+} in the apoplasm (Kinraide, 1998), because Al^{3+} binds more strongly than Ca^{2+} to pectin, a major constituent of cell walls (Rengel and Zhang, 2003) (Figure 2.2C). The displacement of pectin-bound Ca^{2+} would inevitably alter physical properties of cell wall such as extensibility, rigidity, and permeability (Horst, 1995).

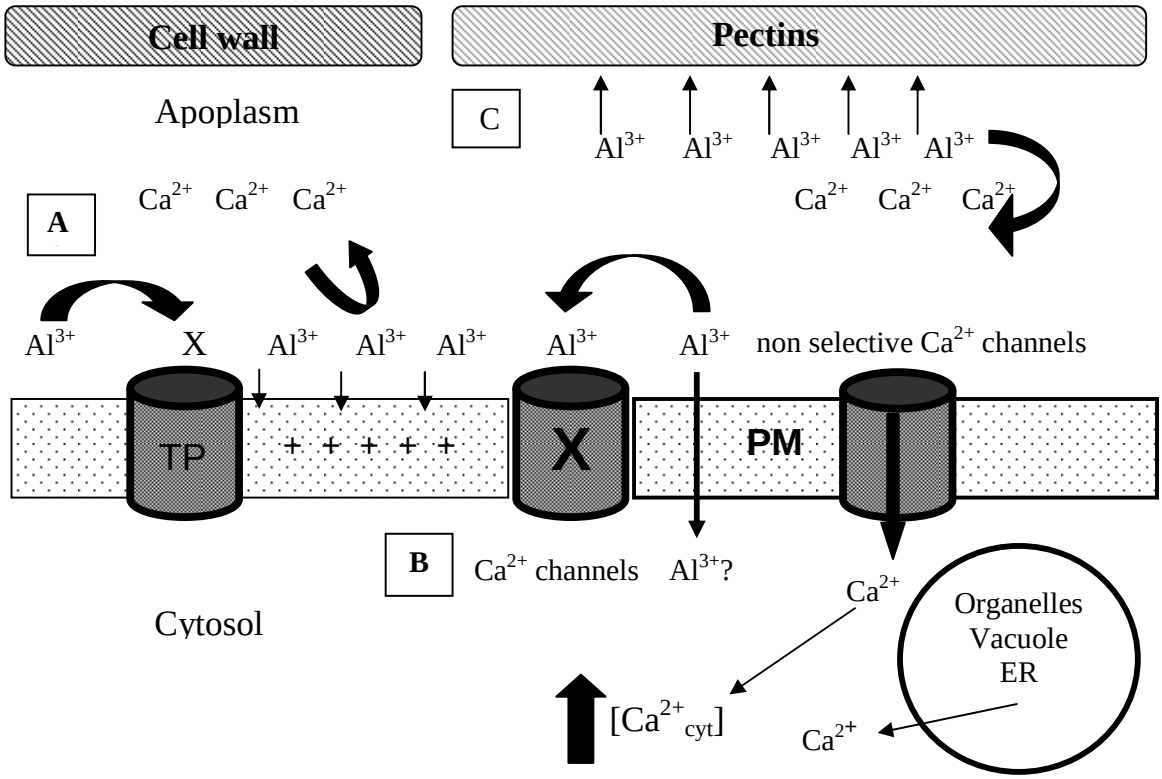

Figure 2.2. Three mechanisms proposed of Ca-Al interactions at cellular level. (A) Inhibition of Ca^{2+} transport via symplasm by Al^{3+}, (B) Disruption of Ca^{2+} homeostasis in cytoplasm by Al^{3+} and (C) Ca^{2+} displacement by Al^{3+} from apoplasm. (ER) Endoplasmatic reticulum, (PM) plasma membrane, (TP) transports protein.

2.3.3 Biochemical Ca and Al interaction in plants

Under normal conditions, physiological processes in higher plants produce relatively small amounts of ROS through successive reduction of O_2 to H_2O, which are a constant threat produced by photosynthetic organisms (Scandalios, 2002; Mittler *et al.*, 2004; Khan *et al.*, 2007). The term ROS includes free radicals as superoxide anion ($*O_2^-$) and hydroxil (*OH), and oxidant molecules such as H_2O_2, singlet oxygen ($^1O_2$) and ozone (O_3) (Guo *et al.*, 2004; Khan *et al.*, 2007). The main sources of ROS in plants are organelles with a highly oxidizing metabolic activity or with an intense rate of electron flow, such as chloroplasts, mitochondria, and peroxisomes (Yamamoto *et al.*, 2002; Mittler *et al.*, 2004). Several biotic or abiotic environmental

stresses induce an increase in ROS production in plants (Foyer and Noctor, 2005), which can cause oxidative damage in different biomolecules, such as lipids, proteins, and nucleic acids (Yamamoto *et al.*, 2003; Guo *et al.*, 2004; 2006).

Heavy metals are an important abiotic factor of environmental stress, that increases ROS production and oxidative stress in plants (Tamás *et al.*, 2005). In acid soils (such as Andisols), high Al^{3+} concentration can triggers an enhancement of ROS in plant cells, accompanied by a strong correlation with oxidative stress (Ma, 2005; Ma *et al.*, 2007). Although, Al itself is not a transition metal and cannot catalyze redox reactions, it is probably has a pro-oxidant function through $*O_2^-$ formation (Yamamoto *et al.*, 2003; Tamás *et al.*, 2005). Aluminum binding to phospholipids induces a rigidity of membranes, facilitates a free radical chain reaction, mediated by Fe (Yamamoto *et al.*, 2002). Therefore, prolonged exposure to Al may be accompanied by enhanced peroxidation of phospholipids and membrane proteins, which could to leading to cell death (Pan *et al.*, 2001; Panda *et al.*, 2003; Šimonovičová *et al.*, 2004). Meriga *et al.* (2004) reported that the primary target of Al-induced increases of ROS in plasma membranes, causing increased peroxidation of phospholipids and proteins. Bóscolo *et al.* (2003), reported to this trivalent ion can induce death in root tip cells in Al-sensitive maize (*Zea mays* L.) cultivars, by increasing the amount of ROS and protein oxidation. Also, they reported controversial results about activities of antioxidant enzymes involved in ROS scavenging.

A number of studies have reported that in parallel to increased ROS levels, Al stress also would induces an enhancement in the antioxidant activity to overcome the deteriorating effects of these toxic species, and improve plant tolerance to Al stress (Guo *et al.*, 2006; Khan *et al.*, 2007). Okamoto *et al.* (2001) reported that tolerance of photosynthetic organisms is mainly related to defense systems rather than prevention of oxidative damage. The ROS scavenging and elimination in plants is regulated by non-enzymatic and enzymatic antioxidant systems, which are highly compartmentalized (Shao *et al.*, 2008). The antioxidant set includes enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX; EC 1.11.1.11), peoxidase (POD; EC. 1.11.1.7), and glutathionereductase (GR), among

others (Ma *et al.,* 2007). In roots of intact plants and cultured cells of *Camelia sinensis* subjected to Al treatments, Ghanati *et al.* (2005) enhanced activities of SOD, CAT, and APX. However, Guo *et al.* (2004) showed greater antioxidative ability in barley root cells subjected to 100 µM Al, when Ca (3 mM) was added to nutrient solution. On the other hand, several non-enzymatic molecular compounds have been reported to play effective antioxidant role such as: ascorbate and glutathione in hydrophilic conditions (Shao *et al.,* 2008), α-tocopherol (Foyer and Noctor, 2005), vitamins C and E (Huang *et al.,* 2005), β-carotene (Stahl and Sies, 2003), (poly) phenols (Prior *et al.,* 2005), salicylic acids (SA), and flavonoids as flavones, isoflavones, flavonones, anthocyanins, and catechins (Mittler *et al.,* 2004; Prior *et al.*,2005).

In plant tolerance to different stresses, Ca^{2+} may also be involved by regulating of antioxidant metabolism (Jiang and Huang, 2001). Generally, Ca^{2+} is considered to act as a secondary messenger in the oxidative stress response plants. Both, Ca^{2+} and Ca^{2+}-binding proteins such as calmodulin, are involved in signaling events associated with ROS sensing through the activation of G proteins and the activation of phospholipids signaling, which results in the accumulation of phosphatidic acid (Foyer and Noctor, 2005). The localization of ROS signals, in specific cell sites, may be similar to that of Ca^{2+} signals in response to many stimuli (Mittler *et al.,* 2004). A cross-talk between Ca^{2+} and ROS originating from cell membrane-bound-NADPH oxidase is also involved in abscisic acid ABA-dependent signal transduction, inducing an effective antioxidant defense and enhancement of antioxidant enzyme as SOD, CAT, APX and GR activities (Jiang and Huang, 2001).

As mentioned above, the amelioration of Al-toxicity has been related to the use of cations such as Ca and Mg (Guo *et al.,* 2004), but the information related to Ca-Al interaction and their effect on antioxidant activity in plants is scarce. In this context, Guo *et al.* (2006) found a stimulated antioxidant enzymatic activity of SOD, POD, and CAT, by Ca addition on barley plants exposed to Al (100 µM). Furthermore, short-term Al-toxicity (5 mM) was mitigated by Ca and Mg addition, by enhancement of antioxidant enzyme activities in Japanese cedar needles (*Cryptomeria japonica* D. Don) growth in acidified nutrient solution (Takami *et al.*

2005). Also, they suggested that Ca/Al ratio was correlated with an increased SOD and CAT activity either with short-term or long-term treatments. Indeed, Mora *et al.* (2008) reported enhanced antioxidant enzymes (POD and APX) application of lime (Calcitic) and P, improving the nutrition, thus, increasing the dry matter and yield of white clover (*Trifolium repens* L.) growing in Andisols. External Ca addition on two grass species, tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass (*Poa pratensis* L.) growing in nutrient solution, increased CAT, APX, and GR activities, reduced lipid peroxidation (LP), raised relative water content (RWC), and improved chlorophyll (Chl) content in leaves (Jiang and Huang, 2001). Also, in maize seedlings Ca^{2+} treatment ($CaCl_2$) allowed to keep relatively higher SOD, CAT, and APX activities, and lower LP levels compared to the non-treated seedlings (Gong *et al.*, 1997). In contrast, other authors report reduction in antioxidant enzyme activity due to Ca addition. For hooky cypress (*Chamaecyparis obtusa*), Ogawa *et al.* (2000) showed that high Ca concentration (5, 12.5, and 25 mM) suppressed antioxidant activity of SOD and CAT, in comparison to Al treatment (5 mM) without Ca treatment. In addition, these authors reported that in parallel to decrease in Ca/Al, there was an increase in activity of these enzymes.

2.3.4 Alleviation of Al-toxicity by Ca^{2+}

There are many reports about the ameliorative effects of Ca on Al-toxicity in different crops growing in acid soils. Several studies have shown that soil pH increases after application of Ca amendments due to the displacement of Al^{3+} and H^{+} by Ca^{2+} from the exchange sorption sites in soil solution (Alva and Sumner, 1988; Mora *et al.* 1999; Mora *et al.*, 2002). It has been shown, that Ca has a beneficial effect on root hair growth and elongation in soybean plants affected by Al^{3+} toxicity (Sanzonowicz *et al.*, 1998). It has also been recognized that Ca/Al molar ratio is a good indicator of Al stress in nutrient solutions (Akaya and Takenaka, 2001; Ritchey and Snuffer, 2002), and may be used to predict acidity effect on plant growth and development. Indeed, it has been demonstrated that there was a 50% risk on Japanese cedar trees growth´s when Ca/Al ratio in soil solution was below 1.0 (Takami *et al.*, 2005). Furthermore, this indicator may not be quite useful for Andisols due to its high OM content, which could be complex Al ions (Takahashi *et al.*, 2006b). Moreover, a

higher concentration of Al in soil solution resulted in reduced foliar concentrations of Ca, Mg, Mn, and Zn, net photosynthesis and cellular respiration, and decreased shoots biomass in red spruce (*Picea rubens* Sarg.) (Schaberg *et al.*, 2000). Also Watanabe and Osaki (2002) reported negative relationships between Al accumulation and other essential minerals in leaves. In addition, Ca played an important role on growth, yield, and fruit quality in different crops such as melon (*Cucumis melo* L.) (Takasu *et al.*, 2006a), highbush blueberry (*Vaccinium corymbosum* L.) (Blatt and McRae, 1997), and yields in wheat (Caires *et al.*, 2002), in a mixed grassland with timothy (*Phleum pratense* L.) and red clover (*Trifolium pretense* L.) (Zheljazkov *et al.*, 2006), Brussels sprouts (*Brassica oleracea* var. gemmifera) (Carter and Cutcliffe, 1990), and perennial ryegrass (*Lolium perenne* L.) (Mora *et al.*, 1999; 2002). Kinraide (1998) reported the Ca ability to alleviate Al^{3+} toxicity at short-term by three proposed mechanisms: 1) Displacement of Al^{3+} by electrostatic effects on the cell surface, most probably by blocking plasma membrane channels to the toxic cation (Kinraide, 1998); 2) Restoration of Ca^{2+} on cell membrane surface with Ca addition, reducing negativity of surface for Al binding (Kinraide *et al.*, 2004); and 3) Interactions between Ca^{2+} and Al^{3+} (Silva *et al.*, 2005). Kinraide (1998) highlighted that in order to inhibit effect of Al^{3+} (1 µmol L^{-1}) a Ca^{2+} concentration approaching 1 mmol L^{-1} is necessary.

2.3.5 Use of Ca as amendment in acid soils

Several studies report the beneficial Ca effects in different crops growing in acid soils (Mora *et al.*, 2002). Currently, Ca application is carried out through different practical alternatives (Campbell *et al.*, 2006; Takasu *et al.*, 2006a,b) including common liming oxides (CaO), hydroxides [$Ca(OH)_2$], silicates of Ca or Ca-Mg, carbonates as calcites ($CaCO_3$), dolomites [$Ca\ Mg(CO_3)_2$], and sulfate salts (Tisdale *et al.*, 1985), as mined gypsum and gypsum by-product such as PG, titanogypsum or red gypsum (Garrido *et al.*, 2003; Illera *et al.*, 2004). Liming is a very common practice, in approximately 22 countries, that are benefitting substantially from well established liming practices (Scott *et al.*, 2000) for restoring available Ca for plants (Mora *et al.*, 1999; 2002), correcting acidic soils (Scott *et al.*,

2000), amelioration of Al and Mn toxicity (Illera *et al.*, 2004), and avoiding metal leaching as cadmium (Cd), copper (Cu), and lead (Pb) in polluted soils (Campbell *et al.*, 2006).

In general, cultivated plant responses to Ca application are positively correlated with an increased DW yield both in shoot and roots, and quality improvement in different crops such as soybean (Caires *et al.*, 2006; Bachiega *et al.*, 2007), blueberries (Blatt and Mc Rae, 1997), wheat (Caires *et al.*, 2002), coffee (Hue, 2005), and tomato (Tuna *et al.*, 2007). However, its use is strongly dependent on the crop type, locality, deep requirement, soil texture, OM content, as well as pH, time, and/or frequency of liming, and the nature and cost of the amendment material (Schuman *et al.*, 1994; Takasu *et al.*, 2006a).

2.4 CALCAREOUS AMENDMENT TYPES

2.4.1 Limes (calcite and dolomite)

The major limestones are $CaCO_3$ and Ca Mg $(CO_3)_2$ (von Willert and Stehouwer, 2003), being $CaCO_3$ associated to various reaction steps with soil water: releasing Ca^{2+} which later can be up taken by roots or lost by water drainage (Tisdale *et al.*, 1985), and HCO^{3-} that increases soil pH (Scott *et al.*, 2000). The main direct benefits of limestone application is pH increase in soils, particularly those having levels below 5.0-5.5 (Mora *et al.*, 2006), and reduction of toxic Al and Mn concentrations (Hue, 2005; Caires *et al.*, 2006). Whereas, indirect benefits could be related to an increased effective cationic exchange capacity (CEC) (de Castro *et al.*, 1999), additional supply of Ca^{2+} as well as Mg^{2+}, if dolomitic limestone are used (Pavan *et al.*, 1984), and enhanced P availability by inactivation of Fe and Al complex (Tisdale *et al.*, 1985). Indeed, a reduction on P adsorption in two Chilean Andisols treated with calcitic and dolomitic limes has been reported by Mora *et al.* 1999. Also, are improved micronutrient availability with adequate range of liming (Tisdale *et al.*, 1985), and amelioration of the nitrification and nitrogen (N) fixation (Campillo *et al.*, 2005). Also, deeper incorporation of lime increases roots

development, resulting in an increased crop production (Sumner *et al.* 1986; Carvalho and van Raij, 1997). Hue (2005) reported that lime amendment was positively correlated with a good growth in roots of coffee plants grown in two acid soils (Andisol and Ultisol) during five months. Mora *et al.* (2002) found a reduction in weed presence in perennial pastures of ryegrass and white clover growing on Andisols. Redente and Richards (1997) added lime plus NPK fertilizer to polluted soils, increasing shoot and root biomass of wheatgrass (*Agropyron spicatum*), and reduced trace element availability and their concentrations in plant tissues. In this context, Garland and Wilkins (1981) found that barley roots subjected to Pb toxicity increased their biomass and length, when Ca concentration was increased from 12 to 72 mM in nutrient solution. Recently, Han *et al.* (2007) showed a significant reduction (20-50%) in Pb concentrations in roots and stems in tea plants growing in acid soils after second year of lime application. However, in watermelon (*Citrullus lanatus*) Locascio and Hochmuth (2002), showed that lime is not necessary for soils with low toxic elements such as Mn and Al, additional Ca from lime [0 to 4.5 ton per hectare (t ha^{-1})], since only a 10% increase was found in the first growing season and no effects for second growing season were found. On the other hand, application of dolomite (4 t ha^{-1}) in a European forest of *Picea abies* increased the development of fresh fine roots and decreased degraded lignin (Rosenberg *et al.*, 2003). Similar results were found earlier by Bakker *et al.* (1999) in a forest of sessile oak (*Quercus petraea* Lieb. M.) limed with $CaCO_3$.

Table 2.1. Effects of PG dose applications on different plant crops in acid soils.

Doses ($t\ ha^{-1}$)	Species	Effects	Authors
2.0	Alfalfa Soybean	Top and root growth Increasing Ca content in plant tissues Decreasing exchangeable Al	Alva and Sumner, 1990
2.5	Subterranean clover	Reduced Al^{3+} concentration in solution and at exchange sites (0-5 cm)	Smith *et al.*, 1994
2.0	Apple tree	Increased root density and decreased Al^{3+} (60 cm depth)	Pavan *et al.*, 1984
0.5	Sugar corn	Improvement of Ca, Mg and SO_4^{2+} uptake by plants Decreasing of Al level	Dam-ampai *et al.*, 2007
10.0	Barley Corn	Negative effects on photoactivity of chloroplast Increased chorophylls a, b, and a+b content	Krutilina *et al.*, 2000
4.0	Melon	Increased dry matter weights Top and root growth	Takasu *et al.*, 2006a

2.4.2 Phosphogypsum

Phosphogypsum by product is available in different regions of the world (Carvalho and van Raij, 1997). Its annual production is estimated to be 5 Mt in India, 27.2 Mt in United States (Florida) and nearly to 2.4 Mt in Brazil (Korcak, 1988; Carvalho and van Raij, 1997; Kumar, 2002). Is a primary by-product of phosphoric acid in the fertilizer industry, its chemical composition of PG varies depending on the phosphate rock used as source in production process (Korcak, 1988; Kumar, 2002). It contains mainly Ca^{2+} and SO_4^{2-}, as well as small amounts of other elements such as P, Si and fluoride (F) (Campbell *et al.*, 2006). Also PG contains trace amounts of barium (Ba), chromium (Cr), Cu, nickel (Ni), zinc (Zn), and some radionuclides not hazardous for handling (Garrido *et al.* 2003).

In agriculture, PG has been used as an alternative for Ca^{2+} and SO_4^{2-} addition for correcting both surface and deep acidity and Al-toxicity in acid soils such as

Andisols, Oxisols and Ultisols (Toma and Saigusa, 1997; Garrido *et al.*, 2003) (Table 2.1), via complex formation with F at pH 4.1-5.3 range (Alva and Sumner, 1988). Also, it can be used alone or in combination with other synthetic organic polymers for preventing runoffs and erosion in agricultural soils exposed to heavy rainstorms (Tang *et al*, 2006). The recommended amounts of PG for use in agriculture vary from 500 to 1000 kg ha^{-1} (Mays and Mortvedt, 1986). Alva and Sumner (1990) found that PG (2 t ha^{-1}) increased Ca content and growth of tops and roots in alfalfa (*Medicago sativa* cv. Hunter River) and soybean cv. Lee crops. These changes were attributed to amelioration of subsoil acidity (60 to 80 cm), ligand exchange among SO_4^{2-} and OH^-, and a decrease in exchangeable Al. In a subterranean clover (*Trifolium subterraneum* L.) pasture growing under acidic soil conditions, PG mixed with $CaCO_3$, and gypsum at rate 2,500 kg ha^{-1} reduced concentration of Al^{3+} in solution and on exchange sites at 0-5 cm, but no significant changes were observed deeper in the soil (Smith *et al.*, 1994). A similar experiment with a raised pH, in wheat var. Jabato growing under greenhouse conditions, was performed by Peregrina-Alonso *et al.* (2006). Pavan *et al.* (1984) showed effects of PG amendment to a depth of 60 cm, obtaining increased roots density and decreased Al in apple trees (*Malus domestica* L.) growing in Brazilian Oxisols. The improvement of Ca, Mg, and SO_4^{2-} uptake by plants and pH correction together with a decreased Al level in acid soil in Thailand were performed on sugar corn (*Zea mays* L.) crops by Dam-ampai *et al.* (2007), obtaining an enhanced DW. In wheat, Mariscal-Sancho *et al.* (2009) studied the effects of PG (1.4 to 84.2 t ha^{-1}) on biomass production and composition. They also found an increase in F, Al and Si at lower of PG rates (0 - 16.8 t ha^{-1}), but not at higher rates (67.3 t ha^{-1}). However, the highest PG rates increased plant Al and F contents, potentially reaching toxic levels for consumers. Similarly, culture of canola (*Brassica rapa* L. cv. Natsurakuten) in pots with an Andisol, were added with PG at 0.30, 0.75, and 1.50 g kg^{-1} over 33 days. Shoot and roots growth increased with a maximum increase of root fresh weight (FW) at dose 0.30 g kg^{-1}. This growth was significantly correlated with an improvement in Ca uptake (Takasu *et al.*, 2006b). Saigusa and Toma (1997) explained that Ca applied as PG had an average movement of 55% in subsoil related to 5% of lime in non-allophanic Andisols.

On the other hand, literature has reported negative toxic effects of PG on soils and plants production. Mays and Mortvedt (1986) applied 0, 22, and 112 t ha^{-1} in soils sown with corn, wheat, and soybean, to know the effects of PG on crop growth and uptake of Cd and radium (Ra). Where demonstrated that, corn production decreased at a high PG rate (112 t ha^{-1}), but wheat and soybeans were not affected. Nutrient elements, Cd, and Ra in grains and soils were not affected by PG application. These experiments revealed that PG origin has an influence on PG radioactive composition. Papastefanou *et al.* (2006) experimented with PG amendments and detected an increase in such radionuclides as radium-226 (^{226}Ra) (derived from a uranium series), from 50 to 479 Becquerel (Bq) kg^{-1} in evaluated rice (*Oryza sativa* L.), and recommended previous PG checking for agricultural purposes. According to the U.S Environmental Protection Agency, controlled PG use is permitted if ^{226}Ra levels are $\leq$10 pCi g^{-1} (Korcak, 1988). An interesting response of photosynthetic apparatus was found by Krutilina *et al.* (2000), when barley and corn seedlings were amended with PG (10t ha^{-1}). Negative effects on the photoactivity of chloroplasts (21.00 and 14.25, respectively) were found, even lower than the control. These effects may be explained by an imbalance of Ca/Mg or Ca/Fe relations in plants that inhibited photosynthetic activity, despite an increase of chlorophyll (Chl) *a* and *b* and Chl *a*+*b* contents in both species compared with the non-amended control.

2.4.3 Gypsum

Calcium sulfate ($CaSO_4$ x H_2O), more commonly known as gypsum, occurs geologically as an evaporate mineral associated with sedimentary deposits (Korcak, 1988; Mandal and Mandal, 2002). Currently, gypsum application may be an interesting option as amendment in soils under acidic conditions, because its most important property (from an agricultural point of view) is its water solubility (2.5 g L^{-1} in water), which is higher than calcite lime (0.5 g L^{-1} in water) (Korcak, 1988). It represents also an important source of Ca^{2+} and sulfur (S) (Bolan *et al.*, 1993) for plant nutrition, and according to some authors, it can improve mineral content in vegetal tissues, such as N, P, K, Ca, Mn, S, and Zn (Caires *et al.*, 2006; Tuna *et al.*, 2007). Gypsum increases subsoil Ca (Caires *et al.*, 2006), decreases subsoil acidity

(Toma *et al.*, 1999), and reduces exchangeable Al (Ritchey and Snuffer, 2002; Hue, 2005), and reduction in metal toxicities has also been documented (Campbell *et al.*, 2006). Calcium sulfate also can reduce the uptake of toxic levels of soil pollutants, such as high selenium (Se) (Mathews and Joost, 1989; Arthur *et al.*, 1993). In respect to physical properties, the benefits of gypsum include increased infiltration (Sahin *et al.*, 2003; Chen *et al.*, 2009), increased soil aggregation (Chen *et al.*, 2009), decreased Na adsorption (Gambaudo, 2004), improved root development (Takahashi *et al.*, 2006a), and decreased soil compactation (Gambaudo, 2004). Other benefits are an increase in the hydraulic conductivity of soil after consecutive gypsum applications (Sahin *et al.*, 2003).

An important gypsum characteristic, is the capacity for reduction of toxic Al and the increase in the Ca status in subsoil (Toma *et al.*, 2005) (Table 2.2), without or only slightly altering pH conditions (Takahashi *et al.*, 2006a). If the pH ranges from 4.5 to 8.4, the addition of gypsum will have no effect on the soil pH (Franzen *et al.*, 2006). It has reported that the reduction of Al exchangeable by gypsum is by precipitation of Al-hydroxy-sulfate minerals or aluminum sulfate ($AlSO_4^+$) formation, considerably less toxic for plants (Saigusa and Toma, 1997; Garrido *et al.*, 2003). For Alva *et al.* (1991), the role of SO_4- in reduction of Al-toxicity is very important in subsoil, where Al is complexed with organic ligands. According to Shamshuddin *et al.* (1991), Al complexing organic ligands after gypsum application in acid soils resulted in an increase of $AlSO_4^+$ and a decrease in Al^{3+} activity, which was correlated with corn yields. Mora *et al.* (1999) reported that gypsum application to ryegrass growing in an Andisol (high OM), resulted in a considerable yield increase and reduction in Al concentration (~50%), without significant changes in soil pH. This minimal effect on pH is very important for crops such as blueberry (*Vaccinium spp*.), which must be developed in acid conditions (pH 4.0-5.2), but that are sensitive to Al-toxicity (Lyrene and Muñoz, 1997). According to Takahashi *et al.* (2006a) in Andisols the Al release by gypsum will depend of OM content, being more effective in soil with a lower humic substance content, also Toma *et al.* (2005) showed that gypsum application in a non-allophanic Andisol was more effective on a soil horizon with lower humus content. Brady *et al.* (1993) studied the effects of three monomeric

Al species, Al^{3+}, $Al(OH)_2^+$, and $A1(OH)^{2+}$, on root growth in soybean amended with calcium sulfate (500-2000 μM), being dose 500 μM who inhibited negative effects of these species on root.

Table 2.2. Effects of gypsum doses (t ha^{-1}) application on different plant crops in acid soils.

Doses (t ha^{-1})	Crop Species	Effects	Authors
2.0	Ryegrass	Yield increase and 50% reduction of Al concentration.	Mora *et al.*,1999
0.5- 4.3	Brussels sprout	Raised Ca and S tissue Marketable yields. Increasing B, Mn, Fe, and Zn in the leaf tissue.	Carter and Cutcliffe, 1990
4.0	Lowbush blueberry	Increased foliar content of N, P, K, S, Mn, and Ca. Length of stem, live buds, and quantity of blossoms.	Sanderson and Eaton, 2004
4.0	Lowbush blueberry	Raised N, K, Ca, Mn, S, and significantly reduced Mg and Fe.	Sanderson, 2004
2.5	Highbush blueberry	Increase in root and leaf Ca content	Korcak, 1992

The effects of gypsum application on different crops in alleviating Al-toxicity has been well studied in soils, as well as at different pH, depths, and OM contents (Favaretto *et al.*, 2006), but these effects are relatively less studied in respect to plant physiology responses. In this context, treatments of gypsum (Ca 0, 25, 75, or 225 μM) on nutrient solution (200 μM Al), increased the mean mineral content in leaves, especially Ca and Zn (mg kg^{-1}), reduced Al, and increased respiration rates (Schaberg *et al.*, 2000). According to Caires *et al.* (2006), surface gypsum applications on soybean resulted in an improved root growth, nutrient contents, water uptake, and S,

P and K content in grains. Bakker (1999) also concluded that gypsum increased the fine root biomass and length of oak growing in acid soils, even four to five years after application. In brussels sprouts grown in low-calcium soil, gypsum application (0.5 - 4.3 t ha^{-1}) raised tissue Ca and marketable yields significantly, as well as increased leaf S, boron (B), Mn, Fe, and Zn content (Carter and Cutcliffe, 1990). On lowbush blueberry (*Vaccinium angustifolium* Ait.), the addition of gypsum (4 t ha^{-1}) plus NPK fertilizer (300 kg ha^{-1}), increased foliar contents of N, P, K, S, Mn, and Ca, stem length, live buds, and blossom quantity (Sanderson and Eaton, 2004). In 2004, Sanderson evaluated the responses in lowbush blueberry to gypsum (4 t ha^{-1}) under field assays. This surface amendment elevated N, K, Ca, Mn, S, and significantly reduced Mg and Fe in comparison to the control. Korcak (1992) reported that gypsum increased root and leaf Ca content in highbush blueberry, which did not affect soil pH significantly. However, Hanson and Berkheimer (2004) reported an increased Ca level in soil, but that these levels in the leaves and fruits of highbush blueberry were not affected.

Little is known about the gypsum effects on biochemical responses in plants affected by the presence of Al-toxicity, there is only information about Ca-Al interactions on biochemical responses (see above). This calls for further research in order to examine such phenomena. However, Guo *et al.* (2006) demonstrated that Ca (0.5, 1.0, and 3.0 mM) in barley seedlings growing in nutrient solution, reduced Al-toxicity efficiently, which is reflected by an increase of root growth, a decrease in Al concentration, malondyaldehide (MDA) content, and increased SOD, POD, and CAT activities compared with Al-only treatment (100μM) (Guo *et al.*, 2006).

2.4.4 Comparative effectiveness of calcareous amendments

Soil pH could affect soil microbial activity and populations, soil chemical reactions, and nutrient availability (Mullen *et al.*, 2007). The progressive acidification of soils by natural and anthropological factors has negative effects on crops, and growers must apply various amendments to sustain their crop production (Wang *et al.*, 2006). In acid soils like Andisols, the amendments depend on conditions like agronomic management, OM content, fertility levels, local climatic conditions, and

crop type (Tisdale *et al.,* 1985). There are a number of studies evaluating the qualities of different amendments in different crops and reporting on the advantages and disadvantages of these treatments.

2.4.5 Surface applications and subsoil effects

Liming is a practice largely used to neutralize the acidity of the surface soil layers, but does not seem to be effective in acidity amelioration at a subsoil level (Carvalho and van Raig, 1997). Moreover, deep limes incorporation requires the implementation of specific equipment and results in higher costs, which makes it unfeasible for use by small farmers (Carvalho and van Raig, 1997). Alternatively, the surface application of gypsum or PG allowing leaching into subsoil has resulted in higher water and nutrient uptake by plant roots (Alva *et al.*, 1988; Sumner, 1993). These effects are attributed to an increase in Ca content and a reduction of Al-toxicity at deeper soil layers (Toma *et al.,* 2005). In addition, both gypsum and PG are more mobile Ca sources than $CaCO_3$ (von Willert and Stehouwer, 2003). In this context, Sumner *et al.* (1986) demonstrated that deep limes incorporation into the soil (100 cm) and surface gypsum application increased root development in alfalfa growing in acid soils.

In alfalfa, application lime have resulted in a complete Al^{3+} precipitation, increased soluble Ca levels, and a 50% increased yields. Surface application of gypsum resulted in 25% increase of yield with a progressive reduction of soluble Al and increased Ca content, creating a similar but lesser effect than liming. Pavan *et al.* (1984) reported that gypsum was more effective in reduction of Al concentration within the 100 cm depth profile, while lime effects were observed only in the upper 20 cm. Caires *et al.* (2006) showed that gypsum ameliorate subsoil pH and Al-toxicity, increasing Ca and S level in wheat leaves. A comparative evaluation of lime, gypsum and PG demonstrated that lime treatment ($CaCO_3$ 2,500 kg ha^{-1}) increased exchangeable Ca and decreased exchangeable Al in the 0-5 cm soil layer, but no significant changes were observed below 5 cm, which suggested limited lime leaching. By contrast, gypsum and PG reduced these values to a 25 cm depth profile (Smith *et al.* 1994). McCray *et al.* (2007) incorporated dolomite limestone (4.0 t ha^{-1})

and PG (10 t ha^{-1}) into the surface soil layer (15 cm). It was shown that PG moved downward much more rapidly than lime, increasing soil solution Ca ion activity to a depth of 80 cm within 5 months of application. Yield responses to PG were attributed to increased root growth below 20 cm, resulting from the increased Ca ion activity over a three-year period. $CaCO_3$ addition in two horizons (A and B) of non-allophanic Andisol in Japan reduced Al amounts complexed organically, as well as exchangeable Al, after 30 days of application (Takahashi *et al.* 2006b). Gypsum (4.3 g kg^{-1} and 8.6 g kg^{-1}) has also improved the root growth of burdock (*Arctium lappa* cv. Kantan) on horizon B of the Andisol (Takahashi *et al.*, 2006a; Figure 2.3).

The amendment plays a role in root disease responses to manageable soil chemical factors, such as pH or Ca saturation. Allmaras *et al.* (1987) evaluated lime and gypsum treatment on a wheat-peas culture rotation and measured the propagated density of *Fusarium solani ssp*. pisi in the 0 to 15 cm soil layer. They found a decrease in the density of propagation (37%) of this fungus species by effect of lime, meanwhile between 15 to 45 cm of depth soil gypsum reduced its propagation density in 22%, therefore concluded that Ca can improve the resistance of the membrane in pea-root to attack by *Fusarium* pathogens, or allowing greater microbial antagonism.

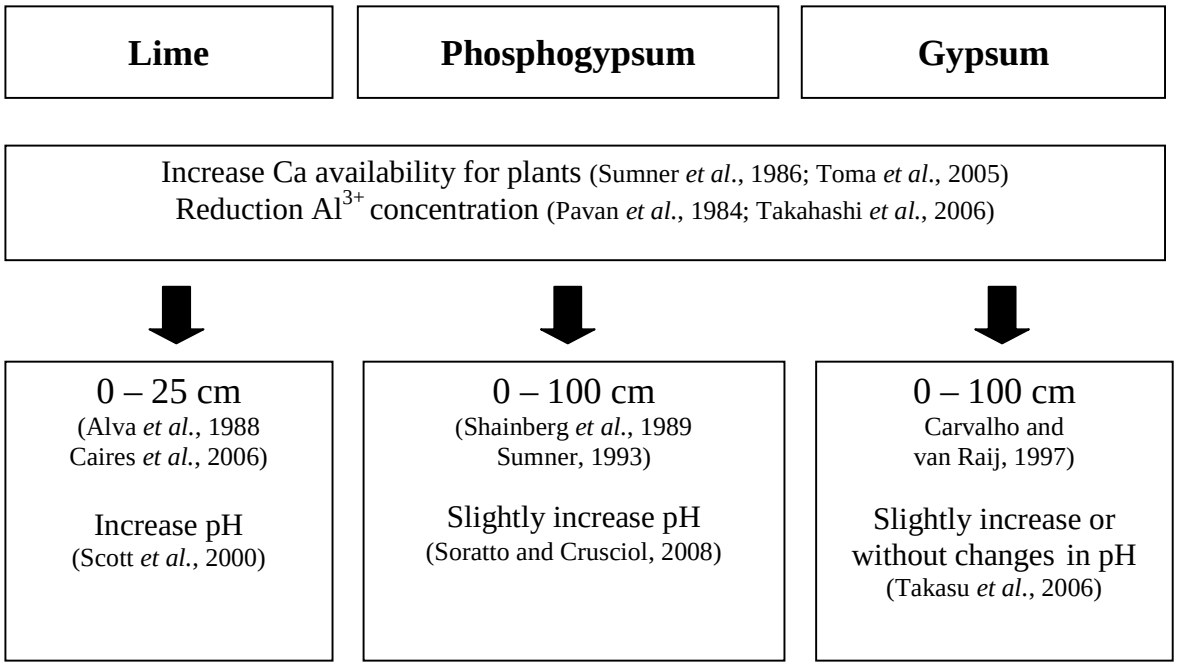

Figure 2.3. Comparative effectiveness related to amendments at different soil layers.

2.4.6 Effects on pH and mineral content

Gypsum treatment does not affect soil pH as much as limestone (Figure 2.3), but results in increased Ca, S, and Mn contents and decreased P and Mg levels (Ritchey *et al.*, 1995). On the other hand, limestone impact on soils is carried out by increasing pH (Scott *et al.*, 2000). Ritchey and Snuffer (2002) concluded that gypsum reduced both Mg in soil solution and orchard grass leaves (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb), but lime (dolomitic limestone) raised Mg ranges in leaves. Application of $CaCO_3$ raised pH from 5.7 to 6.0 in the Andisol and increased foliar and root Ca content in coffee plants (Hue, 2005). By contrast, gypsum did not increase pH, but raised Ca content in leaves (Hue, 2005). Hanson and Berkheimer (2004) added Lime (1,100 kg ha^{-1}) in field assays for five seasons, and the soil pH values increased from 4.2 (1996 season) to 5.0 (2001 season), whereas gypsum amendment (550 kg ha^{-1}) had a slighter effect on pH, increasing it from 4.2 to 4.6 in the same time lapse, while leaf Ca contents did not change significantly.

2.5 CONCLUSIONS AND PERSPECTIVES

The first symptoms of Al^{3+} damage in plant roots growing in acid soils have been well established; however, the effect on the upper parts such as stems, leaves and/or fruits remain a matter of controversy, despite the negative effects of Al^{3+} on photosynthesis, photochemical parameters and cellular respiration have been well established. Al presence in plant tissues has been correlated to decreased leaf nutrient content, especially Ca^{2+} and Mg^{2+}, and damage to the chloroplast and mitochondrial membranes. Al^{3+}-induced damage and toxicity are also related to Ca^{2+} interactions at apoplast and symplast levels and the regulation of cytosolic Ca^{2+} homeostasis. Both cations compete for the active sites of membrane structures to form ligands. This interaction is not fully understood, since each cation inhibits to another one, depending on the state of membrane. The few reports regarding the interaction between Ca^{2+} and Al^{3+} have demonstrated that Ca^{2+} decreases the enzymatic antioxidant activity of SOD, CAT, and POD, concomitant with a decrease in the toxic Al^{3+} levels. Nevertheless, other authors suggest that the antioxidant ability could be favored by the interaction between Ca^{2+} and Al^{3+}. The knowledge of the non-enzymatic antioxidant defense against Al stress is less recognized than the enzymatic one. Further studies are needed to better understand the mechanisms involved in the Ca-Al interactions that affect such physiological and biochemical processes as photosynthesis, respiration, antioxidant activities, signal transduction and cellular homeostasis in plants growing in acid soils like Andisols.

Acknowledgements

We would like thank to FONDECYT Nº11080231 Project for financial support and MECESUP FRO 0601 Scholarship.

2.6 REFERENCES

Abreu Jr., C. H., Muraoka, T., Lavorante, A. F. 2003. Exchangeable aluminum evaluation in acid soils. Sci. Agric. 60, 543 ─ 548.

Akaya, M., Takenaka, C. 2001. Effects of aluminum stress on photosynthesis of *Quercus glauca* Thumb. Plant Soil 237, 137 ─ 146.

Allmaras, R. R., Kraft, J. M., Pikul jr, J. L. 1987. Lime and gypsum effects on pea-root-pathogen inoculum and related factors in a wheat-peas rotation. Agron. J. 79, 439 ─ 445.

Alva, A. K., Kerven, G. L., Edwards, D. G., Asher, C. J. 1991. Reduction in toxic aluminum to plants by sulfate complexation. Soil Sci. 5, 351 ─ 359.

Alva, A. K., Sumner, M. E. 1990. Amelioration of acid soil infertility by phosphogypsum. Plant Soil 128, 127 ─ 134.

Alva, A. K., Sumner, M. E. 1988. Effects of phosphogypsum or calcium sulfate on reactive aluminum in solutions at varying pH. Commun. Soil Sci. Plan. 19, 1715 ─ 1730.

Alvarez, E., Fernández-Marcos, M. L., Monterroso, C., Fernández-Sanjurjo, M. J. 2005. Application of aluminium toxicity indices to soils under various forest species. Forest Ecol. Manage. 211, 227 ─ 239.

Arthur, M. A., Rubin, G., Woodbury, P. B., Weinstein, L. H. 1993. Gypsum amendment to soil can reduce selenium uptake by alfalfa grown in the presence of coal fly ash. Plant Soil 148, 83 ─ 90.

Bachiega, Z., Ferracciú-Alleoni, L. R., Caires, E. F. 2007. Nutrient concentration in soil water extracts and soybean nutrition in response to lime and gypsum applications to an acid Oxisol under no-till system. Nutr. Cycl. Agroecosys. 79, 169 ─ 179.

Bakker, A. J. M., Grath, S. P. M., Reeves, R. D., Smith, J. A. C. 2000. Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. Phytoremediation of Contaminated Soil and Water (Terry, N. & Banuelos, G., Eds.) pp. 85 ─ 107.

Bakker, M. R. 1999. The effect of lime and gypsum applications on a sessile oak (*Quercus petraea* (M.) Liebl.) stand at La Croix-Scaille (French Ardennes) fine root dynamics. Plant Soil 206, 109 ─ 121.

Bakker, M. R., Kerisit, R., Verbist, K., Nys, C. 1999. Effects of liming on rhizosphere chemistry and growth of fine roots and shoots of sessile oak (*Quercus petraea*). Plant Soil 217, 243 ─ 255.

Blamey, F. P. C., Ostatek-Boczynski, Z., Kerven, G. L. 1997. Ligand effects on aluminium sorption by calcium pectate. Plant Soil 192, 269 ─ 275.

Blatt, C. R., McRae, K. H. 1997. Marketable yield response of two lowbush blueberries to preplant applications of gypsum, calcitic, and dolomitic limestones. J. Small Fruit Viticulture 5, 13 ─ 26.

Blokhina, O., Virolainen, E., Fagerstedt, K. V. 2003. Antioxidants, oxidative damage, and oxygen deprivation stress: A review. Ann Bot-London 91, 179 ─ 194.

Bolan, N. S., Syers, J. K., Sumner, M. E. 1993. Calcium-induced sulfate adsorption by soils. Soil Sci. Soc. Am. J. 57, 691 ─ 696.

Bóscolo, P., Menossi, M., Jorge, R. A. 2003. Aluminum-induced oxidative stress in maize. Phytochemistry 62.181 ─ 189.

Brady, D. J., Edwards, D. G., Asher, C. J., Blamey, P. C. 1993. Calcium amelioration of aluminium toxicity effects on root hair development in soybean [*Glycine max* (L.) merr.]. New Phytol. 123, 531 ─ 538.

Bush, D. S. 1995. Calcium regulation in plant cells and its role in signaling. Annu. Rev. Plant Physiol. Plant. Mol. Biol. 46, 95 ─ 122.

Caires, E. F., Churka, S., Garbuio, F. J., Ferrari, R. A., Morgano, M. A. 2006. Soybean yield and quality as a function of lime and gypsum applications. Sci. Agric. 63, 370 ─ 379.

Caires, E. F., Feldhaus, I. C., Barth, G., Garbuio, F. J. 2002. Lime and gypsum application on the wheat crop. Scientia Agric. 59, 357 ─ 364.

Campbell, C. G., Garrido, F., Illera, V., García-González, M. T. 2006. Transport of Cd, Cu and Pb in an acid soil amended with phosphogypsum, sugar foam and phosphoric rock. Appl. Geochem. 21, 1030 ─ 1043.

Campillo, R., Urquiaga, S., Undurraga, P., Pino, I., Boddey, R. M. 2005. Strategies to optimize biological nitrogen fixation in legume/grass pastures in the southern region of Chile. Plant Soil 273, 57 ─ 67.

Carter, M. R., Cutcliffe, J. A. 1990. Effects of gypsum on growth and mineral content of brussels sprouts, and soil properties of Arthic Podzols. Fertilizer Res. 24, 77 ─ 84.

Carvalho, M. C. S., van Raij, B. 1997. Calcium sulphate, phosphogypsum and calcium carbonate in the amelioration of acid subsoils for root growth. Plant Soil 192, 37 ─ 48.

Chen, L., Ramsier, C., Bigham, J., Slater, B., Kost, D., Lee, Y. B., Dick, W. A. 2009. Oxidation of FGD-$CaSO_3$ and effect on soil chemical properties when applied to the soil surface. Fuel 88, 1167 ─ 1172.

Cheng, S., Willmann, M. R., Chen, H., Sheen, J. 2002. Calcium signaling through protein kinases. The arabidopsis calcium-dependent protein kinase gene family. Plant Physiol. 129, 469 ─ 485.

Cronan, C. S., Grigal, D. F. 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. J. Environ. Qual. 24, 209 ─ 226.

Dam-ampai, S., Nilnond, C., Onthong, C. 2007. Effect of some soil amendments on soil properties and plant growth in southern Thailand acid upland soil. Songklanakarin J. Sci. Technol. 29, 117 ─ 131.

Danilov, R. A., Ekelund, N. G. A. 2002. Effects of short-term and long-term aluminium stress on photosynthesis, respiration, and reproductive capacity in a unicellular green flagellate (*Euglena gracilis*). Acta Hydroch. Hydrob. 30, 190 ─ 196.

de Castro, O. M., de Camargo, O. A., Vieira, S. R., Filho, J. V. 1999. Effect of two types of lime on some soil physical attributes of an Oxisol from Brazil. Commun. Soil Sci. Plant Anal. 30, 2183 ─ 2195.

Delhaize, E., Ryan, P. R. 1995. Aluminum toxicity and tolerance in plants. Plant Physiol. 107, 315 ─ 321.

Favaretto, N., Norton, L. D., Joern, B. C., Brouder, S. M. 2006. Gypsum amendment and exchangeable calcium and magnesium affecting phosphorus and nitrogen in runoff. Soil Sci. Soc. Am. J. 70, 1788 ─ 1796.

Foy, C. D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. In F Adams, Ed, Soil Acidity and Liming. American Society of Agronomy, Madison, Wi, 57 ─ 97.

Foy, C. D., Chaney, R. L., White, M. C. 1978. The physiology of metal Al-toxicity in plants. Annu. Rev. Plant Physiol. 29, 511 ─ 566.

Foyer, C. H., Noctor, G. 2005. Oxidant and antioxidant signalling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 28, 1056 ─ 1071.

Franzen, D., Rehm, G., Gerwing, J. 2006. Effectiveness of gypsum in the North-central region of the U. S. Extension Service. North Dakota State University. 4p.

Gambaudo, S. 2004. Calidad del yeso natural para uso agrícola. Inta – Estación experimental agropecuaria Rafaela. Información técnica cultivos de verano. Campaña 2006. Publicación miscelánea N º 106.

Garland, C. J., Wilkins, D. A. 1981. Effect of calcium on the uptake and toxicity of lead in *Hordeum vulgare* L. and *Festuca ovina* L. New Phytol. 87, 581 ─ 593.

Garrido, F., Illera, V., Vizcaíno, C., García-González, M. T. 2003. Evaluation of industrial by-products as soil acidity amendments: chemical and mineralogical implications. Eur. J. Soil Sci. 54, 411 ─ 422.

Ghanati, F., Morita, A., Yokota, H. 2005. Effects of aluminum on the growth of tea plant and activation of antioxidant system. Plant Soil 276, 133 ─ 141.

Gilroy, S., Bethke, P. C., Jones, R. L. 1993. Calcium homeostasis in plants. J. Cell Sci. 106, 453 ─ 462.

Gong, M., Chen, S. N., Song, Y. Q., Li, Z. G. 1997. Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. Aus. J. Plant Physiol. 24, 371 ─ 379.

Guo, T., Chen, Y., Zhang, Y., Jin, Y. 2006. Alleviation of Al-toxicity in barley by addition of calcium. Agr. Sci.-China 5, 828 ─ 833.

Guo, T., Zhang, G., Zhou, M., Wu, F., Chen, J. 2004. Effects of aluminum and cadmium toxicity on growth and antioxidant enzyme activities of two barley genotypes with different al resistance. Plant Soil 258, 241 ─ 248.

Han, W. Y., Shi, Y. Z., Ma, L. F., Ruan, J. Y., Zhao, F., J. 2007. Effect of liming and seasonal variation on lead concentration of tea plant (*Camellia sinensis* (L.) O. Kuntze). Chemosphere 66, 84 ─ 90.

Hanson, E. J., Berkheimer, S. F. 2004. Effect of soil calcium applications on blueberry yield and quality. J. Small Fruits Rev. 3, 133 ─ 139.

Hede, A. R., Skovmand, B., López-Cesati, J. 2001. Acid soil and aluminum toxicity, In: M.P. Reynolds, J.J. Ortiz-Monasterio, and A. Mchab (eds.). Application of physiology in wheat breeding. International Maize and Wheat Improvement Center p. 172 ─ 182.

Hepler, P. K. 2005. Calcium: A central regulator of plant growth and development. Plant Cell 17, 2142 ─ 2155.

Homann, P. H. 2002. Chloride and calcium in Photosystem II: from effects to enigma. Photosynth. Res. 73, 169 ─ 175.

Horst, W. J. 1995. The role of the apoplast in aluminium toxicity and resistance of higher
plants: a review. Z. Pflänzenernähr. Bodenk. 158, 419 ─ 428.

Hoshino, A. A., Boni, T. A., Prioli, J. A., Pereira Bravo, J., Pinto Priori, S. M. A. 2000. Changes caused by aluminum in protein and carbohydrate contents in the apex of maize seminal roots. Acta Sci. 22, 877 ─ 882.

Huang, D., Ou, B., Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, 1841 ─ 1856.

Huang, J. W., Shaff, J. E., Grunes, D. L., Kochian, L. V. 1992. Aluminum effects on calcium fluxes at the root apex of aluminum-tolerant and aluminum-sensitive wheat cultivars. Plant Physiol. 98, 230 ─ 237.

Hue, N. V. 2005. Responses of coffee seedlings to calcium and zinc amendments to two Hawaiian acid soils. J. Plant Nutr. 27, 261 ─ 274.

Iamarino, M., Terribile, F. 2008. The importance of andic soils in mountain ecosystems: a pedological investigation in Italy. Eur. J. Soil Sci. 59, 1284 ─ 1292.

Illera, V., Garrido, F., Vizcayno, C., García-González, M. T. 2004. Field application of industrial by-products as Al-toxicity amendments: chemical and mineralogical implications. Eur. J. Soil Sci. 55, 681 ─ 692.

Jiang, Y., Huang, B. 2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J. Exp. Bot. 52, 341 ─ 349.

Jones, D. L., Kochian, L. V., Gilroy, S. 1998. Aluminum induces a decrease in cytosolic calcium concentration in by-2 tobacco cell cultures. Plant Physiol. 116, 81 ─ 89.

Jones, D. L., Kochian, L. V. 1997. Aluminum interaction with plasma membrane lipids and enzyme metal binding sites and its potential role in al cytotoxicity. Febs. Lett. 400, 51 ─ 57.

Kameyama, K., Miyamoto, T. 2008. Measurement of solid phase permittivity for volcanic soils by time domain reflectometry. Eur. J. Soil Sci. 59, 1253 ─ 1259.

Kinraide, T. B., Pedler, J. F., Parker, D. R. **2004.** Relative effectiveness of calcium and magnesium in the alleviation or rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium, and low pH. Plant Soil 259, 201 ─ 208.

Kinraide, T. H. 1998. Three mechanisms for the calcium alleviation of mineral toxicities. Plant Physiol. 118, 513 ─ 520.

Khan, N. A., Samiullah, S., Singh, S., Nazar, R. 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J. Agron. Crop Sci. 193, 435 ─ 444.

Kleber, M., Jahn, R. 2007. Andosols and soils with andic properties in the German soil taxonomy. J. Plant Nutr. Soil Sci. 170, 317 ─ 328.

Kochian, L. V., Pineros, M. A., Hoekenga, O. A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274, 175 ─ 195.

Kochian, L. V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 237 ─ 260.

Korcak, R. F. 1992. Short-term response of blueberry to elevated soil calcium. J. Small Fruit Viticulture. 1, 9 ─ 20.

Korcak, R. F. 1988. Fluidized bed material applied at disposal levels: effects on an apple orchard. J. Am. Soc. Hort. Sci. 113, 189 ─ 193.

Krutilina, V. S., Polyanskaya, S. M., Goncharova, N. A., Letchamo, W. 2000. Effects of zeolite and phosphogypsum on growth, photosynthesis and uptake of Sr, Ca and Cd by barley and corn seedlings. J. Environ. Sci. Health 35, 15 ─ 29.

Kumar, S. 2002. A perspective study on fly ash–lime–gypsum bricks and hollow blocks for low cost housing development. Constr. Build. Materials 16, 519 ─ 525.

Kumar Roy, A., Sharma, A., Talukder, G. 1988. Some aspects of aluminum toxicity in plants. Bot. Rev. 54, 145 ─ 178.

Langer, H., Cea, M., Curaqueo, G., Borie, F. 2009. Influence of aluminum on the growth and organic acid exudation in alfalfa cultivars grown in nutrient solution. J. Plant Nutr. 32, 618 ─ 628.

Lecourieux, D., Ranjeva, R., Pugin, A. 2006. Calcium in plant defence-signalling pathways. New Phytol. 171, 249 ─ 269.

Lidon, F., Barreiro, M. 2002. An overview into aluminum toxicity in maize. Bulg. J. Plant Physiol. 28, 96 ─ 112.

Lindberg, S., Strid, H. 1997. Aluminium induces rapid changes in cytosolic pH and free calcium and potassium concentrations in root protoplasts of wheat (*Triticum aestivum*). Physiol. Plantarum 99, 405 ─ 414.

Locascio, S. J., Hochmuth, C. J. 2002. Watermelon production as influenced by lime, gypsum, and potassium. Hortscience 37, 322 ─ 324.

Lyrene P. M., Muñoz, C. 1997. Blueberry production in Chile. J. Small Fruit Viticulture 5, 1 ─ 20.

Ma, B., Wan, J., Shen, Z. 2007. H_2O_2 production and antioxidant responses in seeds and early seedlings of two different rice varieties exposed to aluminum. Plant Growth Regul. 52, 91 ─ 100.

Ma, J. F. 2005. Physiological mechanisms of Al resistance in higher plants. Soil Sci. Plant Nutr. 61. 609 ─ 612.

Ma, Q., Rengel, Z., Kuo, **J. 2002.** Aluminium toxicity in rye (*Secale cereale*): root growth and dynamics of cytoplasmic Ca^{2+} in intact root tips. Ann. Bot. 89, 241 ─ 244.

Mandal, P. K., Mandal, T. K. 2002. Anion water in gypsum ($CaSO_4$ $2H_2O$) and hemihydrate ($CaSO_4$ $1/2H_2O$). Cement Concrete Res. 32, 313 ─ 316.

Mariscal-Sancho, I., Espejo, R., Peregrina, F. 2009. Potentially toxic effects of phosphogypsum on palexerults in western Spain. Soil Sci. Soc. Am. J. 73, 146 ─ 153.

Mathews, B. W., Joost, R. E. 1989. Use of sulfate salts to reduce subsoil aluminum toxicity: a review. J. Haw. Pac. Agri. 2, 24 ─ 30.

Mays, D. A., Mortvedt, J. J. 1986. Crop response to soil applications of phosphogypsum. J Environ. Qual. 15, 78 ─ 81.

Mc Cray, J. M., Sumner, M. E., Radcliffe, D. E., Clark, R. L. 2007. Soil Ca, Al, acidity and penetration resistance with subsoiling, lime and gypsum treatments. Soil Use Manage. 7, 193 ─ 199.

Meriga, B., Reddy, B. K., Rao, K. R., Reddy, L., Kishor, P. B. K. 2004. Aluminium-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). J. Plant Physiol. 161, 63 ─ 68.

Mittler R., Vanderauwera, S., Gallery, M., van Breusegem, F. 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9, 490 ─ 498.

Mora, M. L., Pinilla, L., Rosas, A., Cartes, P. 2008. Selenium uptake and its influence on the antioxidative system of white clover as affected by lime and phosphorus fertilization. Plant Soil 303, 139 ─ 149.

Mora, M. L., Cartes, P., Nuñez, P., Salazar, M., Demanet, R. 2007. Movement of NO_3^--N and NH_4^+-N in an Andisol and its influence on ryegrass production in a short term study. R. C. Suelo Nutr. Veg. 7, 46 ─ 64.

Mora, M. L., Alfaro, M. A., Jarvis, S. C., Demanet, R., Cartes, P. 2006. Soil aluminum availability in Andisols of Southern Chile and its effect on forage production and animal metabolism. Soil Use Manage. 22, 95 ─ 101.

Mora, M. L., Cartes, P., Demanet, R., Cornforth, I. S. 2002. Effects of lime and gypsum on pasture growth and composition on an acid Andisol in Chile, South America. Commun. Soil Sci. Plant Anal. 33, 2069 ─ 2081.

Mora, M. L., Schnettler, B., Demanet, R. 1999. Effect of liming and gypsum on soil chemistry, yield, and mineral composition of ryegrass grown in an acidic Andisol. Commun. Soil Sci. Plant Anal. 30, 1251 ─ 1266.

Mossor-Pietraszewska, T. 2001. Effect of aluminium on plant growth and metabolism. Acta Biochim. Pol. 3, 673 ─ 686.

Mullen, R., Lentz, E., Watson, M. 2007. Soil acidity and liming for agronomic production. Extension Factsheet. 6p.

Nanzyo, M., Shoji, S., Dahlgren, R. A. 1993. Physical characteristics of volcanic ash soils. In volcanic ash soils – genesis, properties and utilization. Eds. S Shoji, M Nanzyo and R A Dahlgren, pp. 189 ─ 207. Elsevier, Amsterdam.

Ogawa, T., Matsumoto, C., Takenaka, C., Tezuka, T. 2000. Effect of Ca on Al-induced activation of antioxidant enzymes in the needles of hinoki cypress (*Chamaecyparis obtusa*). J. For. Res. 5, 81 ─ 85.

Okamoto, O. K., Pinto, E., Latorre, L. R., Bechara, E. J. H., Colepicolo, P. 2001. Antioxidant modulation in response to metal-induced oxidative stress in algal chloroplasts. Arch. Environ. Contam. Toxicol. 40, 18 ─ 24.

Ono, T. A., Rompel, A., Mino, H., Chiba, N. 2001. Ca^{2+} function in photosynthetic oxygen evolution studied by alkali metal cations substitution. Biophys. J. 81, 1831 ─ 1840.

Pan, J. W., Zhu, M. Y., Chen, H. 2001. Aluminum-induced cell death in root-tip cells of barley. Env. Exp. Bot. 46, 71 ─ 79.

Panda, S. K., Singha, L. B., Khan, M. H. 2003. Does aluminium phytotoxicity induce oxidative stress in greengram (*Vigna radiata*)?. Bulg. J. Plant Physiol. 29, 77 ─ 86.

Papastefanou, C., Stoulos, S., Ioannidou, A., Manolopoulou, M. 2006. The application of phosphogypsum in agriculture and the radiological impact. J. Environ. Radioactiv. 89, 188 ─ 198.

Pavan, M. A., Bingham, F. T., Peryea, F. J. 1984. Influence of calcium and magnesium salts on acid soil chemistry and calcium nutrition of apple. Soil Sci. Soc. America J. 51, 1526 ─ 1530.

Peixoto, H. P., da Matta, F. M., da Matta, J. C. 2002. Responses of the photosynthetic apparatus to aluminum stress in two sorghum cultivars. J. Plant Nutr. 25, 821 ─ 832.

Peregrina-Alonso, F., Santano-Arias, J., Ordónez-Fernández, R., González-Fernández, P., Espejo-Serrano, R. 2006. Agronomic implications of the supply of lime and gypsum by-products to palexerults from western Spain. Soil Sci. 171, 65 ─ 81.

Plieth, C. 2005. Calcium: Just another regulator in the machinery of life?. Ann. Bot. 96, 1 ─ 8.

Poschenrieder, C., Gunsé, B., Corrales, I., Barceló, J. 2008. A glance into aluminum toxicity and resistance in plants. Sci. Total Environ. 400, 356 ─ 368.

Poovaiah, B. W., Glenn, G. M., Reddy, A. S. N. 1988. Calcium and fruit softening: physiology and biochemistry. Hort. Rev. 10, 107 ─ 152.

Prior, R. L., Wu, X., Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53, 4290 ─ 4302.

Redente, E. F., Richards, J. L. 1997. Effects of lime and fertilizer amendments on plant growth in smelter impacted soils in Montana. Arid Land Res. Manage. 11, 353 ─ 366.

Reich, P. B., Oleksyn, J., Tjoelker, M. G. 1994. Relationship of aluminum and calcium to net CO_2 exchange among diverse Scots pine provenances under pollution stress in Poland. Oecologia 97, 82 ─ 92.

Rengel, Z., Zhang, W. H. 2003. Role of dynamics of intracellular calcium in aluminum-toxicity syndrome. New Phytol. 159, 295 ─ 314.

Rengel, Z. 1996. Uptake of aluminium by plant cells. New Phytol. 134, 389 ─ 406.

Rengel, Z. 1992a. Disturbance of cell Ca^{2+} toxicity syndrome homeostasis as a primary trigger of Al. Plant Cell Environ. 15, 931 ─ 938.

Rengel, Z. 1992b. Role of calcium in aluminum toxicity. New Phytol. 121, 499 ─ 513.

Reyes-Díaz, M., Alberdi, M., Mora, M. L. 2009. Short-term aluminum stress differentially affects the photochemical efficiency of photosystem II in highbush blueberry genotypes. J. Amer. Soc. Hort. Sci. 134, 14 ─ 21.

Ritchey, K. D., Snuffer, J. D. 2002. Limestone, gypsum, and magnesium oxide influence restoration of an abandoned Appalachian pasture. Agron. J. 94, 830 ─ 839.

Ritchey, K. D., Feldhake, C. M., Clark, R. B., de Sousa, D. M. G. 1995. Improved water and nutrient uptake from subsurface layers of gypsum-amended soils. p. 157 ─ 181. In D.L. Karlen *et al.* (ed.) agricultural utilization of urban and industrial by-products. Spec. Publ. 58. asa, cssa, and sssa, madison, wi.

Rosenberg, W., Nierop, K. G. J., Knicker, H., de Jager, P. A., Kreutzer, K., Wei, T. 2003. Liming effects on the chemical composition of the organic surface layer of a mature Norway spruce stand (*Picea abies* [L.] Karst.). Soil Biol. Biochem. 35, 155 ─ 165.

Rout, G. R., Samantara, S., Das, **P. 2001.** Aluminium toxicity in plants: a review. Agronomie 21, 3 ─ 21.

Ryan, P. R., Delhaize, E. 2010. The convergent evolution of aluminium resistance in plants exploits a convenient currency. Funct. Plant Biol. 37, 275 ─ 284.

Ryan, P. R., Reid, R. J., Smith, F. A. 1997. Direct evaluation of the Ca^{2+}-displacement hypothesis for Al-toxicity. Plant Physiol. 113, 1351 ─ 1357.

Ryan, P. R., Kochian, L. V. 1993. Interaction between aluminum toxicity and calcium uptake at the root apex in near-isogenic lines of wheat (*Triticum aestivum* L.) differing in aluminum tolerance. Plant Physiol. 102, 975 ─ 982.

Sahin, U., Oztas, T., Anapali, O. 2003. Effects of consecutive applications of gypsum in equal, increasing, and decreasing quantities on soil hydraulic conductivity of a saline-sodic soil. J. Plant Nutr. Soil Sci. 166, 621 ─ 624.

Saigusa, M., and Toma, M. 1997. Mechanism of reduction of exchangeable aluminum by gypsum application. Soil Sci. Plant Nutr. 43, 343 ─ 349.

Samac, D. A., Tesfaye, M. 2003. Plant improvement for tolerance to aluminum in acid soils– a review. Plant Cell Tiss. Org. 75, 189 ─ 207.

Sanders, D., Pelloux, J., Brownlee, C., Harper, J. F. 2002. Calcium at the crossroad of signalling. Plant Cell 14, 401 ─ 407.

Sanderson, K. 2004. Gypsum as an organic amendment in lowbush blueberry production. sustainable production systems. Crops and Livestock Research Centre, Charlottetown, PE. 2004F-05E.

Sanderson, K. R., Eaton, L. J. 2004. Gypsum—An alternative to chemical fertilizers in lowbush blueberry production. J. Small Fruits Rev. 3, 57 ─ 71.

Sanzonowicz, C., Smyth, T. J., Israel, D. W. 1998. Calcium alleviation of hydrogen and aluminum inhibition of soybean root extension from limed soil into acid subsurface solutions. J. Plant Nutr. 21, 785 ─ 804.

Scandalios, J. G. 2002. The rise of ROS. Trends Biochem. Sci. 27, 483 ─ 486.

Schaberg, P. G., Tilley, J. W., Hawley, G. J., Dehayes, D. H., Bailey, S. W. 2006. Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. Forest Ecol. Manage. 223, 159 ─ 169.

Schaberg, P. G., Dehayes, D. H., Hawley, G. J., Strimbeck, G. R., Cumming, J. R., Murakami, P. F., Borer, C. H. 2000. Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. Tree Physiol. 20**,** 73 ─ 85.

Schuman, G. E., Depuit, E. J., Roadifer, K. M. 1994. Plant responses to gypsum amendment of sodic bentonite mine soil. J. Range Manage. 47, 206 ─ 209.

Scott, B. J., Ridley, A. M., Conyers, M. K. 2000. Management of soil acidity in long-term pastures of south-eastern Australia: a review. Aust. J. Exp. Agr. 40, 1173 ─ 1198.

Shamshuddin, J., Che fauziah, I., Sharifuddin, H. A. H. 1991. Effects of limestone and gypsum application to a Malaysian Ultisol on soil solution composition and yields of maize and groundnut. Plant Soil 134, 45 ─ 52.

Shao, H. B., Chu, L. Y., Shao, M. A., Jaleel, C. A., Mi, H. M. 2008. Higher plant antioxidants and redox signaling under environmental stresses. CR. Soc. Biol. 331, 433 ─ 441.

Silva, I. R., Ferrufino, A., Sanzonowicz, C., Smyth, T. J., Israel, D. W., Carter jr, T. E. 2005. Interactions between magnesium, calcium, and aluminum on soybean root elongation. R. Bras. Ci. Solo 29, 747 ─ 754.

Šimonovičová, M., Tamás, L., Huttová, J., Mistrík, I. 2004. Effect of aluminium on oxidative stress related enzymes activities in barley roots. Biol. Plantarum 48, 261 ─ 266.

Smith, C. J., Peoples, M. B., Keerthisinghe, G., James, T. R., Garden, D. I., Tuomi, S. S. 1994. Effect of surface applications of lime, gypsum and phosphogypsum on the alleviating of surface and subsurface acidity in a soil under pasture. Aust. J. Soil Res. 32, 995 ─ 1008.

Sparks, D. I. 2004. Advances in agronomy. University of Chicago. USA, 440p.

Stahl, W., Sies, H. 2003. Antioxidant activity of carotenoids. Mol. Aspects Med. 24, 345 ─ 351.

Sumner, M. E. 1993. Gypsum and acid soils: the world scene. Adv. Agron. 51, 1 ─ 32.

Sumner, M. E., Shahandeh, H., Bouton, J., Hammel, J. 1986. Amelioration of an acid soil profile through deep liming and surface application of gypsum. Soil Sci. Soc. Am. J. 50, 1254 ─ 1258.

Takahashi, T., Nanzyo, M., Hiradate, S. 2007. Aluminum status of synthetic Al–humic substance complexes and their influence on plant root growth. Soil Sci. Plant Nutr. 53, 115 ─ 124.

Takahashi, T., Ikeda, Y., Nakamura, H., Nanzyo, M. 2006a. Efficiency of gypsum application to acid Andosols estimated using aluminum release rates and plant root growth. J. Soil Sci. Plant Nutr. 52, 584 ─ 592.

Takahashi, T., Ikeda, T., Fujita, K., Nanzyo, M. 2006b. Effect of liming on organically complexed aluminum of nonallophanic Andosols from northeastern Japan. Geoderma 130, 26 ─ 34.

Takami, C., Takenaka, C., Tezuka, T. 2005. Mitigation of aluminum toxicity by calcium and magnesium in japanese cedar (*Cryptomeria japonica*). J. For. Res. 10, 9 ─ 14.

Takasu, E., Yamada, F., Shimada, N., Kumagai, N., Hirabayashi, T., Saigusa, M.. 2006a. Effect of phosphogypsum application on the chemical properties of Andosols, and the growth and Ca uptake of melon seedlings. Soil Sci. Plant Nutr. 52, 760 ─ 768.

Takasu, E., Yamada, H., Shimada, N., Yoshida, Y., Maigusa, M. 2006b. Effect of phosphogypsum application on the growth and Ca uptake of komatsuna (*Brassica rapa* L.) cv. Natsurakuten. Soil Sci. Plant Nutr. 52, 251 ─ 255.

Tamás, L., Budíková, S., Huttovà, J., Mistrík, I., Šimonovičová, M., Siroka, B. 2005. Aluminum-induced cell death of barley-root border cells is correlated with peroxidase- and oxalate oxidase-mediated hydrogen peroxide production. Plant Cell Rep. 24, 189 ─ 194.

Tang, Z., Lei, T., Yu, J., Shainberg, I., Mamedov, A. I., Ben-hur, M., Levy, G. J. 2006. Runoff and interrill erosion in sodic soils treated with dry PAM and phosphogypsum. Soil Sci. Soc. Am. J. 70, 679 ─ 690.

Terry, N., Huston, R. P. 1975. Effects of calcium on the photosynthesis of intact leaves and isolated chloroplasts of sugar beets. Plant Physiol. 55, 923 ─ 927.

Tisdale, S. L., Nelson, W. L., Beaton, J. D. 1985. Soil fertility and fertilizers. Mac Millan Publishing Company, New York, Fourth Edition, chapter 8, pp 329 ─ 339.

Toma, M., Saigusa, M., Gafoku, N., Sumner, M. E. 2005. Effects of gypsum on amelioration of subsoil acidity in Andisols. J. Integr. Field Sci. 2, 69 ─ 71.

Toma, M., Sumner, M. E., Weeks, G., Saigusa, M. 1999. Long-term effects of gypsum on crop yield and subsoil chemical properties. Soil Sci. Soc. Am. J. 39, 891 ─ 895.

Toma, M., Saigusa, M. 1997. Effects of phosphogypsum on ameliorating strongly acid non-allophanic Andosols. Plant Soil 192, 49 ─ 55.

Tuna, A. L., Kaya, C., Ashraf, M., Altunlu, H., Yokas, I., Yagmur, **B. 2007.** The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ. Exp. Bot. 59, 173 ─ 178.

Von Willert, F. J., Stehouwer, R. C. 2003. Compost, limestone, and gypsum effects on calcium and aluminum transport in acidic minespoil. Soil Sci. Soc. Am. J. 67, 778 ─ 786.

Wang, J. P., Harsh, R., Guo-Ping, Z., Neville, M., Zhou, M. X. 2006. Aluminium tolerance in barley (*Hordeum vulgare* L.): physiological mechanisms, genetics and screening methods. J. Zhejiang Univ. Science B. 7, 769 ─ 787.

Watanabe, T., Osaki, M. 2002. Mechanisms of adaptation to high aluminum condition in native plant species growing in acid soils: A review. Commun. Soil Sci. Plant Anal. 33, 1247 ─ 1260.

White, P., Broadley, M. R. 2003. Calcium in plants. Ann. Bot. 92, 487 ─ 511.

Yakimova, E. T., Kapchina-Toteva, V. M., Weltering, E. J. 2007. Signal transduction events in aluminum-induced cell death in tomato suspension cells. J. Plant Physiol. 164, 702 ─ 708.

Yamamoto, Y., Kobayashi, Y., Devi, S. R., Rikiishi, S., Matsumoto, H. 2003. Oxidative stress triggered by aluminum in plant roots. Plant Soil 255, 239 ─ 243.

Yamamoto, Y., Kobayashi, Y., Devi, S. R., Rikiishi, S., Matsumoto, H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. Plant Physiol. 128, 63 ─ 72.

Yocum, C. F. 2008. The calcium and chloride requirements of the O_2 evolving complex. Coordin. Chem. Rev. 252, 296 ─ 305.

Zhang, X. B., Liu, P., Yang, Y. S., Gen-Di, X. 2007. Effect of Al in soil on photosynthesis and related morphological and physiological characteristics of two soybean genotypes. Bot. Studies 48, 435 ─ 444.

Zhang, W. H., Rengel, Z. 1999. Aluminium induces an increase in cytoplasmic calcium in intact wheat root apical cells. Aust. J. Plant Physiol. 26, 401 ─ 409.

Zheljazkov, V. D., Astatkie, T., Caldwell, C. D., Macleod, J., Grimmett, M. 2006. Compost, manure, and gypsum application to timothy/red clover forage. J. Environ. Qual. 35, 2410 ─ 2418.

Chapter 3. *Mitigation of Al toxicity through the Regulation of Ca/Al Ratios in Highbush Blueberry (Vaccinium corymbosum L.) using Calcium Sulfate*

Paper submitted to Journal of Agronomy and Crop Science 2012

MITIGATION OF Al TOXICITY THROUGH THE REGULATION OF Ca/Al RATIOS IN *Vaccinium corymbosum* L USING CALCIUM SULFATE

Meriño-Gergichevich C.[1], Alberdi, M.[2,3] and Reyes-Díaz M. [2,3*] and N. Huner[4]

[1]Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

[2]Departamento de Ciencias Químicas y Recursos Naturales; Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera, Temuco, Chile.

[3]Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Temuco, Chile.

[4]Department of Biology, University of Western Ontario, London, Ontario, N6A 5B7 Canada

[*]Corresponding author: Marjorie Reyes Díaz.

Departamento de Ciencias Químicas y Recursos Naturales,
Facultad de Ingeniería, Ciencias y Administración,
Universidad de La Frontera
Casilla 54-D
Temuco (Chile)
Telephone: (56-45) 325493
Fax: (56-45) 325050
e-mail: reyesm@ufro.cl

ABSTRACT

The effect of $CaSO_4$ on Ca/Al molar ratio (Ca/Al) and on chemical, physiological and biochemical features in highbush blueberry (*Vaccimium corymbosum* L.*)* was investigated. Cultivars with contrasting tolerance to Al (Legacy, Al-tolerant and Bluegold, Al-sensitive) were grown for 15 days in a nutrient solution containing $CaSO_4$ concentrations (2.5, 5, and 10 mM) and Al (100 and 200 μM). Afterwards, leaf and roots were harvested to evaluate calcium (Ca) and aluminium (Al) concentrations, lipid peroxidation and enzymatic and non-enzymatic antioxidant responses. In addition, photosynthetic performance was determined. In both cultivars, Ca content and Ca/Al increased up to ~100% and 180%, respectively by adding $CaSO_4$ concomitant with a reduction in foliar Al in both Legacy and Bluegold ($r=-0.80$; $P\leq0.001$ and $r=-0.74$; $P\leq0.001$, respectively). A high Ca/Al had a positive effect on photochemical parameters in both cultivars ($P\leq0.05$) as well as in the reduction of oxidative stress and increase of total phenols and SOD, particularly in Legacy. Furthermore, *V. corymbosum* develops well in acid soils, where Ca/Al ratio is typically low; $CaSO_4$ amendment, mainly at 5 and 10 mM, may represent an effective alternative to application in Chilean acid soils, as Ca source and reduction of toxic Al especially in Legacy.

Keywords: *Acid soil, Aluminum toxicity, molar ratio, Calcium sulfate, blueberry.*

3.1 INTRODUCTION

At soil pH ≤ 5.5, toxic aluminium (Al^{3+}) is a main stress factor for several cultivated plants. The effects of Al toxicity have been studied more in roots than in upper organs (Kochian et al. 2005, Ryan and Delhaize 2010), because Al effects are first manifested in the roots (Delhaize and Ryan, 1995, Ryan and Delhaize 2010), reducing growth and nutrient uptake capacity, mainly Ca (Rengel and Robinson 1989, Poschenrieder et al. 2008). Moustakas et al. (1995) pointed out that Al^{3+} stress affects photosynthesis as result of a partial inhibition of photosynthetic electron transport rate (ETR) and closure of reaction centers in photosystem II (PSII). Furthermore, other photochemical parameters as the maximum quantum yield (Fv/Fm) and the effective quantum yield of PSII (ΦPSII) also decreased as a consequence of Al^{3+} stress in blueberry cultivars (Reyes-Díaz et al. 2009, 2010). Another negative effect of toxic Al on plants is the increase in reactive oxygen species (ROS), which induce oxidative stress and lipid peroxidation of cell biomembranes, which may result in cell death (Yamamoto et al. 2002, Ma et al. 2007). Studies have reported that a rise in the antioxidant activity is induced to mitigate the oxidative stress that enhances plant tolerance to Al^{3+} (Guo et al. 2006, Shao et al. 2008). Antioxidant enzymatic (e.g. superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Blokhina et al. 2003, Khan et al. 2007) and non-enzymatic systems (polyphenols, including flavonoids, anthocyanins and phenols) (Prior et al. 1998, Prior et. al. 2005) are frequently associated with a reduction in oxidative stress (Ma et al. 2007, Shao et al. 2008).

Calcium is a crucial regulator for growth and development of plants (Hepler 2005). It is important for many functions such as structural support and environmental signal

perception (Schaberg et al. 2006), being involved in plant tolerance to different stresses by regulating the antioxidant metabolism (Jiang and Huang 2001, Cheng et al. 2002). Inadequate levels of foliar Ca can also reduce the photosynthetic performance of plants, especially the water spplitting reaction in PSII (Ghanotakis and Yocum 1990, Vrettos et al. 2001; Miqyass et al. 2007, Yocum 2008).

The interaction between Ca and Al is probably the most important factor affecting Ca uptake and transport in plants grown in acid soils (Zhang and Rengel 1999, Rengel and Zhang 2003, Schaberg et al. 2006) because these two cations compete for the active sites of soil and plant roots (Ryan et al. 1997, Kinraide 1998, Kinraide et al. 2004). Moreover, the degree of Al^{3+} stress is strongly correlated with Ca/Al rather than to the Al concentration in soil or nutrient solution (Cronan and Grigal 1995). This molar ratio in soil nutrient solution and plant tissues has been suggested as one of the best expressions for assessing Al toxicity in plants (Cronan and Grigal 1995, Brunner et al. 2002). The value of this molar ratio able to indicate the Al^{3+} toxicity risk is highly variable depending on the soil solution, plant species and organs. For example, Boudot et al. (1994) reported that a soil solution with a Ca/Al lower than 1 or 2 would be in serious danger of Al^{3+} toxicity for *Picea abies* and *Fagus silvatica*, whereas Truman et al. (1986) from experiments in nutrient solution reported that Ca/Al ratios below 10.5 indicated risk of Al^{3+} toxicity. In *Pinus radiata* fine roots values of 0.2 and 0.1 Ca/Al had an estimated 50% and 80% Al^{3+} toxicity risk, respectively (Cronan and Grigal, 1995). For leaves, these authors reported that a Ca/Al ratio less than 12.5 would indicate a 50% risk of Al^{3+} toxicity and a Ca/Al lower than 6.5 a 75% risk.

To reduce subsoil Al toxicity in acid soils and enhance Ca^{2+} availability for plants, the addition of calcareous amendments (e.g. calcite lime and/or gypsum) has been studied (Fenn and Gobran 1999, Caires et al., 2002, Ritchey and Snuffer 2002, Toma et al. 2005, Caires et al. 2006). The effectiveness for reducing Al toxicity to a greater or lesser extent depends on the Ca source and the crop species used (Takahashi et al. 2006a, Bachiega et al. 2007, Meriño-Gergichevich et al. 2010). Nevertheless, the effects of calcareous amendments on radical and foliar Ca/Al in fruit crops have been scarcely considered, in particular those relating to $CaSO_4$ (Sanderson et al. 1995, Hanson and Berkheimer 2004). Currently, $CaSO_4$ application may be an interesting option as an amendment in acid soils due to its high Al^{3+} ameliorative effect on subsoil (Toma et al. 2005) and the increase in Ca contents of soil solutions (Bachiega et al. 2007). Hence, $CaSO_4$ represents an important source of Ca in addition to sulfur (S) for plant nutrition (Toma et al. 2005, Zheljazkov et al. 2007). Toma et al. (1999) suggested that an increase in exchangeable Ca and S after $CaSO_4$ application increased the yield up to 50% in corn (*Zea mays* L.) and alfalfa (*Medicago sativa* L.). It can also improve the contents of nitrogen (N), phosphorus (P), potassium (K), manganese (Mn), zinc (Zn), and the Ca/Al in plant tissues and thereby the plant productivity (Caires et al. 2006; Tuna et al. 2007). Stout and Priddy (1996) mentioned that an amendement with $CaSO_4$ augmented the yield in 21% concomitant with an increase of ~ 45% in the Ca/Al in alfalfa roots compared to the non-amended ones. It is remarkable that this amendment can reduce Al toxicity in acid soils without altering the soil pH necessary for a good growth and development of some crops such as commercial berries (Gough 1997, Mora et al. 2002, Takahashi et al. 2006a, b).

Blueberry is an important crop in Chile (Prodorutti et al. 2007, Espinoza et al. 2009) due to its richness in antioxidants, which are beneficial to human health. In southern Chile blueberry is mainly cultivated in soils with a pH from 3.5 to 5.5. Although this crop species is well adapted to this soil type, it is sensitive to Al toxicity induced by acidity, decreasing its viability and productivity (Yang et al. 1996, Blatt and McRae 1997, Suzuki et al. 1999). Therefore, the aim of this study was to ascertain the effect of $CaSO_4$ on Ca/Al molar ratio (Ca/Al) and on chemical, physiological and biochemical features in two highbush blueberry cultivars with contrasting Al tolerance hydroponically grown under phytotoxic aluminum.

3.2 MATERIAL AND METHODS

3.2.1 Plant material and experimental conditions

The study was carried out in a greenhouse at the Universidad de La Frontera, Temuco, La Araucanía Region, Chile. Two highbush blueberry cultivars frequently cultivated in southern Chile (Guerrero 2006) and with contrasting Al tolerance (Legacy and Bluegold, Al-tolerant and Al-sensitive, respectively) (Reyes-Díaz et al. 2009, 2010, Inoztroza-Blancheteau et al. 2011) were used in this study. One-year-old plants growing in a substrate of 1 oat: 1 shell sawdust: 1 pine needless by volume were provided by commercial farm Berries San Luis, located in Lautaro, La Araucanía Region, Chile. Plant roots were washed with abundant deionized water (<1 microsiemens) and then transferred to plastic pots (four plants per pot) and filled with 10 L of Hoagland nutrient solution (Hoagland and Arnon 1950) for conditioning for seven days. The Hoagland nutrient solution composition was as follows: $Ca(NO_3)$ 2 mM, KNO_3 3 mM, $MgSO_4$ 1 mM, KH_2PO_4 0.1 mM, H_3BO_3 22 μM, $MnSO_4$ 2 μM,

NH_4NO_3 1 mM, $(NH_4)_6Mo_7O_{24}$ 0.07 μM, $ZnSO_4$ 1.6 μM, $CuSO_4$ 0.4 μM, and Fe-EDTA 20 μM. After conditioning, plants were transferred to a Hoagland hydroponic solution containing increased $CaSO_4$ concentrations (2.5, 5 and 10 mM) and Al, as aluminum chloride ($AlCl_3$), in 100 and 200 μM, for 15 days. The treatments were: (i) nutrient solution alone (control); (ii) nutrient solution plus 100 μM $AlCl_3$ (100Al); (iii) nutrient solution plus 200 μM $AlCl_3$ (200Al); (iv) nutrient solution plus 100 μM $AlCl_3$ plus 2.5 mM $CaSO_4$ (100Al+2.5G); (v) nutrient solution plus 200 μM $AlCl_3$ plus 2.5 mM $CaSO_4$ (200Al+2.5G); (vi) nutrient solution plus 100 μM $AlCl_3$ plus 5 mM $CaSO_4$ (100Al+5G); (vii) nutrient solution plus 200 μM $AlCl_3$ plus 5 mM $CaSO_4$ (200Al+5G); (viii) nutrient solution plus 100 μM $AlCl_3$ plus 10 mM $CaSO_4$ (100Al+10G); (ix) nutrient solution plus 200 μM $AlCl_3$ plus 10 mM $CaSO_4$ (200Al+10G). Every pot had a water pump to aerate the nutrient solution. The pH was adjusted daily to 4.5 with 0.1 M HCl or 1M NaOH and measured with a high-accuracy portable pH meter (model pH-0.13; Hi-Tech-Instruments, Shanghai, China). The chemical speciation of solution was calculated using the computer speciation program GEOCHEM-EZ, by which chemical species such as free ions, soluble complexes, and chelates were calculated (Shaff et al. 2010) (see in appendices).

The greenhouse conditions were: 25/20ºC (day/night), 16/8 h light regimen (light/dark, respectively), relative air humidity of 70%, and photosynthetic photon flux density (PPFD) mean of 300 μmol photons m^{-2} s^{-1}. At the end of experiment, chemical, physiological and biochemical analyses of roots and leaves were performed as indicated below. At 15d, plants of different treatments were harvested; one half

was used for chemical analyses and another half was stored at -80ºC in a freezer (REVCO®, model ULT 1386-5-V41, USA) until biochemical analysis.

3.2.2 Chemical analyses

Contents of Ca and Al were determined in the leaves and roots according to the method described by Sadzawka et al. (2007), using a simultaneous multi-element atomic absorption spectrophotometer (model UNICAM 969 Atomic absorption Spectrometer, England).

3.2.3 Physiological determinations

3.2.3.1 Net Photosynthesis

Net photosynthesis of attached leaves was performed by using an infrared gas analyzer (IRGA) (Licor LI-6400 XTP) between 9:00 am and 11:00 am. Using a PPFD of 300 µmol photons m^{-2} s^{-1}, external air was scrubbed with CO_2 and mixed with a supply of pure CO_2, resulting in a reference concentration of 360 ppm of CO_2, with a flow rate of 200 mL min^{-1} and 80% external relative humidity. The temperature inside the leaf chamber was 22ºC (Reyes-Díaz et al., 2009).

3.2.3.2 Chlorophyll fluorescence parameters

In order to determine the photochemical efficiency of PSII in leaves, the basic protocol of Reyes-Díaz et al. (2009) adapted to blueberries was followed. Attached leaves were dark adapted for 20 minutes, and chlorophyll fluorescence was measured using a portable pulse-amplitude modulated fluorimeter (FMS 2; Hansatech Instruments, Norfolk, UK). Minimal fluorescence (F_0) was determined by applying a weak-modulated light (0.4 µmol m^{-2} s^{-1}) and maximum fluorescence (Fm) was

induced by a short pulse (0.8 s) of saturating light (9,000 μmol m^{-2} s^{-1}). After 10s, actinic light (300 μmol m^{-2} s^{-1}) was turned on to obtain fluorescence parameters during steady-state photosynthesis had been reached to determine the maximum fluorescence in light-adapted leaves (Fm') and steady-state fluorescence (Fs). Finally, the actinic light was turned off and a 5-s far-red (FR) pulse was applied immediately to obtain minimum fluorescence in light-adapted leaves (F_0'). Different fluorescence parameters of PSII were determined: maximum quantum yield (Fv/Fm), effective quantum yield (ΦPSII) and electron transport rate (ETR). ΦPSII was calculated as (Fm'– Fs)/Fm' and ETR as PPFD x 0.5 x ΦPSII x 0.84 (Maxwell and Johnson 2000).

3.2.4 Biochemical analyses

3.2.4.1 Lipid peroxidation

The level of lipid peroxidation (LP) was expressed as malondialdehyde acid (MDA) content (nmol MDA g^{-1} FW) and determined as 2-thiobarbituric acid reactive-substances (TBARS), according to the modified protocol by Du and Bramlage (1992).

3.2.4.2 Radical scavenging activity

Fresh leaves and root samples were frozen in liquid nitrogen, powdered in a mortar, and homogenized with 1mL of 80% (v/v) methanol, centrifuged to 10,000 rpm x 5 min (4ºC), and then the supernatant was collected (~500 μL) and stored at -80°C until analysis (Li et al. 2007).

3.2.4.3 Superoxide dismutase activity

The Superoxide dismutase (SOD) activity in leaves and roots was assayed by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium

(NBT) method described by Oberley and Spitz (1986). The samples were homogenized in 1mL buffer extraction (phosphate 0.1M pH 7.0) and centrifuged at 13,000 rpm x 15 min (4ºC). Afterwards, extracts were mixed with 10 mM EDTA, 260 mM methionine, 322 μM NBT. Finally, riboflavin was added and the test tubes were illuminated for 15 minutes and the absorbance of the samples was measured at 560 nm in an UV-VIS spectrophotometer. Non-illuminated and illuminated reactions without supernatant were used as controls. One SOD unit was defined as the amount of enzyme corresponding to 50% inhibition of the NBT reduction (Donahue et al. 1997). The SOD activity was expressed as U mg protein^{-1}. Enzyme activity was expressed on protein basis. Total protein was estimated according to Bradford (1976) using bovine serum albumin (BSA) as the standard.

3.2.4.4 Total phenols content

Total phenol content (TPC) was determined following the method of Slinkard and Singleton (1997) using the Folin-Ciocalteu reagent. The absorbance of samples was measured in a UV-VIS spectrophotometer at 765 nm and expressed as μg chlorogenic acid equivalents (CAE) per g^{-1} FW.

3.2.5 Experimental design and statistical analyses

The experimental design corresponded to a factorial split-plot with four replicates each. In this design the treatments are randomly assigned to groups of experimental units called blocks or repetitions, maintaining the variability between experimental units inside a block, maximizing the differences between blocks (Little and Hills 1978). Values corresponded to the mean of four replicates for each cultivar, treatments and chemical, physiological and biochemical determinations. For

normality and equal variance all data were tested by Kolmogorov-Smirnov and Levene test respectively. A two-way analysis of variance (ANOVA) test was used, where the factors are two cultivars and nine treatments, and a Tukey test with a significance level at $P \leq 0.05$ was considered for comparison of values with significant differences. All statistical analyses were performed with Sigma Stat 3.1 (SPSS® Inc., Chicago, IL, USA).

3.3 RESULTS

3.3.1 Effects of $CaSO_4$ on mineral content and Ca/Al molar ratios

After 15d of experiment, Bluegold leaves showed at least two-fold higher Ca contents than Legacy in all treatments ($P \leq 0.001$), whereas the roots of Legacy presented in average 67% higher Ca content than Bluegold ($P \leq 0.05$; Table 3.1). Neither Legacy nor Bluegold showed any significant decrease in foliar Ca levels at 100 and 200Al treatments compared to the non-treated plants. By contrast, the same treatments significantly reduced the root Ca content (~50%) compared to the control in both cultivars, independent of their Al tolerance ($P \leq 0.05$). When Al-treated Bluegold plants were supplied with $CaSO_4$ at 5 and 10 mM, foliar Ca content increased significantly (between 24 and 55%) in comparison to the control plants, while root Ca content increased up to 127% in Legacy (Table 3.1). In leaves, Bluegold exhibited a strong enhanced Al content (~1,900%), when the plants were subjected to 100 and 200Al in comparison to the non-treated control plants ($P \leq 0.05$). Nevertheless, $CaSO_4$ at 5 and 10 mM significantly reduced foliar Al content in Legacy (~3-fold) and Bluegold (~6-fold), whereas root Al content was significantly

decreased by 5 mM $CaSO_4$ supply in Bluegold compared to control plants. In both cultivars a statistically significant interaction between cultivar and treatment were observed either in leaves or roots for Al contents ($P \leq 0.001$).

Molar ratios between Ca and Al were calculated from their concentrations in the nutrient solution and tissues (leaves and fine roots) of blueberry plants grown in hydroponic experiment. A positive relation was observed between Ca/Al molar ratio of nutrient solution and foliar Ca/Al (fCa/Al) of Bluegold ($r=0.91$; $P=0.005$) and Ca/Al molar ratio of roots (rCa/Al) in Legacy ($r=0.80$; $P=0.015$). The fCa/Al and rCa/Al of Legacy and Bluegold significantly increased with 2.5, 5 and 10 mM $CaSO_4$ compared to the non-amended plants (Table 3.1). Foliar Ca/Al was correlated positively with the Ca content of leaves in Legacy ($r=0.48$; $P=0.016$), and Bluegold ($r=0.38$; $P=0.049$), and negatively with foliar Al content (Legacy $r=-0.80$ and Bluegold $r=-0.74$; $P \leq 0.001$). For Legacy, no differences in the fCa/Al were found in the Al supply treatments (100 and 200Al) compared to the control, but in Bluegold fCa/Al decreased more than 63% ($P \leq 0.05$; Table 3.1). A statistically significant relation between rCa/Al and Ca content was found (Legacy $r=0.921$ and Bluegold $r=0.63$; $P \leq 0.05$), although with Al contents a negative correlation was found only in Bluegold ($r=-0.71$; $P \leq 0.001$). For both fCa/Al and rCa/Al ratios significant interactions between cultivars and $CaSO_4$ treatment were found ($P \leq 0.001$).

3.3.2 Photosynthetic performance of PSII under $CaSO_4$ treatments

3.3.2.1 Net photoynthesis

In Legacy, $CaSO_4$ treatments did not show any significant effect on net photosynthesis compared to the control plants (Table 3.2). However, 200Al caused a significant reduction of net photosynthesis (15%) compared to the control plants ($P \leq 0.001$) and no relationship between fCa/Al and net photosynthesis was observed. In Bluegold, plants subjected to 100Al and 200Al for 15 days showed a significant decrease (~14%) in net photosynthesis, concomitant with lower fCa/Al. In both cultivars, a reduced photosynthesis by Al was recovered with $CaSO_4$ at same level of control plants, although 200Al+10G the amendment performance did not recover the negative effects caused by Al in Bluegold, showing a lower net photosynthesis than the control ($P \leq 0.050$). Foliar and root Al content in Bluegold were negatively correlated with net photosynthesis ($r = -0.47$; $P = 0.05$ and $r = -0.70$; $P \leq 0.001$, respectively).

3.3.2.2 Photochemical efficiency of PSII

There was practically no variation in the Fv/Fm in cultivars under exposure of treatment, remaining between 0.7-0.8 (data not shown), which is the normal value ranged for healthy leaves (Björkman and Demmig 1987). Frequently, the ΦPSII and ETR increased in Legagy by $CaSO_4$ addition compared to the control ($P \leq 0.05$), while Bluegold exhibited lower ΦPSII and ETR with minor changes in all treatments (Figure 3.1 A,B). Foliar Ca/Al was correlated directly with ΦPSII and ETR (around $r = 0.65$; $P \leq 0.001$ and $r = 0.48$; $P = 0.011$ for Legacy and Bluegold, respectively). The foliar Al content was negatively correlated with ΦPSII and ETR, in Legacy and Bluegold (around $r = -0.65$ and -0.41; $P \leq 0.05$), respectively.

Table 3.1. Mineral concentration in leaves and roots of blueberry cultivars subjected to different levels of Al and $CaSO_4$ in nutrient solution after 15 days. Values are means of four measurements ± S.E. Different lowercase letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same cultivar. Uppercase letter indicates differences ($P \leq 0.05$) between cultivars for the same treatment.

Cultivar	Treatment	Mean mineral concentrations (mg kg^{-1} DW)				Ca/Al molar ratio	
		Ca		Al			
		leaves	roots	leaves	roots	leaves	roots
Legacy	Control	6,200±43^{Bab}	5,927±713^{Ac}	66±6^{Ac}	120±0.2^{Bc}	2.60±0.20^{Bcd}	0.35±0.03^{Bcd}
	100Al	5,627±152^{Bb}	3,616±177^{Ad}	784±17^{Ba}	7,065±101^{Aa}	1.90±0.01^{Ad}	0.28±0.01^{Ad}
	200Al	5,130±117^{Bb}	2,979±123^{Ad}	868±9^{Ba}	6,315±744^{Aa}	1.65±0.04^{Ad}	0.24±0.01^{Ad}
	100Al+2.5G	5,812±397^{Bb}	6,075±72^{Ac}	463±20^{Bab}	6,570±132^{Aa}	3.24±0.14^{Acd}	0.49±0.04^{Ac}
	200Al+2.5G	4,420±205^{Bb}	4,687±221^{Acd}	330±11^{Bb}	5,541±884^{Aab}	3.82±0.05^{Ac}	0.36±0.01^{Bcd}
	100Al+5G	6,597±431^{Bab}	5,021±122^{Ac}	267±34^{Ab}	5,530±90^{Aab}	7.61±0.03^{Bb}	0.49±0.00^{Bc}
	200Al+5G	9,150±291^{Ba}	6,139±25^{Ac}	306±73^{Ab}	5,685±456^{Aab}	10.08±0.90^{Aa}	0.62±0.02^{Bb}
	100Al+10G	9,028±196^{Ba}	10,232±964^{Ab}	369±23^{Ab}	3,890±10^{Ab}	8.44±0.59^{Bab}	1.59±0.03^{Aa}
	200Al+10G	7,903±18^{Ba}	13,462±137^{Aa}	268±39^{Bb}	4,616±955^{Ab}	6.70±0.01^{Bb}	1.51±0.07^{Aa}
Bluegold	Control	12,295±702^{Abc}	1,937±89^{Bb}	77±2^{Ad}	79±0.4^{Cc}	4.53±0.12^{Ae}	0.64±0.02^{Ad}
	100Al	10,708±386^{Ac}	1,414±150^{Bc}	1,518±92^{Aa}	1,665±256^{Ba}	1.74±0.07^{Af}	0.24±0.03^{Ae}
	200Al	10,930±608^{Ac}	951±35^{Bc}	1,286±34^{Ab}	1,464±62^{Ba}	2.26±0.12^{Af}	0.18±0.00^{Ae}
	100Al+2.5G	15,452±147^{Aa}	2,623±77^{Ba}	1,406±28^{Aab}	165±7^{Bd}	2.90±0.21^{Aef}	4.31±0.32^{Bb}
	200Al+2.5G	15,204±132^{Aa}	2,946±144^{Ba}	1,005±11^{Ac}	652±43^{Bc}	4.38±0.22^{Ae}	1.21±0.09^{Ac}
	100Al+5G	12,332±703^{Abc}	2,913±82^{Ba}	242±22^{Ae}	156±0.4^{Bd}	13.77±0.04^{Ab}	5.15±0.04^{Aa}
	200Al+5G	17,177±1394^{Aa}	1,565±14^{Bb}	207±14^{Ae}	394±14^{Bcd}	8.39±0.42^{Bd}	1.08±0.04^{Ac}
	100Al+10G	17,860±404^{Aa}	1,857±43^{Bb}	265±43^{Ae}	1,036±21^{Bb}	15.99±0.73^{Aa}	0.47±0.00^{Bd}
	200Al+10G	19,084±1201^{Aa}	1,604±18^{Bbc}	457±44^{Ae}	1,461±200^{Ba}	11.29±0.61^{Ac}	0.50±0.08^{Bd}

Table 3.2. Net photosynthesis (μmol CO_2 m^{-2} s^{-1}) in two highbush blueberry cultivars (Legacy and Bluegold) under Al and $CaSO_4$ treatments. Values represent the mean of four replicates ± S.E. Different lowercase letters indicate statistically significant differences (P≤0.05) between treatments for the same cultivar. Different uppercase letters show differences (P≤0.05) between cultivars for the same treatment. Measurements were obtained under relative air humidity (RH) of 70%, and photosynthetic photon flux density (PPFD) average of 300 μmol photons m^{-2} s^{-1}.

Cultivar	Treatment	Net photosynthesis (μmol CO_2 m^{-2} s^{-1})
Legacy	Control	8.45± 0.54Aab
	100Al	7.82± 0.20Ab
	200Al	7.19± 0.11Ac
	100Al+2.5G	8.91± 0.38Aa
	200Al+2.5G	7.95± 0.36Bab
	100Al+5G	8.62± 0.45Aa
	200Al+5G	7.91± 0.49Aab
	100Al+10G	8.84± 0.44Aa
	200Al+10G	7.70± 0.23Ab
Bluegold	Control	8.83±0.38Aa
	100Al	7.62±0.13Ab
	200Al	7.72±0.17Ab
	100Al+2.5G	9.03±0.11Aa
	200Al+2.5G	9.06±0.20Aa
	100Al+5G	9.05±0.17Aa
	200Al+5G	8.70±0.18Aa
	100Al+10G	9.22±0.34Aa
	200Al+10G	7.47±0.15Ab

3.3.3 Lipid peroxidation and antioxidant plant responses

At the end of the experiment, Bluegold leaves exhibited up to (~47%) higher foliar LP than Legacy in 100Al ($P \leq 0.001$, Figure 3.2A). Interactions between cultivar and Al treatment were found for the LP ($P \leq 0.001$). A negative relationship between foliar LP and fCa/Al was observed for Legacy ($r = -0.39$; $P = 0.04$) and Bluegold ($r = -0.75$; $P \leq 0.001$), whereas foliar Al content and LP was significantly correlated in both cultivars ($r = 0.44$ for Legacy and $r = 0.64$ for Bluegold). In roots, the increased LP obtained by Al treatments was reduced by $CaSO_4$ addition in both cultivars, compared to the control plants ($P \leq 0.05$) (Figure 3.2A). In Legacy a negative relationship between rCa/Al and LP ($r = -0.55$; $P = 0.003$) was found. Calcium and LP in roots were also negatively correlated in Legacy ($r = -0.54$; $P = 0.003$) and Bluegold ($r = -0.57$; $P = 0.019$), while in the latter cultivar the Al content was correlated with LP ($r = 0.60$; $P = 0.001$).

Radical scavenging activity (RSA) of Legacy and Bluegold leaves displayed a significant reduction under the highest Al treatment compared to the control leaves, whereas $CaSO_4$ did not increase RSA significantly (Figure 3.2B). In the roots, $CaSO_4$ only improved RSA at 100Al+2.5G and 100Al+5G in Legacy, while a decrease was observed at 200Al in comparison to the non-treated plants. Bluegold exhibited a correlation between the Al content and RSA in roots ($r = 0.52$; $P = 0.004$).

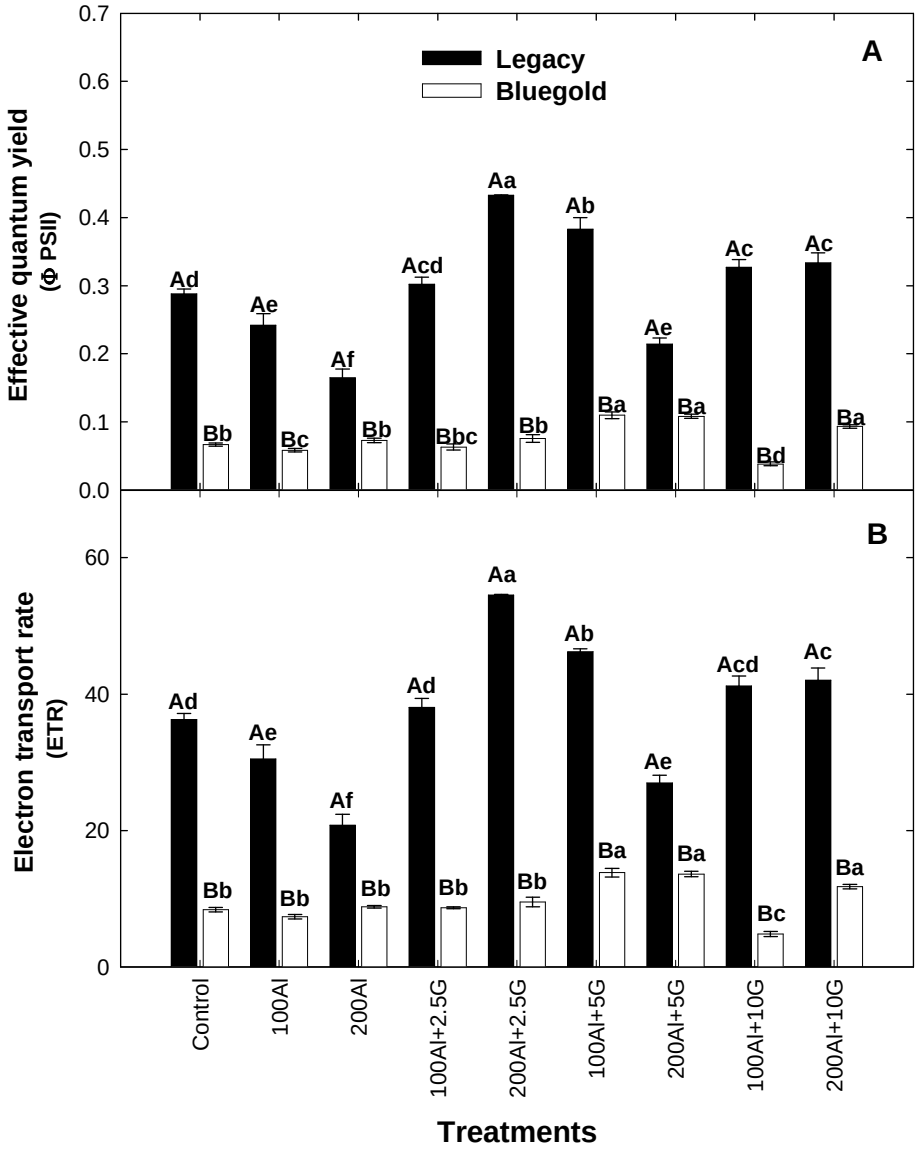

Figure 3.1. Changes in effective quantum yield (ΦPSII) (A) and electron transport rate (ETR) (B) of two highbush blueberry cultivars grown in nutrient solution (15d) under Al and $CaSO_4$ treatments. Values represent the mean of four replicates ±S.E. Different lowercase letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same cultivar indicate statistically significant differences. Different upper case letters show differences ($P \leq 0.05$) between cultivar for the same treatment.

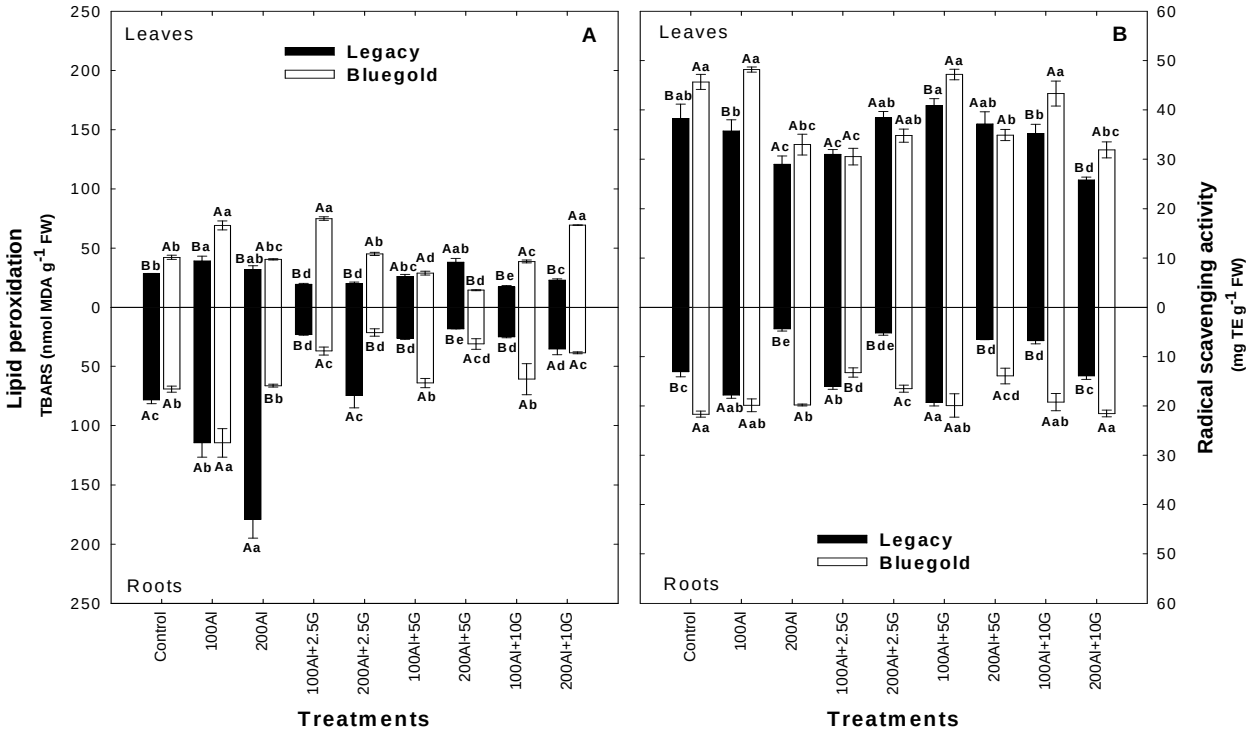

Figure 3.2. (A) Lipid peroxidation as malondialdehyde content (nmol MDA g^{-1} FW) and (B) radical scavenging activity (mg TE g^{-1} FW), in leaves and roots of two highbush blueberry cultivars grown in nutrient solution (15d) under Al and $CaSO_4$ treatments. Values represent the ,mean of four replicates ± S.E. Different lowercase letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same cultivar. Different uppercase letters show differences ($P \leq 0.05$) between cultivars for the same treatment.

The superoxide dismutase (SOD) activity in leaves was significantly increased in Legacy under 200Al+5G ($P \leq 0.001$) concomitant with the highest fCa/Al achieved under this treatment (Table 3.1, Figure 3.3). A correlation between fCa/Al and SOD activity were found ($r=0.79$; $P \leq 0.001$). By contrast, the enzyme activity in Legacy was significantly reduced by 200Al (Figure 3.3) and negatively correlated with foliar Al content ($r=-0.52$; $P=0.005$). Bluegold exhibited higher leaf SOD activity than Legacy ($P \leq 0.001$) mainly under Al treatment ($P \leq 0.05$, Figure 3.3). In roots, Legacy showed a higher SOD activity than Bluegold in the most treatments ($P \leq 0.001$), showing a significant SOD increase in treatments with predominant Al toxicity. A relation was found between root Al content and SOD activity ($r=0.46$; $P=0.014$), whereas Ca content and rCa/Al were negatively correlated with SOD activity ($r=-0.57$ and $r=-0.49$, respectively) in the roots. In Bluegold, all treatments reduced SOD activity in respect to the control plants ($P \leq 0.05$, Figure 3.3).

A significant interaction between cultivar and treatments for Total phenol content (TPC) was established. Legacy and Bluegold plants subjected to toxic Al exhibited no increased TPC compared to the non-treated plants ($P>0.05$), but a $CaSO_4$ (5 mM) allowed a significant enhancing in both cultivars (Figure 3.4). In Legacy, TPC was significantly correlated with fCa/Al ($r=0.47$), Ca content ($r=0.42$), and negatively correlated with Al content ($r=-0.70$). Bluegold showed no clear increase in TPC related to an improved fCa/Al.

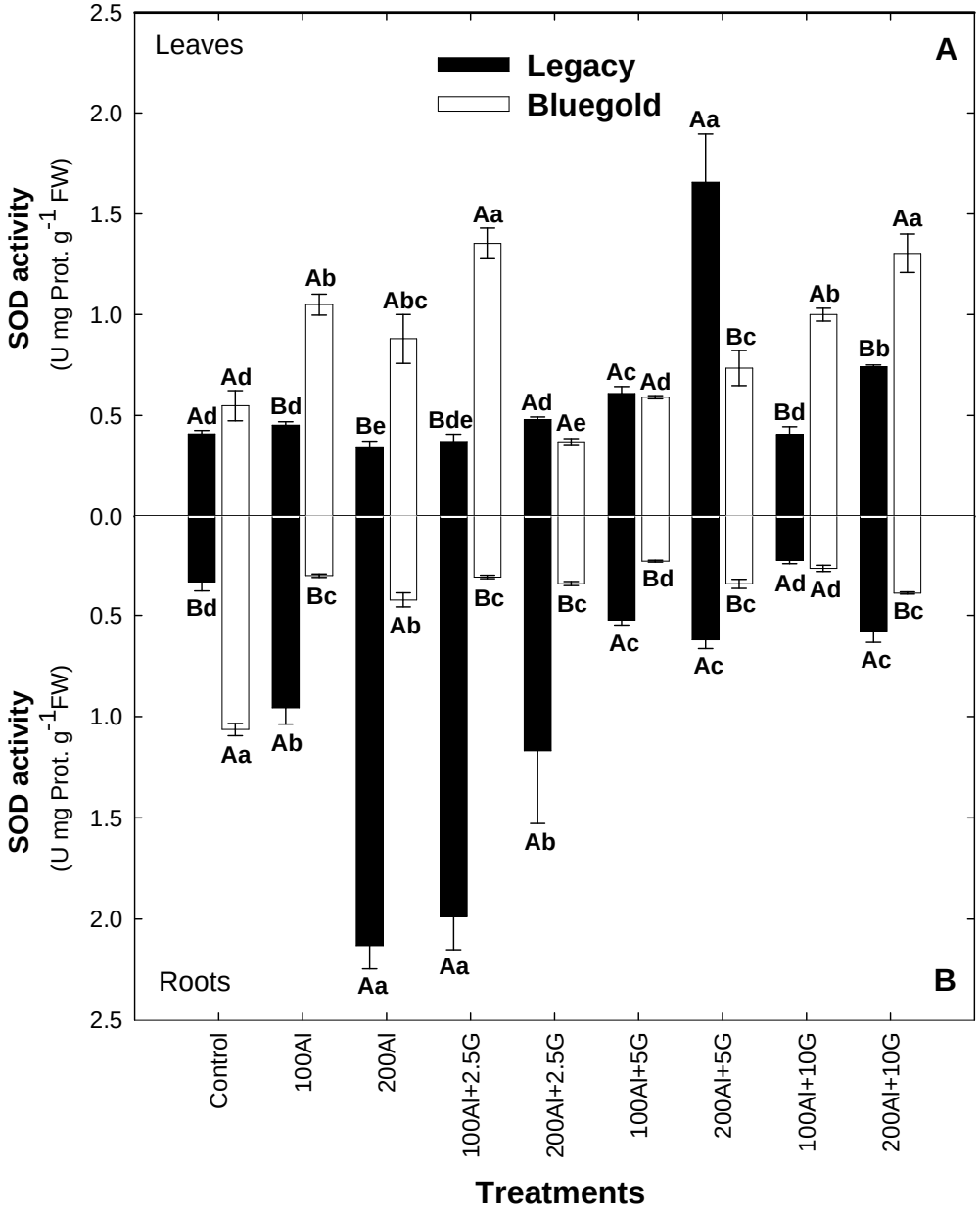

Figure 3.3. Superoxide dismutase (U mg Prot. g^{-1} FW) activity in leaves (A) and roots (B) of two highbush blueberry cultivars grown in nutrient solution (15d) under Al-toxicity and $CaSO_4$ treatments. Values represent the average of four replicates ± SE. Different lowercase letters indicate statistically significant differences ($P \leq 0.05$) among $CaSO_4$ treatments for the same cultivar. Different uppercase letters show differences ($P \leq 0.05$) between cultivars for the same treatment.

3.4 DISCUSSION

In this study, we informed about the effectiveness of $CaSO_4$ amendment added to an acid substrate in improving the constraints induced by toxic Al^{3+} in *V. corymbosum* cultivars. It was showed that the effectiveness of this amendement on Al toxicity depends of the dose of amendent applied and the cultivar. Field studies guided by Hanson and Berkheimer (2004), reported foliar Ca concentration from 3,000 to 4,000 mg kg^{-1} DW, in Ca-amended blueberry plants. This is generally consistent with the results obtained for the Al-tolerant cultivar (Legacy) under $CaSO_4$, while higher Ca contents (up to two-fold higher) for the Al-sensitive cultivar Bluegold were observed (Table 3.1). However, in the roots, Legacy presented up to eight-fold higher Ca concentration than Bluegold. Therefore, Ca concentration in these species depends on the cultivar, Ca uptake efficiency and the organ involved. Rout et al. (2001) reported that Al-sensitive wheat cultivars (*Triticum aestivum* L.) subjected to toxic Al exhibited less efficiency in root uptake, translocation and use of Ca and other nutrients, than the Al-tolerant cultivar. In this way, we found that the increased Ca content was concomitant with Al tissue reduction in both cultivars, with Legacy exhibiting a significantly higher Al concentration in roots (Table 3.1), which suggest that this cultivar could be an Al accumulator plant. According to Chenery (1949) plants containing equal to or more than 1000 mg Al kg^{-1} DW may be considered Al-accumulators and those with less than 1000 mg Al kg^{-1} DW as non Al-accumulators. By contrast, Reyes-Díaz et al. (2010) found in a long term Al-toxicity treatment that Legacy roots showed a 40% lower Al accumulation than Bluegold. Guo et al. (2006) reported that in barley (*Hordeum vulgare* L.) plants subjected to Al toxicity (100 μM) the addition of Ca (from 0.5 to 3.0 mM) efficiently reduced Al contents in tissues by

reducing Al uptake. Suzuki et al. (1999) reported that Al-induced injuries to shoots in highbush blueberry decreased with the coexistence of Ca at appropriate values.

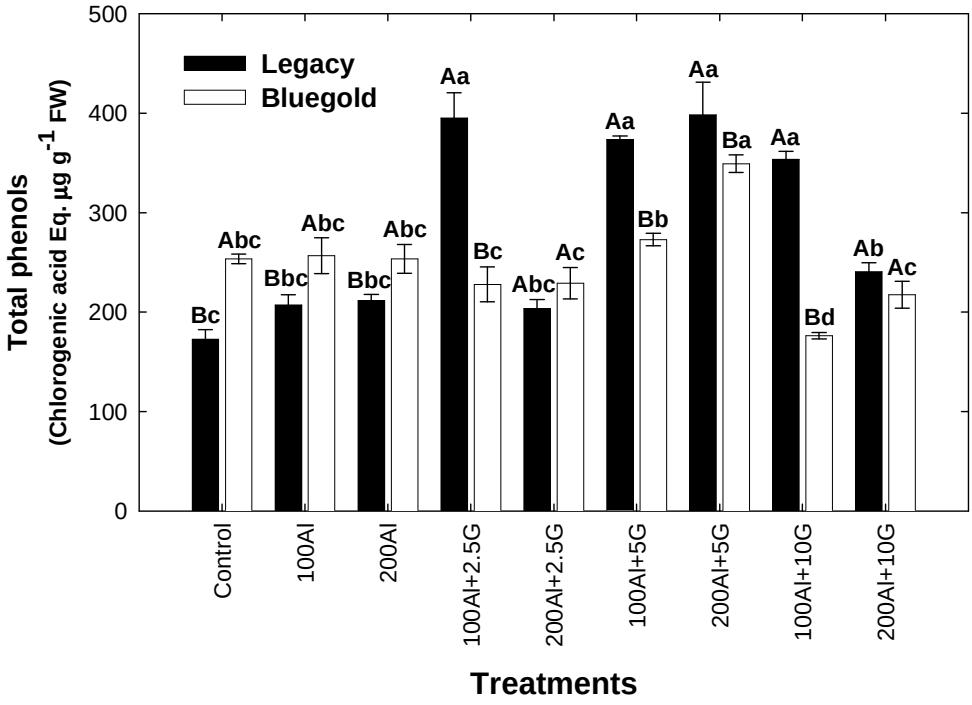

Figure 3.4. Total phenols content (µg CAE g^{-1} FW) in leaves of two highbush blueberry cultivars grown in nutrient solution (15d) under Al and $CaSO_4$ treatments. Values represent the average of four replicates ± SE. Different lowercase letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same cultivar. Different uppercase letters show differences ($P \leq 0.05$) between cultivars for the same treatment.

Bearing in mind the suggestions of Cronan and Grigal (1995) and Alvarez et al. (2005), about the importance of the Ca/Al molar ratios in woody species to evaluate the level of Al toxicity in plants, our results revealed that the application of $CaSO_4$ to nutrient solution with toxic Al increased this ratio in both cultivars with different Al-tolerances, Bluegold exhibiting a higher fCa/Al (from 1.74 to 15.99) than

Legacy (from 1.65 to 10.08) (Table 3.1; P≤0.001). Weber-Blaschke and Rehfuess (2002) studied the effect of different Ca sources on fCa/Al on *Fraxinus excelsior*, including $CaSO_4$ in a dose of 2000 kg ha^{-1}, and found a rise in the fCa/Al ratio up to 41(63%) under the effect of $CaSO_4$ compared to the non-amended control. In the present study the fCa/Al ratio as indexes of Al toxicity in both cultivars, which were negatively correlated with foliar Al concentration (around r=─0.80). Notwithstanding this, only Bluegold displayed a significant correlation with foliar Ca content (r=0.38; P=0.049), suggesting that a reduction in Al uptake (by the addition of $CaSO_4$) was the main effect of improved molar ratio. For fine roots (≤1mm), the rCa/Al of our studied cultivars under the both levels of applied Al treatments lie in the range between 0.1 and 0.2, presenting a high risk (50 to 80%) of Al stress (Cronan and Grigal 1995). It should be noted that by adding $CaSO_4$ these root ratios were raised up to 1.6 in Legacy and 5.15 in Bluegold, indicating a clear positive effect of $CaSO_4$ on the risk reduction of root Al stress, mainly in the sensitive cultivar (Bluegold). Weber-Blaschke and Rehfuess (2002) in roots of *F. excelsior* reported a ratio of 0.76 for $CaSO_4$-fertilized plants versus 0.10 in the control plants. Borken *et al.* (2007) reported similar ratios in Norway spruce [*Picea abies* (L.) Karst] roots when they evaluated rCa/Al in this species standing on humic and mineral soil horizons.

There is a paucity of information about Ca/Al molar ratio and physiological processes as photosynthesis has been scarcely reported. A net photosynthesis reduced by Al toxicity and nutrient deficiency could be considered a limiting factor related to the reduction of dry matter production (Reich et al. 1994, Giertych et al. 1997). In sugar maple (*Acer saccharum*), growing in acidic and nutrient imbalanced conditions, St. Clair et al. (2005) found a positive correlation between this foliar ratio and CO_2

exchange rate, and St. Clair et al. (2008) reported that lower ratios were more correlated with impaired photosynthesis. However, in our study fCa/Al of both cultivars was not correlated with net photosynthesis, whereas in the Al-sensitive Bluegold rCa/Al and root Al content were positively and negatively correlated with photosynthesis ($r=0.42$; $P=0.025$ and $r=-0.70$; $P\leq0.001$, respectively). Thus, these results have shown that Al perturbation in nutrient uptake by roots of the Al-sensitive cultivar was more associated with a decreased photosynthesis. Nonetheless, improved fCa/Al was correlated with ΦPSII (Legacy $r=0.66$; $P\leq0.001$ and Bluegold $r=0.48$; $P=0.011$) and ETR (Legacy $r=0.63$; $P\leq0.001$ and Bluegold $r=0.47$; $P=0.012$). We proposed that the increase in the photochemical parameters, due to an improved fCa/Al, could be associated to better efficiency in the light-driven oxidation of water in PSII, using Ca as cofactor (Vander Meulen et al. 2004, Yocum 2008, Chen et al. 2010), and a reduction of Al-content in the leaves. Reyes-Díaz et al. (2009, 2010) found that a higher Al accumulation in the leaves of blueberry genotypes reduced the photochemical efficiency of PSII.

The LP is early evidence of oxidative stress in plants, subjected to several environmental stresses. In this way, Al content in tissues has been associated with LP causing damage in biological membranes (Yamamoto et al. 2002, Bóscolo et al. 2003). Our study showed a concomitant increase of Al content in tissues with LP, mainly in Bluegold leaves and roots. Pereira et al. (2010) showed that cucumber (*Cucumis melo*) plants increased their MDA conent by almost 90% when the Al concentration in the nutrient solution was 100 μM, suggesting that Al sorption affects the conformation of the Ca pectates, which seem interact with carboxylate groups. Our findings demonstrated a significant Ca effect on the drop in MDA in roots, and

there were in fact correlations among these parameters (r=─0.54; P=0.003 and r=─0.44; P=0.019 in Legacy and Bluegold respectively). This might be explained as a response to increased antioxidant activity from adding Ca (Jiang and Huang 2001, Guo et al. 2006). The increased LP triggered by the Al content was also mitigated by an improved Ca/Al as the result of a better balance between Ca and Al mediated by the addition of $CaSO_4$, particularly at 5 and 10 mM treatments.

Plants have evolved strategies such as antioxidant compounds and enzymes to protect them against oxidative stress induced by environmental factors as phytotoxic Al stress (Lin and Kao 2000, Wang and Ballington 2007). Blueberries in particular, are recognized by their higher antioxidant activity than other vegetables (Wang et al. 2010). Reyes-Díaz et al. (2010) and Inoztroza-Blancheteau et al. (2011) associated the antioxidant activity with the degree of Al tolerance in blueberry cultivars subjected to Al toxicity. In our study, the sensitive cultivar Bluegold exhibited higher foliar and root RSA (measured by DPPH method) than tolerant Legacy ($P \leq 0.001$, Figure 3.2B). The $CaSO_4$ (5 mM)-enhanced RSA in both cultivars, showing similar values than control, despite Al stress in nutrient solution. Although RSA was not related with LP produced by Ca and Al imbalance, an improved rCa/Al triggered an increase in RSA in Bluegold. Moreover, when fCa/Al was enhanced, changes in TPC levels were more associated with the Al tolerance in Legacy and Al sensitivity in Bluegold, respectively. A significant positive correlation was also found between TPC and RSA in Legacy leaves (r=0.42; P=0.025). Other authors also reported positive correlations between TPC and RSA in *Smilax excelsa* leaves (r=0.67; P≤0.05) (Ozsoy et al. 2008) and in tea and herbal infusions (r=0.71; P≤0.05) (Horžić et al., 2009). According to Howard et al. (2003) and Ribera et al. (2010), the content

of these metabolites in blueberries plants may depend on genotypic and environmental factors. In our experiment, however, TPC in the cultivars showed no differences among control plants and phytotoxic Al treatments (100Al and 200Al). Ghanatti et al. (2005) reported that the phenolic content in tea (*Camellia sinensis* L.) plants was reduced by the treatment with Al (400 μM) in comparison with non-treated plants.

Superoxide dismutase is the most effective and first-line antioxidant enzyme to avoid LP by catalyzing the conversion of superoxide anion to H_2O_2 (Bóscolo et al. 2003). A statistically significant correlations between SOD with Al in roots of Legacy was found (r=0.46) and but no for LP (r=0.23). In Bluegold leaves and roots LP was negatively correlated with Al contents (r=−0.64 and r=−0.64), although no relationships with SOD activity were observed. Therefore, the degree of Al tolerance could be related to higher SOD activity in roots of tolerant cultivar, however Legacy showed a higher LP than Bluegold in roots, indicating other mechanism to avoid Al toxicity. Calcium sulfate treatment (particularly 5 mM) activated enzyme activity in both Legacy leaves and roots and in Bluegold leaves. Guo et al. (2006) reported an enhancement SOD activity in barley after 3.0 mM Ca amendment was added to an Al-toxified nutrient solution.

In highbush blueberry cultivars with contrasting degrees of Al tolerance, $CaSO_4$ was an effective amendment for improve the calcium contents concomitant with a reduccion of aluminum contents, showing an important contribution to mineral balance in the Al- sensitive Bluegold. The improvement of Ca/Al molar ratio in leaves, by adding $CaSO_4$ mainly at 5 and 10 mM, allowed a higher photochemical parameters (ΦPSII and ETR) in the Al-tolerant and Al-sensitive cultivars, probably

due to a decrease on lipid peroxidation caused by Al toxicity. This reduction in oxidative stress resulted from an increased radical scavenging activity, total phenols content and superoxide activity related to the Ca/Al molar ratio in leaves. With respect to antioxidant compounds, Legacy (Al-tolerant) showed a close relationship between total phenolic content and superoxide dismutase activity with foliar Ca/Al molar ratio, showing efficient mechanisms in this cultivar to mitigate oxidative stress caused by aluminum. Molar ratios in leaves from 6 to 10 in Legacy, and 10 to 14 for Bluegold showed are adequated to ameliorate toxicity by this toxicant. However, more studies about the effects of adding $CaSO_4$ to Al saturated soil on other physiological and biochemical features are necessary.

Acknowledgements

This study was supported by FONDECYT Nº 11080231 and the Comisión Nacional de Ciencia y Tecnología de Chile (CONICYT) for Doctoral Thesis Support AT-24100159 and Congress Attendance Scholarship. The authors would like to thank Berries San Luis, Lautaro, Región of La Araucanía, Chile. C. Meriño-Gergichevich wishes to gratefully acknowledge the support of the MECESUP FRO 0601 Maintenance Scholarship, as well as Ximena Garrido Ramírez and Jessica Yañez Delgado for their technical assistance.

3.5 REFERENCES

Alvarez, E., M. L. Fernández-Marcos, C. Monterroso, and M. J.Fernández-Sanjurjo, 2005: Application of aluminium toxicity indices to soils under various forest species. Forest Ecol. Manage. 211, 227 ─ 239.

Bachiega, Z., L. R. Ferracciú Alleoni, and E. F. Caires, 2007: Nutrient concentration in soil water extracts and soybean nutrition in response to lime and gypsum applications to an acid Oxisol under no-till system. Nutr. Cycl. Agroecosys. 79, 169 ─ 179.

Björkman, O., and B. Demmig, 1987: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170, 489 ─ 504.

Blatt, C. R., and K. H. McRae, 1997: Marketable yield response of two lowbush blueberries to preplant applications of gypsum, calcitic and dolomitic limestones. J. Small Fruit Viticulture 5, 13 ─ 26.

Blokhina, O., E. Virolainen, and K. V. Fagerstedt, 2003: Antioxidants, oxidative damage, and oxygen deprivation stress: A review. Ann. Bot.─ London 91, 179 ─ 194.

Borken, W., G. Kossmann, and E.Matzner, 2007: Biomass, morphology and nutrient contents of fine roots in four Norway spruce stands. Plant Soil 292, 79 ─ 93.

Bóscolo, P., M. Menossi, and R. A. Jorge, 2003: Aluminum-induced oxidative stress in maize. Phytochemistry 62, 181 ─ 189.

Boudot, J. P., T. Becquer, D. Merlet, and J. Rouiller, 1994: Aluminium toxicity in declining forests: a general overview with a seasonal assessment in a silver fir forest in the Vosges Mountains (France). Ann. Sci. For. 51, 27 ─ 51.

Bradford, M. M., 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248 ─ 254.

Brunner, I., S. Brodbeck, and L. Walthert, 2002: Fine root chemistry, starch concentration, and “vitality” of subalpine conifer forests in relation to soil pH. Forest Ecol. Manage. 165, 75 ─ 84.

Caires, E. F., I. C. Feldhaus, G. Barth, and F. J. Garbuio, 2002: Lime and gypsum application on the wheat crop. Sci. Agric. 59, 357 ─ 364.

Caires, E. F., S. Churka, F. J. Garbuio, R. A. Ferrari, and M. A. Morgano, 2006: Soybean yield and quality as a function of lime and gypsum applications. Sci. Agric. 63, 370 ─ 379.

Chen, L. S., Y. P. Qi, H. X. Jiang, L. T. Yang, and G. H. Yang, 2010: Photosynthesis and photoprotective systems of plants in response to aluminum toxicity. Afr. J. Biotechnol. 9, 9237 ─ 9247.

Chenery, E. M., 1949: Aluminium in the plant world. II. Monocotyledons and gymnosperms; III. Cryptograms. Kew Bull. 4, 463 ─ 473.

Cheng, S., M. R. Willmann, H. Chen, and J. Sheen, 2002: Calcium signaling through protein kinases. The arabidopsis calcium-dependent protein kinase gene family. J. Plant Physiol. 129, 469 ─ 485.

Close, D. C., C. L. Beadle, P. H. Brown, G. K. and Holz, 2000: Cold-induced photoinhibition affects establishment of *Eucalyptus nitens* (Deane and Maiden) and *Eucalyptus globulus* Labill. Trees – Struct. Funct. 15, 32 ─ 41.

Cronan, C. S., and D. F. Grigal, 1995: Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. J. Environ. Qual. 24, 209 ─ 226.

Delhaize, E., and P. R. Ryan, 1995: Aluminum toxicity and tolerance in plants. J. Plant Physiol. 107, 315 ─ 321.

Donahue, J. L., C. Moses Okpodu, C. I. Cramer, E. A. Grabau, and R. G. Alscher, 1997: Responses of Antioxidants to Paraquat in Pea Leaves. Plant Physiol. 113, 249 ─ 257.

Du, Z., and W. J. Bramlage, 1992: Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food Chem. 40, 1566 ─ 1570.

Espinoza, J. G., E. X. Briceño, E. R. Chávez, J. R. Úrbez-Torres, and B. A.Latorre, 2009: *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. Plant Dis. 93, 1187 ─ 1194.

Fenn, L. B., and G. R. Gobran, 1999: Willow tree productivity on fertilizer solutions containing various Ca/Al ratios. Nutr. Cycl. Agrosys. 53, 121 ─ 131.

Ghanati, F., A. Morita, and H. Yokota, 2005: Effects of aluminum on the growth of tea plant and activation of antioxidant system. Plant Soil 276, 133 ─ 141.

Ghanotakis, D. F., and C. F. Yocum, 1990: Photosystem II and the oxygen-evolving complex. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 255 ─ 276.

Giertych, M. J., L. O. De Temmerman, and L. Rachwal, 1997: Distribution of elements along the length of Scots pine needles in a heavily polluted and a control environment. Tree Physiol. 17, 697 ─ 703.

Gough, R. E., 1997: Blueberries-North and South. J. Small Fruit Viticulture 4, 71 ─ 106.

Guerrero, J. A., 2006: Capacidad antioxidante, fenoles y antocianinas totales e inhibición de *Botrytis cinerea* Pers. Ex Fr. por extractos crudos de fruta de cultivares de arándano alto (*Vaccinium corymbosum* L.) según localidad de la zona Sur de Chile. PhD Thesis. Universidad Austral de Chile, Valdivia, Chile.

Guo, T., Y. Chen, Y. Zhang, and Y. Jin, 2006: Alleviation of Al toxicity in barley by addition of calcium. Agr. Sci.-China 5, 828 ─ 833.

Hanson, E. J., and S. F. Berkheimer, 2004: Effect of soil calcium applications on blueberry yield and quality. Small Fruits Rev. 3, 133 ─ 139.

Hepler, P. K., 2005: Calcium: A central regulator of plant growth and development. Plant Cell 17, 2142 ─ 2155.

Hoagland, D. R., and D. I. Arnon, 1950: Water-culture method for growing plants without soil. Calif Agric Exp. Stn. Circ. 347, University of California, Berkeley, CA.

Horžić, D., D. Komes, A. Belščak, K. Kovačević Ganić, D. Iveković, and D. Karlović, 2009: The composition of polyphenols and methylxanthines in teas and herbal infusions. Food Chem. 115, 441 ─ 448.

Howard, L. R., J. R. Clark, and C. Brownmiller, 2003: Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. J. Sci. Food Agric. 83, 1238 ─ 1247.

Inostroza-Blancheteau, C., M. Reyes-Díaz, F. Aquea, A. Nunes-Nesi, M. Alberdi, P. Arce-Johnson, 2011: Biochemical and molecular changes in response to aluminium-stress in highbush blueberry (*Vaccinium corymbosum* L.). Plant Physiol. Bioch. 49, 1005 ─ 1012.

Jiang, Y., and B. Huang, 2001: Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J. Exp. Bot. 52, 341 ─ 349.

Khan, N. A., S. Samiullah, S. Singh, and R. Nazar, 2007: Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J. Agron. Crop Sci. 193, 435 ─ 444.

Kinraide, T. H., 1998: Three mechanisms for the calcium alleviation of mineral toxicities. J. Plant Physiol. 118, 513 ─ 520.

Kinraide, T. B., J. F. Pedler, and D. R. Parker, 2004: Relative effectiveness of calcium and magnesium in the alleviation or rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium, and low pH. Plant Soil 259, 201 ─ 208.

Kochian, L. V., M. A. Pineros, and O. A. Hoekenga, 2005: The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274, 175 ─ 195.

Lawlor, D. W., 1993: Photosynthesis. 2nd edition. ISBN 0-582-086574. 317 p.

Li, X. M., X. L. Li, and A. G. Zhou, 2007: Evaluation of antioxidant activity of the polysaccharides extracted from *Licium barbarum* fruits in vitro. Eur. Polym. J. 43, 448 ─ 497.

Lin, C. C., and C. H. Kao, 2000: Effect of NaCl stress on H_2O_2 metabolism in rice leaves. J. Plant Growth Regul. 30, 151 ─ 155.

Little, T. M., and Y. F. J. Hills, 1978: Métodos estadísticos para la investigación en la agricultura. Editorial Grillas, México D.F., México. 270p

Ma, B., J. Wan, and Z. Shen, 2007: H_2O_2 production and antioxidant responses in seeds and early seedlings of two different rice varieties exposed to aluminum. J. Plant Growth Regul. 52, 91 ─ 100.

Maxwell, K., and G. N. Johnson, 2000: Chorophyll fluorescens- a practical guide. J. Exp. Bot. 345, 659 ─ 668.

Meriño-Gergichevich, C., M. Alberdi, A. G. Ivanov, and M. Reyes-Díaz, 2010: Al^{3+}-Ca^{2+} interaction in plants growing in acid soils: Al-phytotoxicity response to calcareous amendments. J. Soil. Sci. Plant Nutr. 10, 217 ─ 243.

Miqyass, M., H. J. van Gorkom, and C. F. Yocum, 2007: The PSII calcium site revisited. Photosynth. Res. 92, 275 ─ 287.

Mora, M. L., P. Cartes, R. Demanet, and I. S. Cornforth, 2002: Effects of lime and gypsum on pasture growth and composition on an acid Andisol in Chile, South America. Commun. Soil Sci. Plant Anal. 33, 2069 ─ 2081.

Moustakas, M., G. Ouzounidou, P. E. Eleftherios, and R. Lannoye, 1995: Aluminum effect on photosynthesis and elemental uptake in an aluminum-tolerant and non-tolerant wheat cultivar. J. Plant Nutr. 18, 669 ─ 683.

Oberley, L. W., and D. R. Spitz, 1986: Nitroblue tetrazolium. In Handbook of Methods for Oxygen Radical Research. Edited by Grenwald, R.A. pp. 217 ─ 220. CRC Press, Boca Raton, Florida.

Ozsoy, N., A. Can, R. Yanardag, and N. Akev, 2008: Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem. Toxicol. 110, 571 ─ 583.

Pereira, L. B., A. Melazzo, C. Mazzanti, J. F. Gonçalves, D. Cargnelutti, L. A. Tabaldi, A. G. Becker, N. Spanholi Calgaroto, J. Gomes Farias, V. Battisti, B. Bohrer, F. T. Nicoloso, V. Morsch, and M. R. C. Schetinger, 2010: Aluminum-induced oxidative stress in cucumber. Plant Physiol. Bioch. 48, 683 ─ 689.

Poschenrieder, C., B. Gunsé, I. Corrales, J. Barceló, 2008: A glance into aluminum toxicity and resistance in plants. Sci. Total Environ. 400, 356 ─ 368.

Prior, R. L., G. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer, and M. Mainland, 1998: Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 46, 2686 − 2693.

Prior, R. L., X. Wu, and K. Schaich, 2005: Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53, 4290 ─ 4302.

Prodorutti, D., I. Pertot, L. Giongo, and C. Gessler, 2007: Highbush blueberry: cultivation, protection, breeding and biotechnology. Eur. J. Plant Sci. Biotechnol. 1, 44 ─ 56.

Reich, P. B., J. Oleksyn, and M. G. Tjoelker, 1994: Relationship of aluminum and calcium to net CO_2 exchange among diverse Scots pine provenances under pollution stress in Poland. Oecologia 97, 82 ─ 92.

Rengel, Z., and D. L. Robinson, 1989: Aluminum effects on growth and macronutrient uptake by annual ryegrass. Agron. J. 81, 208 ─ 215.

Rengel, Z., and W. H. Zhang, 2003: Role of dynamics of intracellular calcium in aluminum-toxicity syndrome. New Phytol. 159, 295 ─ 314.

Reyes-Díaz, M., M. Alberdi, M. L. Mora, 2009: Short-term aluminum stress differentially affects the photochemical efficiency of photosystem II in highbush blueberry genotypes. J. Amer. Soc. Hort. Sci. 134, 14 ─ 21.

Reyes-Díaz, M., C. Inostroza-Blancheteau, R. Millaleo, E. Cruces, C. Wulff-Zottele, M. Alberdi, and M. L. Mora, 2010: Long-term aluminum exposure effects on physiological and biochemical features of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 135, 212 ─ 222.

Ribera, A. E., M. Reyes-Díaz, M. Alberdi, G. E. Zuñiga, and M. L. Mora, 2010: Antioxidant compounds in skin and pulp of fruits change among genotypes and maturity stages in highbush blueberry (*Vaccinium corymbosum* L.) grown in southern Chile. J. Soil. Sci. Plant Nutr. 10, 509 ─ 536.

Ritchey, K. D., and J. D. Snuffer, 2002: Limestone, gypsum, and magnesium oxide influence restoration of an abandoned Appalachian pasture. Agron. J. 94, 830 ─ 839.

Rout, G. R., S. Samantara, and P. Das, 2001: Aluminium toxicity in plants: a review. Agronomie 21, 3 ─ 21.

Ryan, P. R., R. J. Reid, and F. A. Smith, 1997: Direct evaluation of the Ca^{2+}-displacement hypothesis for Al toxicity. J. Plant Physiol. 113, 1351 ─ 1357.

Ryan, P. R., and E. Delhaize, 2010: The convergent evolution of aluminium resistance in plants exploits a convenient currency. Funct. Plant Biol. 37, 275 ─ 284.

Sadzawka, A., R. Grez, M. Carrasco, and M. Mora, 2007: Métodos de análisis de tejidos vegetales. 53p. Santiago, Chile, Comisión de Normalización y Acreditación Sociedad Chilena de la Ciencia del Suelo.

Sanderson, K. R., M. R. Carter, and J. A. lvany, 1995: Effects of gypsum on yield and nutrient status of native lowbush blueberry. Can. J. plant Sci. 26, 361 ─ 366.

Schaberg, P. G., J. W. Tilley, G. J. Hawley, D. H. Dehayes, and S. W. Bailey, 2006: Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. Forest Ecol. Manag. 223, 159 ─ 169.

Shaff, J., B. A. Schultz, E. J. Craft, R. T. Clark, and L. V. Kochian, 2010: Geochem EZ: a chemical speciation program with greater power and flexibility. Plant Soil 330, 207 ─ 214.

Shao, H. B, L. Y. Chu, M. A. Shao, C. A. Jaleel, and H. M. Mi, 2008: Higher plant antioxidants and redox signaling under environmental stresses. C. R. Biologies 331, 433 ─ 441.

Slinkard, K., and V. L. Singleton, 1997: Phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticulture 28, 49 ─ 55.

St. Clair, S. B., J. E. Carlson, and J. P. Lynch, 2005: Evidence for oxidative stress in sugar maple stands growing on acidic, nutrient imbalanced forest soils. Oecologia 145, 258 ─ 269.

St. Clair, S. B., W. E. Sharpe, and J. P. Lynch, 2008: Keys interaction between nutrient limitation and climatic factors in temperate forests: a synthesis of the sugar maple literature. Can. J. For. Res. 38, 401 ─ 414.

Stout, W. L., and W. E. Priddy, 1996: Use of flue gas desulfurization (FGD) by-product gypsum on alfalfa. Commun. Soil Sci. Plant Anal. 27, 2419 ─ 2432.

Suzuki, A., A. Miura, and K. Aoba, 1999: Effects of aluminum, calcium, and phosphate on shoot growth, viability, and aluminum distribution in the root apices in highbush blueberry cultured *in vitro*. J. Japan Soc. Hort. Sci. 68, 937 ─ 941.

Takahashi, T., Y. Ikeda, H. Nakamura, and M. Nanzyo, 2006a: Efficiency of gypsum application to acid Andosols estimated using aluminum release rates and plant root growth. J. Soil Sci. Plant Nutr. 52, 584 ─ 592.

Takahashi, T., T. Ikeda, K. Fujita, and M. Nanzyo, 2006b: Effect of liming on organically complexed aluminum of nonallophanic Andosols from northeastern Japan. Geoderma 130, 26 ─ 34.

Toma, M., M. E. Sumner, G. Weeks, and M. Saigusa, 1999: Long-term effects of gypsum on crop yield and subsoil chemical properties. Soil Sci. Soc. Am. J. 39, 891 ─ 895.

Toma, M., M. Saigusa, N. Gafoku, and M. E. Sumner, 2005: Effects of gypsum on amelioration of subsoil acidity in Andisols. JIFS 2, 69 ─ 71.

Tuna, A. L., C. Kaya, M. Ashraf, H. Altunlu, I. Yokas, and B. Yagmur, 2007: The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ. Exp. Bot. 59, 173 ─ 178.

Truman, R. A., F. R. Humphreys, and P. J. Ryan, 1986: Effect of varying solution ratios of Al to Ca and Mg on the uptake of phosphorus by *Pinus radiate*. Plant Soil 96, 109 ─ 123.

Vander Meulen, K. A., A. Hobson, and C. F. Yocum, 2004: Reconstitution of the photosystem II Ca^{2+} binding site. Biochim. Biophys. Acta 1655, 179 ─ 183.

Vrettos, J. S., D. A. Stone, and G. W. Brudvig, 2001: Quantifying the ion selectivity of the Ca^{2+} site in photosystem II: Evidence for direct involvement of Ca^{2+} in O formation. Biochemistry 40, 7937 ─ 7945.

Wang, S. Y., H. Chen, and M. Ehlenfeldt, 2010: Variation in antioxidant enzyme activities a nonenzyme component among cultivars of rabitteye blueberries (*Vaccinium ashei* Reade) and *V. ashei* derivates. Food Chem. (2010), doi: 10.1016/j.foodchem.2010.11.126.

Wang, S. Y., and J. R. Ballington, 2007: Free radical scavenging capacity and antioxidant enzyme activity in deerberry (*Vaccinium stamineum* L.). LWT 40, 1352 ─ 1361.

Weber-Blaschke, G., and K. E. Rehfuess, 2002: Correction of Al toxicity with European ash (*Fraxinus excelsior* L.) growing on acid soils by fertilization with Ca and Mg carbonate and sulfate in pot experiments. Forest Ecol. Manage. 167, 173 ─ 183.

Yamamoto, Y., Y. Kobayashi, S. R. Devi, S. Rikiishi, and H. Matsumoto, 2002: Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. J. Plant Physiol. 128, 63 ─ 72.

Yang, W. Q., B. Goulart, and K. Demchak, 1996: The effect of aluminium and media on the growth of mycorrhizal and nonmycorrhizal highbush blueberry plantlets. Plant Soil 183, 301 ─ 308.

Yocum, C. F., 2008: The calcium and chloride requirements of the O_2 evolving complex. Coordin. Chem. Rev. 252, 296 ─ 305.

Zhang, W. H., and Z. Rengel, 1999: Aluminium induces an increase in cytoplasmic calcium in intact wheat root apical cells. Aust. J. Plant Physiol. 26, 401 ─ 409.

Zheljazkov, V. D., T. Astatkie, C. D. Caldwell, J. Macleod, and M. Grimmett, 2006: Compost, manure, and gypsum application to timothy/red clover forage. J. Environ. Qual. 35, 2410 ─ 2418

Chapter 4. *Calcium sulfate improve physiological and biochemical performance of highbush blueberry to elevated toxic soil aluminum*

Paper in preparation

CALCIUM SULFATE IMPROVE PHYSIOLOGICAL AND BIOCHEMICAL PERFORMANCE OF HIGHBUSH BLUEBERRY TO ELEVATED TOXIC SOIL ALUMINUM

Meriño-Gergichevich, C.[1], Alberdi, M.[2,3], Reyes-Díaz, M. [2,3] and Horst, W. J.[4]

[1]Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

[2]Center of Plant-Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile.

[3]Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile.

[4]Institute of Plant Nutrition, Leibniz University Hannover, Herrenhaeuser Strasse 2, 30419 Hannover, Germany.

[*]Corresponding author: Marjorie Reyes Díaz.
Departamento de Ciencias Químicas y Recursos Naturales,
Facultad de Ingeniería, Ciencias y Administración,
Universidad de La Frontera
Casilla 54-D
Temuco (Chile)
Telephone: (56-45) 325493
Fax: (56-45) 325050
e-mail: reyesm@ufro.cl

ABSTRACT

Highbush blueberry (*Vaccinium corymbosum* L.) is well adapted to acid soils (pH≤5.5). However, it is sensitive to aluminum (Al^{3+}), which is released under acid conditions. To ameliorate its toxicity, without alter soil pH, a common agronomical practice is the use of calcium sulfate (gypsum, $CaSO_4$). The calcium sulfate effect on chemical, physiological (growth, water content, and photochemical performance), and biochemical (lipid peroxidation, antioxidant capacity, and antioxidant compounds) features were studied in this species growing under Al^{3+} saturation. Legacy (Al-tolerant) and Bluegold (Al-sensitive) cultivars were grown for 60 days in an Andisol with high Al-saturation, that was amended with calcium sulfate at doses of 0 (G0), 700 (1), 1400 (2), 2800 (4) mg kg^{-1} soil. Calcium sulfate improved Ca/Al molar ratio and nutrient contents, plant growth, as well as photochemical parameters, carotenoids contents, and relative water content (RWC) in leaves, especially in tolerant cultivar. However, the amendment did not show clear effects on chlorophyll contents (Chl) and leaf water potential (Ψ_l). The Ca/Al molar ratio was related to a decreased lipid peroxidation (LP) in both cultivars, whereas radical scavenging activity (RSA), anthocyanins (TAN), and antioxidant enzymes were directly increased by this ratio. It was concluded that calcium sulfate can be an effective amendment to ameliorate Al^{3+} toxicity in highbush blueberry, mainly in the Al tolerant cultivar. Moreover, we observed that different Ca/Al molar ratio could be established to improve the physiological and biochemical performance in this species, depending on the cultivar.

Keywords: Aluminum, antioxidant, Ca/Al molar ratio, fluorescence parameters, gypsum, *Vaccinium corymbossum*.

4.1 INTRODUCTION

In Chile, highbush blueberry, is a significant commercial fruit crop, due to its richness in antioxidants and the high market price of its fruits (Guerrero, 2006; 2010), with over 7,000 hectares (ha) planted until 2010 (ODEPA, 2011). This species demands acid soils for its growth (Trehane, 2004), which are provided in south-central Chile by Andisols, volcanic soils origin, with high organic matter (OM) content, low available phosphorus (P) content, and high acidity (pH≤5.5) (Nanzyo *et al.*, 1993; Mora *et al.*, 2002). It is known that, mainly due to acid conditions, high rainfall, OM mineralization, and cation bases lixiviation, Al^{3+} is probably the main stress factor for several cultivated plants in acid soils (Foy, 1984; Zhang and Rengel, 1999; Poschenrieder *et al.*, 2008). In Andisols of southern Chile, Al^{3+} levels >0.1 cmol+/ kg have been reported by van Lierop (1990) and Mora *et al.* (2004), although Mora *et al.* (1999b) reported values over 1.5 cmol+/ kg at soil pH 4.5.

In spite, a lot of reports exist about the effects of Al^{3+} in roots, less is known about their effects on leaves. Regarding roots it is showed that affect its growth and capacity for water and nutrients uptake, especially calcium (Ca), P, and magnesium (Mg) (Rengel and Robinson, 1989; Barceló and Poschenrieder, 2002; Poschenrieder *et al.*, 2008). For leaves of pea (*Pisum sativum* L.), Watanabe and Osaki (2002) found a negative relationship between Al accumulation and essential minerals such as Ca, P, and Mg, diminishing their growth and yield. In some plants Al toxicity could lead to a reduction in photosynthetic pigments and photochemical reactions of photosystem II (PSII) and carbon dioxide (CO_2) assimilation (Peixoto *et al.*, 2002; Wang *et al.*, 2006). Similarly, Reyes-Díaz *et al.* (2009) reported a reduction in the photochemical traits of PSII, when compared contrasting Al-resistance blueberry cultivars subjected to toxic Al (0-100μM). This reduction was more evident in the sensitive cultivar. In another study, Reyes-Díaz *et al.* (2010) found that photochemical parameters in blueberry were more affected in Al sensitive cultivar concomitant with higher LP than resistant cultivar. This, could be explained by the oxidative stress triggered by Al^{3+}, by an increment of reactive oxygen species (ROS) in cellular organelles as chloroplasts as reported by Kochian *et al.* (2005) and Khan *et al.* (2007). Otherwise, oxidative stress induces LP in biological membranes, which leads to breakdown of

their structure and function (Yamamoto *et al.*, 2002; Yamamoto *et al.*, 2003; Ma *et al.*, 2007). In parallel to enhanced ROS levels, both roots and leaves, may increase the antioxidant activity to ameliorate the deleterious effects of ROS, improving the tolerance of plants to stresses as Al toxicity (Guo *et al.*, 2006; Shao *et al.*, 2008). The antioxidant set includes enzymatic and non-enzymatic compounds, with capacity to remove and scavenge ROS (Shao *et al.*, 2008).

It has been reported that to revert Al stress in crop stands on acid soils, Ca addition efficiently reduce Al toxicity, improving their nutrients and water availability (Toma *et al.*, 2005; Takahashi *et al.*, 2006). Calcium is a crucial regulator of growth and development in plants, with important functions as structural support and environmental signal perception, being involved in plant tolerance to different stresses, by regulating the antioxidant metabolism (Jiang and Huang, 2001; Cheng *et al.*, 2002; Hepler, 2005; Schaberg *et al.*, 2006). Currently, external Ca applications for Al^{3+} amelioration in acid soils, are carried out through different agronomical practices including the application of calcareous amendments (Campbell *et al.*, 2006) such as carbonates as calcites ($CaCO_3$) and calcium sulfate or gypsum ($CaSO_4$) (Tisdale *et al.*, 1985; Garrido *et al.*, 2003; Illera *et al.*, 2004).

It has been widely demonstrated the effectiveness of calcium sulfate to reduce Al toxicity risk on crops (Alva *et al.*, 1991; Carvalho and van Raig, 1997, Mora *et al.*, 1999a; 2002; Caires *et al.*, 2006; Takahashi *et al.*, 2006). This amendment represents an important source of Ca and sulfur (S) for plant nutrition (Mathews and Joost, 1989; Bolan *et al.*, 1993), improving contents of Ca, S, P, potassium (K), manganese (Mn), and zinc (Zn) in vegetal tissues (Caires *et al.*, 2006; Tuna *et al.*, 2007). Besides, this amendment did not greatly modify soil pH necessary soil condition for a good growth and development of some crops as highbush blueberries (Gough, 1997; Lyrene and Munoz, 1997; Mora *et al.*, 1999a). Traditionally, the effects of gypsum on blueberry crops have been studied from a mineral content point of view and to conferring firmness to fruits (Korcak, 1988, Blatt and Mc Rae, 1997). Angeletti *et al.* (2010) reported that surface gypsum application in highbush blueberry cultivars showed an increased Ca content, delayed postharvest softening and reduced loss of weight in fruits. However, little is reported about gypsum effect on physiological and

biochemical features in blueberries, particularly about the photosynthetic performance and antioxidant systems. Moreover, the effects of gypsum on physiological and biochemical processes in highbush blueberry plants stand on an acidic Andisol with high Al saturation are still little known. Therefore, the aim of this study was to known wether gypsum amendment counteract the deleterious effects of toxic Al on physiological and biochemical processes in blueberry cultivars growing in an Al-saturated Andisol.

4.2 MATERIAL AND METHODS

4.2.1 Plant material

The assay was carried out in a greenhouse of Instituto de Agroindustria in Universidad de La Frontera, Temuco, Region of La Araucanía, Chile. Two highbush blueberry (Legacy and Bluegold) cultivars frequently cultivated in southern Chile (Guerrero, 2006) were considered in this study. One year-old plants growing in a solid substrate (1 oat: 1 shell sawdust: 1 pine needles by volume) were provided from nursery of Berries San Luis, Lautaro, Región de La Araucanía, Chile (38º 29` S, 72º 23`W). These cultivars were chosen for their contrasting degree of tolerance to Al toxicity, according with chemical, physiological, biochemical, and molecular studies performed by Reyes-Díaz *et al.* (2009 and 2010) and Inostroza-Blancheteau *et al.* (2011) who reported that Legacy was a more Al-resistant cultivar to Al toxicity than Bluegold.

4.2.2 Plant culture

Highbush blueberry plants were established during 60 days (d) in polyethylene pots (3 L) containing an acidified Andisol, from Southern Chile, Gorbea Series (Typic Hapludands) (CIREN, 2002) was used for this assay. It was provided by Semillas Baer, Cajón, Temuco, Region of La Araucanía, Chile. Chemical features of this soil, important for this work stand out their acidity, high percentage of Al-saturation and nutrient content are showed in Table 4.1. Soil samples from the top 20

cm depth were collected from cultivated areas, and afterwards dried, sieved, and then moistened at field condition. Calcium sulfate amendment was carefully added to the soil sample, mixed and then incubated, according to protocol performed by Mora *et al.* (1999a). The applied treatments were: i) soil without amendment (control), ii) soil control plus calcium sulfate at 700 mg kg^{-1} soil, iii) soil control plus calcium sulfate at 1400 mg kg^{-1} soil and iv) soil control plus calcium sulfate at 2800 mg kg^{-1} soil (subsequently referred to as G0, G1, G2 and G4 respectively). Each plant received a fertilization equivalent to 33.7 mg N kg^{-1} soil, applied as urea, 67 mg P kg^{-1} soil, as triple superphosphate, and commercial fertilizer Sulpomag at 371.6 mg kg^{-1} soil was used to applied equivalents of 67.5 mg K kg^{-1} soil, 67.5 mg Mg kg^{-1} soil, 81.0 mg S kg^{-1} soil. The application of boron (B) was 2.7 mg kg^{-1} soil as Boronat, zinc (Zn) and copper (Cu), both in doses of 1.3 mg kg^{-1} soil, as $ZnSO_4$ and $CuSO_4$ were added to soil, respectively. Each treatment had 10 replicates in a completely randomized design. The experiment was conducted in the greenhouse where growth conditions were: 25/20ºC (day/night), 16/8 h light regimen (light/dark, respectively), relative air humidity of 70%, and photosynthetic photon flux density (PPFD) average of 300 μmol photons m^{-2} s^{-1}.

4.2.3 Plant growth and chemical determinations

4.2.3.1 Dry matter and plant growth

At harvest (60d), five randomly plant were divided in roots, stems, and leaves, and dried by placing them in a 70 ºC forced-air oven for 48 h to determine dry matter (DM). For growth determination the longest shoots and primary roots of five plants of each treatment were measured and recorded (at initial and final time).

4.2.3.2 Mineral content in tissues

After 60d, contents of Ca, Mg, K, Na, and Al were determined according to the method described by Sadzawka *et al.* (2007), using a simultaneous multi-element atomic absorption spectrophotometer (model UNICAM 969 Atomic absorption Spectrometer, England). For S and P determination, turbidimetric and colorimetric methods respectively, described by Sadzawka *et al.* (2007) were followed. The

conten oft S was measured at 440 nm, and P content at 466 nm, both measurements were carried out in an UV-VIS spectrophotometer (UNICO® 2800 UV/VIS, Spain).

4.2.4 Physiological determinations

4.2.4.1 Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured using a pressure chamber PMS (model 1000, Instrument Co., Corvallis, Ore.), following the recommendations of Hsiao (1990). Measurements were made at midday, between 13:30 and 15:30h, on shaded leaves enclosed at least one hour (h) in plastic bags laminated with aluminum foil.

4.2.4.2 Relative water content (RWC)

For RWC, a Smart and Bingham`s protocol (1974) was followed. A composite sample of leaf discs was taken and fresh weight (FW) was determined, followed by flotation on water for up to 4h. The turgid weight (TW) was then recorded, and the leaf tissue was subsequently on-dried (48h) to a constant weight at about 85ºC (DW). After, RWC was calculated by

$$[(FW–DW)/(TW–DW)] \times 100$$

4.2.4.3 Chlorophyll fluorescence parameters of PSII

In order to determine the photochemical efficiency of PSII in leaves, the basic protocol of Reyes-Díaz *et al.* (2009) adapted to blueberries was followed. Attached leaves of two blueberry cultivars were dark adapted for 20 min and fluorescence was measured using a portable pulse-amplitude modulated fluorimeter (FMS 2; Hansatech Instruments, Norfolk, UK). Fluorescence parameters of PSII such as: photochemical maximum quantum yield (Fv/Fm), photochemical effective quantum yield (ΦPSII), electron transport rate (ETR), non photochemical quenching (NPQ) (Maxwell and Johnson, 2000) were determined at 0, 30, and 60d.

4.2.4.4 Photosynthetic pigments

At 60d, total chlorophylls (Chl*a* and *b*) and carotenoids, were extracted with 96% ethanol, measured by a spectrophotometer (UNICO® 2800 UV/VIS, Spain) at 663, 646, and 470 nm. Pigment concentrations were calculated according to Lichtenthaler and Welburn (1983).

Table 4.1 Initial soil chemical properties of Andisol Gorbea Series, used in soil assay before calcium sulfate treatment (*).

Chemical properties	Values
N (mg kg^{-1})	32.67
Olsen-P (mg kg^{-1})	20.33
K (mg kg^{-1})	70.70
pH (H_2O)	4.60
Organic matter (%)	13.67
K (cmol+ kg^{-1})	0.18
Na (cmol+ kg^{-1})	0.03
Ca (cmol+ kg^{-1})	0.20
Mg (cmol+ kg^{-1})	0.04
Al (cmol+ kg^{-1})	1.23
Al saturation (%)	68.40
CEC (cmol+ kg^{-1})	1.68
$\sum$ Bases (cmol+ kg^{-1})	0.45
B (ppm)	0.29
Zn (ppm)	0.36
Cu (ppm)	0.77
Fe (ppm)	26.24
Mn (ppm)	5.72
S (ppm)	31.00
Al ext. (ppm)	1050.00

*Methodology: P 8.5 (0lsen); available S: Ca extraction (H_2P0_4) 20.01 mol L^{-1}; Ca, Mg, K and Na exchangeable: extraction with $CH_3\,COONH_4$ 1 mol L^{-1}. At pH 7.0; exchangeable Al: extraction with KCI 1 mol L^{-1}; CEC: Ca+ Mg+ K+ Na+ Al exchangeable, Al saturation: (Al exchangeable x 100)/ CEC. Analyzed by Plant and Soil Laboratory, Instituto de Agroindustria, Universidad de La Frontera, Temuco, Chile.

4.2.5 Biochemical determinations

4.2.5.1. Lipid peroxidation

Lipid peroxidation (LP) in tissues was expressed as malondialdehyde acid (nmol MDA g^{-1} FW) content and determined by 2-thiobarbituric acid (TBA) reactive-metabolites, according with protocol of Heath and Parker (1968), modified by Du and Bramlage (1992). Samples were measured in UV-VIS spectrophotometer (UNICO® 2800 UV/VIS, Spain) at 440, 532, and 600 nm to correct the interference induced by TBARS-sugar complexes.

4.2.5.2 Radical scavenging activity

The free radical scavenging activity (RSA) of tissues material from the two cultivars were tested through the 2.2 diphenyl-2 picrylhydrazyl ($DPPH^{-}$) method (Li *et al.*, 2007) with minor modifications. The reaction took 8 min and the absorbance was measured at 515 nm, in a spectrophotometer (UNICO® 2800 UV/VIS, Spain).

4.2.5.3 Total phenols content

Total phenol contents (TPC) was done following Slinkard and Singleton`s method (1977). The TPC were established with the Folin-Ciocalteu reagent and absorbance was measured in a spectrophotometer (UNICO® 2800 UV/VIS, Spain) at 765 nm. A regression equation, using chlorogenic acid as standard, was used for calculate TPC.

4.2.5.4 Total anthocyanins

The quantification of anthocyanins content (TAN) in leaves was determined according to the protocol modified by Close *et al.* (2000) with minor modifications. The homogenized samples were centrifuged at 3,000 rpm and supernatant was measured at 530 and 657 nm in an UV-VIS spectrophotometer (UNICO® 2800 UV/VIS, Spain).

4.2.5.5 Total flavonoids

Total flavonoid contents (TFA) were determined in leaves of two highbush blueberry evaluated following the method described by Zhuang *et al.* (1992). Total flavonoids from extracts were measured in a spectrophotometer (UNICO® 2800 UV/VIS, Spain) at 510 nm.

4.2.5.6 Superoxide dismutase and catalase activity

The superoxide dismutase (SOD, EC. 1.15.1.1) activity in leaves and roots was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) through the method described by Oberley and Spitz (1986). The SOD activity was expressed as U mg^{-1} protein. The activity of catalase (CAT, EC. 1.11.1.6) was assayed according to the method of Aebi (1983). The rate of hydrogen peroxide (H_2O_2) decomposition was measured at 240 nm (extinction coefficient of 0.036 mM^{-1} cm^{-1}). The enzyme activities were expressed on protein basis. Total protein (TP) was estimated according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

4.2.6 Experimental design and statistical analyses

The experiment was arranged on a split-plot design with two cultivars x four treatments (G, G1, G2 and G4) x six replicates x 3 evaluation times (0, 30 and 60d) for the photochemical parameters. Chemical and biochemical determinations were carried out at end of experiment (60d). To minimize any positional effects pots with plants were changed in position every day. For statistical analyses, reported values correspond to the average of six individual replicates for each cultivar, calcium sulfate treatments, and time. All data passed the normality and equal variance by Kolmogorov-Smirnov and Levene test respectively. Data for chlorophyll fluorescence were subjected to a two-ways Analysis of Variance (ANOVA) (where the factors were calium sulfate treatments and exposition time) for Chemical and biochemical analysis to a two-ways ANOVA (where the factors were calcium sulfate treatment and cultivar). A Tukey test was used to identify those values with significant

differences. Both analyses were performed with Sigma Stat 2.0 software (SPSS, Chicago, IL). Differences between the values were considered significant at $P \leq 0.05$.

4.3 RESULTS

4.3.1 Plant growth

Shoots of cultivars in soil amended with calcium sulfate (see in appencices) exhibited an increase up to ~20% after 60d in comparison to control plants ($P \leq 0.05$, Table 4.2). The maximum shoot increases in the treatment G1 were observed for Legacy ($P \leq 0.001$) and at G4 for Bluegold ($P \leq 0.001$) compared to control. Calcium sulfate amendment had positive effects on root length, where Legacy showed significant increases in plants grown at G1, G2 and G4, whereas in roots of Bluegold the three calcium doses showed significantly increases. In the non-amendment soil, growth of roots decreased in Legacy (61%) and in Bluegold (92%), while calcium application did overcome this trend ($P \leq 0.05$; Table 4.2).

The results obtained from this experiment show that calcium sulfate significantly increased total and root DM in Bluegold compared to stressed plants, whereas Legacy in G2 exhibited higher total, root, and shoot DM ($P \leq 0.05$; Table 4.3). In relation to shoot:root ratio no statistically differences in Legacy were observed among treatments, meanwhile in Bluegold this ratio was significantly decreased by 45, 100 and 52% in comparison to G0 for treatments G1, G2 and G3 respectively (Table 4.3).

4.3.2 Mineral content in leaves and roots

Foliar S contents did not change in Legacy and Bluegold, an enlarged S content in roots with G2 treatment was observed in Bluegold compared to the control ($P \leq 0.05$). In both cultivars root P content at G4 (Legacy) and at G1 and G2 were enhanced in comparison to the control ($P \leq 0.001$; Table 4.4). Calcium sulfate significantly increased foliar Ca content up to 30% and 20% in Legacy and Bluegold respectively in comparison to the control. Legacy roots under G4 treatment, showed

higher Ca content (~30%) than in plants stand on control soil (P=0.028). In both organs, Bluegold showed higher Ca contents (~30%) than Legacy under all treatments (P≤0.001; Table 4.4). However, Mg content of roots was decreased up to 54% under calcium sulfate application in Legacy (P≤0.001), whereas in roots and leaves of Bluegold a bigger Mg content under G1 was observed when compared with non amendment treatment (P≤0.050). The amendment increased K content in leaves of Legacy at G4 (P≤0.001), and significantly decreased foliar and root Na contents (up to 50%) in Legacy and Bluegold. On the other hand, in leaves of Legacy and Bluegold Al was decreased up to 27% and 23% respectively by calcium sulfate addition (G2 and G4)in respect to the control (P≤0.05; Table 4.4). Nonetheless, this significant Al reduction was more outstanding in roots that showed up to 89% and 46% in Legacy and Bluegold respectively, at the highest calcium sulfate dose (G4). Statistically significant differences between cultivars were found in regard to Al accumulation of plants growing in Andisol with high Al saturation, showing Legacy higher Al content than Bluegold especially in leaves (56%).

4.3.3 Leaf water relations

The leaf water potential (Ψ_{leaf}) in Legacy .were 45 and 33% higher than Bluegold at G0 and G1 (P≤0.05), whereas in G2 and G4 no statistically differences were observed (Figure 4.1A). Both cultivars significantly diminished Ψ_{leaf}l until G2 remaining constant (~ ─0.4 MPa) until higher calcium sulfate treatment (G4) compared to control (G0). In plants under G2 and G4 were observed more negative Ψ_{leaf} than G0 for Legacy (up to 66% in G2), whreas in Blueogl all treatment with calcium sulfate reduced up to 20% Ψ_{leaf} in comparison to control (Figure 4.1A). On regard to RWC Bluegold was not affected by calcium sulfate addition (P>0.050). Nonetheless, Legacy raised its relative water content (RWC) in plants grown in soil amended with calcium sulfate treatments G1, G2 and G4 (P≤0.05). Statistically significant differences between cultivars were established in all treatments of calcium sulfate to soil where Legacy plants were ground RWC (Figure 4.1B).

4.3.4 Photochemical efficiency of PSII and photosynthetic pigments

In Legacy at 30d and 60d ΦPSII and ETR were significantly increased (~150%) in all calcium sulfate treatments, in comparison to control. In Bluegold, ΦPSII and ETR raised up to 125% in comparison to plants subjected to Al saturated without amendment (Table 4.5). Non photochemical quenching, which represent the energy dissipation as heat relative to dark-adapted state, showed to the end of experiment a large reduction in plants of Legacy treated with calcium sulfate doses ($P \leq 0.05$). In Bluegold, the fall of NPQ was slight, keeping higher NPQ in control plants than in amended plants ($P \leq 0.05$, Table 4.5).

Chlorophyll *a* content in Legacy was significantly increased by G1 (24%) and G2 (34%) treatments, whereas no changes in Chl*b* contents were observed in relation to the control plants. Bluegold showed significant reductions in Chl*a* and Chl*b* contents, at the highest calcium sulfate dose ($P \leq 0.05$; Table 4.6). Legacy, but no Bluegold, exhibited an increased Chl*a+b* in amended plants compared to non-amended plants ($P \leq 0.05$; Table 4.6), and Chl *a/b* was reduced in Legacy at calcium sulfate doses of G2 and G4 ($P \leq 0.05$), meanwhile in Bluegold an increment of this ratio occurred under G4 ($P = 0.013$; Table 4.6). Carotenoids content in Legacy were raised at 63%, 73%, and 119% by treatments G1, G2, and G4 respectively compared with the control soil ($P \leq 0.05$). Bluegold did not showed significant changes in respect to the control ($P > 0.05$,Table4.6).

Table 4.2. Effects of calcium sulfate addition on shoot and roots growth (cm) for two highbush blueberry cultivars grown in an Al-saturated Andisol. Values represent the average of six replicates ± S.E.

Treatments	Legacy				Bluegold			
	Shoot		Root		Shoot		Root	
	Time (days)							
	0	60	0	60	0	60	0	60
G0	41.5±2.3Aa	42.5±0.3Ca	12.0±0.5Ba	12.5±0.3Ba	50.0±1.2Aa	53.0±0.7Ca	16.0±0.1Aa	16.5±0.7Ba
G1	46.0±2.7Ab	66.5±3.5Aa	14.0±0.2ABb	16.0±0.9Aa	51.0±2.0Ab	68.0±0.9ABa	15.0±0.1Ab	21.5±0.5Aa
G2	41.0±1.9Ab	50.0±0.3Ba	13.0±0.6Ba	14.5±0.3Aa	46.0±0.6Bb	66.0±2.6Ba	15.0±0.3Ab	21.0±0.2Aa
G4	43.0±0.7Ab	56.0±2.0Ba	15.5±0.8Ab	17.0±0.6Aa	53.0±0.1Ab	71.2±1.4Aa	15.0±0.2Ab	21.0±0.1Aa

*Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between time 0 and 60d for the same calcium sulfate treatment. Different upper case letters show differences ($P \leq 0.05$) between calcium sulfate treatments for the same time (d).

Table 4.3. Effects of calcium sulfate addition on dry mass and shoot:root ratios for two highbush blueberry cultivars grown in Al-saturated Andisol. Values represent the average of six replicates ± S.E.

Cultivar	Treatments	Total DM	Root DM	Shoot DM	Shoot:root ratio
			g plant^{-1}		
Legacy	G0	3.88Ab	2.54Ab	1.35Ab	0.53Aa
	G1	4.35Aab	2.62Aab	1.73Aa	0.66Ba
	G2	5.01Aa	3.20Aa	1.81Aa	0.57Ba
	G4	4.47Aab	2.82Aab	1.66Aab	0.58Ba
Bluegold	G0	2.93Bc	1.77Bc	1.16Bb	0.65Aa
	G1	3.92Ab	2.73Ab	1.20Ba	0.43Bb
	G2	4.70Aa	3.54Aa	1.24Ba	0.35Bb
	G4	4.04Aab	2.83Ab	1.21Ba	0.43Bb

*Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between calcium treatments for the same cultivar. Different upper case letters show differences ($P \leq 0.05$) between cultivars for the same calcium sulfate treatment.

Table 4.4. Effects of calcium sulfate addition (mg kg^{-1} soil) on mineral content for two blueberry cultivars grown in Al-saturated Andisol. Values represent the average of six replicates ± S.E.

		Legacy				Bluegold			
		Calcium sulfate treatments							
Mineral content (g kg^{-1} DW)		G0	G1	G2	G4	G0	G1	G2	G4
Leaves	S	0.94 ± 0.08^{Ba}	0.97 ± 0.04^{Ba}	0.92 ± 0.02^{Ba}	1.00 ± 0.04^{Aa}	1.09 ± 0.02^{Aa}	1.29 ± 0.02^{Aa}	$1.10\pm0,03^{Aa}$	1.12 ± 0.03^{Aa}
	P	0.79 ± 0.01^{Ba}	0.82 ± 0.04^{Ba}	0.67 ± 0.01^{Bb}	0.77 ± 0.02^{Ba}	0.92 ± 0.01^{Aa}	0.89 ± 0.02^{Aa}	0.91 ± 0.03^{Aa}	0.90 ± 0.01^{Aa}
	Ca	4.21 ± 0.12^{Bc}	5.08 ± 0.21^{Bb}	4.44 ± 0.05^{Bbc}	5.96 ± 0.13^{Ba}	6.78 ± 0.19^{Ac}	7.46 ± 0.43^{Ab}	6.94 ± 0.16^{Abc}	8.55 ± 0.12^{Aa}
	Mg	3.05 ± 0.39^{Aa}	3.64 ± 0.19^{Ba}	3.34 ± 0.23^{Ba}	3.10 ± 0.45^{Ba}	3.09 ± 0.02^{Ac}	4.48 ± 0.05^{Aa}	4.01 ± 0.50^{Ab}	3.80 ± 0.85^{Ab}
	K	9.66 ± 0.05^{Ab}	9.68 ± 0.48^{Ab}	8.68 ± 0.11^{Ab}	11.40 ± 0.19^{Aa}	8.70 ± 0.02^{Ba}	8.90 ± 0.29^{Aa}	8.81 ± 0.27^{Aa}	8.48 ± 0.24^{Ba}
	Na	0.49 ± 0.01^{Aa}	0.49 ± 0.01^{Aa}	0.26 ± 0.02^{Ac}	0.33 ± 0.02^{Ab}	0.29 ± 0.01^{Ba}	0.28 ± 0.03^{Ba}	0.25 ± 0.01^{Ab}	0.20 ± 0.01^{Bc}
	Al	0.71 ± 0.04^{Aa}	0.66 ± 0.01^{Aa}	0.54 ± 0.02^{Ab}	0.52 ± 0.02^{Ab}	0.44 ± 0.01^{Ba}	0.36 ± 0.04^{Bab}	0.35 ± 0.02^{Bb}	0.34 ± 0.01^{Bb}
	Ca/Al	4.33 ± 0.01^{Bc}	5.19 ± 0.14^{Bb}	5.54 ± 0.15^{Bb}	7.69 ± 0.02^{Ba}	10.65 ± 0.15^{Ad}	15.59 ± 0.16^{Ab}	12.82 ± 0.01^{Ac}	16.36 ± 0.18^{Aa}
Roots	S	0.85 ± 0.07^{Aa}	0.97 ± 0.07^{Aa}	0.91 ± 0.02^{Ba}	0.88 ± 0.02^{Aa}	0.63 ± 0.03^{Bb}	0.63 ± 0.02^{Bb}	1.17 ± 0.70^{Aa}	0.88 ± 0.08^{Ab}
	P	0.96 ± 0.02^{Ab}	0.99 ± 0.01^{Bb}	0.87 ± 0.04^{Bc}	1.12 ± 0.04^{Aa}	0.97 ± 0.01^{Ab}	1.12 ± 0.04^{Aa}	1.22 ± 0.04^{Aa}	1.00 ± 0.03^{Bb}
	Ca	1.26 ± 0.07^{Bb}	1.51 ± 0.05^{Bab}	1.31 ± 0.17^{Bb}	1.87 ± 0.13^{Ba}	2.10 ± 0.10^{Aa}	2.37 ± 0.04^{Aa}	2.23 ± 0.01^{Aa}	2.18 ± 0.09^{Aa}
	Mg	1.13 ± 0.02^{Aa}	0.81 ± 0.02^{Ab}	0.51 ± 0.03^{Ac}	0.84 ± 0.05^{Ab}	0.49 ± 0.04^{Bb}	0.61 ± 0.02^{Ba}	0.57 ± 0.01^{Ab}	0.56 ± 0.04^{Bb}
	K	3.97 ± 0.01^{Aa}	3.85 ± 0.02^{Aa}	4.12 ± 0.12^{Aa}	3.88 ± 0.07^{Aa}	4.07 ± 0.01^{Aa}	3.78 ± 0.17^{Aa}	3.48 ± 0.11^{Bb}	$3.87\pm0.22A^{ab}$
	Na	0.25 ± 0.01^{Ba}	0.16 ± 0.01^{Bc}	0.19 ± 0.01^{Bb}	0.11 ± 0.01^{Bd}	0.34 ± 0.02^{Aa}	0.35 ± 0.01^{Aa}	0.24 ± 0.01^{Ab}	0.17 ± 0.01^{Ac}
	Al	1.61 ± 0.22^{Aa}	0.85 ± 0.01^{Ab}	0.13 ± 0.01^{Bc}	0.18 ± 0.01^{Ac}	0.71 ± 0.01^{Bb}	0.97 ± 0.07^{Aa}	0.78 ± 0.05^{Ab}	0.38 ± 0.01^{Ac}
	Ca/Al	0.50 ± 0.02^{Bd}	1.20 ± 0.20^{Bc}	7.20 ± 0.32^{Ab}	8.22 ± 0.18^{Aa}	1.89 ± 0.01^{Ab}	1.74 ± 0.10^{Ab}	1.80 ± 0.02^{Bb}	3.75 ± 0.03^{Ba}

*Different lower case letters indicate statistically significant differences ($P\leq0.05$) between calcium treatments for the same cultivar. Different upper case letters show differences ($P\leq0.05$) between cultivars for the same calcium sulfate treatments.

**Analyzed by Plant and Soil Laboratory, Instituto de Agroindustria, Universidad de La Frontera, Temuco, Chile.

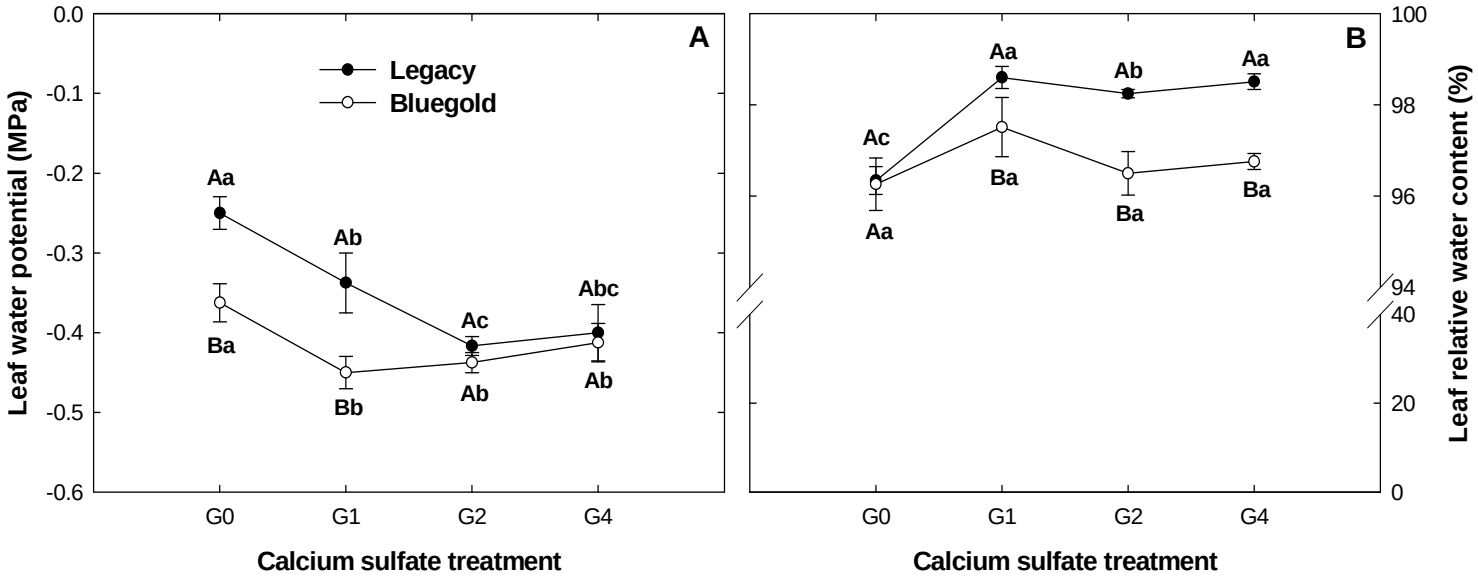

Figure 4.1. (A) Changes in leaf water potential (Ψ_{leaf}) and (B) relative water potential (RWC) in leaves of two highbush blueberry cultivars grown in an Al saturated Andisol amended with calcium sulfate. Values represent the average of six replicates ± SE. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between calcium sulfate treatments for the same cultivar. Different upper case letters show differences ($P \leq 0.05$) between cultivars for the same treatment.

Table 4.5. Photochemical and non-photchemical parameters of PSII in two highbush blueberry cultivars grown in an Al saturated Andisol amended with calcium sulfate for different times (0, 30 and 60 d). Values represent the mean of four replicates

		Legacy			Bluegold		
Time (d)	Treatments	ΦPSII	ETR	NPQ	ΦPSII	ETR	NPQ
0	G0	0.210±0.009Aa	24.460±0.579Ba	1.10±0.040Ca	0.264±0.003Ba	33.312±0.297Ba	2.103±0.005Aa
	G1	0.212±0.010Ca	24.456±0.547Ca	1.074±0.056Ba	0.268±0.034Ca	32.428±0.347Ca	2.201±0.070Aa
	G2	0.215±0.007Ca	24.480±0.480Ca	1.132±0.049Ba	0.256±0.022Ca	34.389±0.503Ca	2.206±0.035Aa
	G4	0.216±0.012Ba	24.564±0.603Ca	1.152±0.079Ba	0.261±0.018Ba	34.867±0.295Ca	2.168±0.005Aa
30	G0	0.421±0.001Ac	54.091±0.126Ad	2.075±0.002Aa	0.346±0.004Ab	43.625±0.612Ac	1.929±0.003Ba
	G1	0.436±0.002Ab	51.726±0.744Ac	1.851±0.007Ab	0.341±0.002Ba	42.830±0.211Bb	1.843±0.002Bb
	G2	0.457±0.005Aa	55.032±0.576Ab	1.604±0.009Ac	0.330±0.002Ba	45.817±0.213Ba	1.658±0.003Bb
	G4	0.463±0.006Aa	58.352±0.795Aa	1.853±0.027Ab	0.279±0.001Bc	44.017±0.152Ba	1.881±0.006Bb
60	G0	0.220±0.002Ac	27.750±0.168Ac	0.971±0.020Ba	0.431±0.003Ab	54.207±0.346Ac	1.625±0.001Ba
	G1	0.328±0.002Ba	41.369±0.271Ba	0.868±0.005Cb	0.436±0.001Aa	54.931±0.239Ab	1.631±0.002Cc
	G2	0.262±0.005Bb	29.256±0.484Bb	0.581±0.007Cc	0.433±0.004Aa	54.547±0.217Ab	1.686±0.005Bb
	G4	0.230±0.002Bc	28.967±0.738Bbc	0.408±0.006Cd	0.450±0.003Aa	56.665±0.269Aa	1.630±0.002Cc

±S.E.

*Different lower case letters indicate statistically significant differences (P≤0.05) among treatments for the same treatment. Different upper case letters show differences (P≤0.05) between cultivar for the same time (d).

4.3.5 Lipid peroxidation, radical scavenging activity, and antioxidant compounds

4.3.5.1 Lipid peroxidation

In plants stand on in Al-saturated soil (control), leaves of Legacy showed a higher LP than Bluegold ($P \leq 0.001$, Figure 4.4A). In contrast in both cultivars LP declined strongly (~80%) with G2 treatment respect to the control plants ($P \leq 0.001$). Contrarily, LP of control roots of Bluegold where a 30% higher LP than in Legacy roots was found. The major dose of calcium sulfate did not reduced significantly the LP of Legacy roots, whereas Bluegold exhibited a significantly reduction in LP (Figure 4.4A).

4.3.5.2 Radical scavenging activity

An increased RSA in leaves of the two cultivars under calcium sulfate amendment, in comparison those without amendment was exhibited (Figure 4.4B). In Legacy an augment up to ~80% RSA under G2 treatment in respect to control plants was evidenced ($P \leq 0.001$), while in the other treatments no statistically differences between them (G1 and G4) were observed ($P=0.857$). Notwithstanding this, these both treatments were statistically significant different in respect to non-amendment plants. Generally Bluegold leaves had higher RSA than Legacy ($P \leq 0.05$; Figure 4.4B). On the other hand, RSA of roots of Bluegold increased at G2 and G4 compared to non amended control plants ($P \leq 0.05$), being enhancements of 37 and 19% respectively. Roots of Legacy with G2 treatment had higher RSA values (~58%) when compared with roots growing in the Al- saturated soil. The calcium sulfated addition showed a better effect in Legacy RSA root than in Bluegold at both G1 and G2 ($P \leq 0.001$, Figure 4.4B).

Table 4.6. Photosynthetic pigment concentrations (mg kg^{-1} FW) in two highbush blueberry cultivars grown in an Al-saturated Andisol amended with calcium sulfate for 60d. Values represent the mean of six replicates ±S.E. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) among treatments for the same cultivar. Different upper case letters show differences ($P \leq 0.05$) between cultivar for the same treatment.

Cultivar	Treatments	Chl*a*	Chl*b*	Chl*a*+*b*	Chl*a*/*b*	Carotenoids
Legacy	G0	0.574±0.051Bb	0.248±0.020Aa	0.623±0.030Bb	2.082±0.079Ba	0.081±0.007Bc
	G1	0.541±0.056Aa	0.209±0.011Ab	0.677±0.002Ba	2.250±0.161Aa	0.133±0.011Ab
	G2	0.556±0.052Aa	0.273±0.017Aa	0.730±0.027Aa	1.782±0.060Bb	0.141±0.004Ab
	G4	0.459±0.010Aab	0.260±0.004Aa	0.689±0.018Ab	1.650±0.033Bb	0.178±0.011Aa
Bluegold	G0	0.375±0.035Aa	0.225±0.028Aa	0.730±0.040Aa	2.483±0.143Ab	0.104±0.010Aab
	G1	0.467±0.009Aa	0.235±0.008Aa	0.762±0.072Aa	2.428±0.086Ab	0.110±0.004Ba
	G2	0.504±0.037Aa	0.243±0.014Aa	0.741±0.019Aa	2.563±0.089Ab	0.109±0.005Ba
	G4	0.429±0.015Ab	0.166±0.002Bb	0.625±0.012Ab	2.759±0.022Aa	0.097±0.000Bb

*Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between calcium treatments for the same cultivar. Different upper case letters show differences ($P \leq 0.05$) between cultivars for the same calcium sulfate treatment.

4.3.5.3 Total phenols, anthocyanins, and flavonoid contents

In both cultivars, an increased TPC in leaves were observed, concomitant with higher calcium sulfate doses (G2 and G4) compared to the control plants ($P \leq 0.05$, Figure 4.5A,B). The enhanced TPC represented up to ~123% in Bluegold plants treated with G2 and G4 ($P \leq 0.001$). The levels of TAN in leaves of cultivars grown in control soil were increased up to ~70% in Legacy ($P \leq 0.001$) and ~40% in Bluegold ($P \leq 0.001$), by the higher calcium sulfate addition (G4) (Figure 4.5B). Concerning the cultivars, TPC values of plants growing in soils free of amendment were higher in Bluegold than in Legacy ($P \leq 0.001$). After 60d under acid and Al toxicity conditions (control) Bluegold showed higher TAN than Legacy ($P = 0.037$), although calcium sulfate applied at 4000 allowed an increased TAN in Legacy, reaching ~37% above Bluegold ($P \leq 0.001$). The TFA in Legacy was diminished up to 30%, under calcium sulfate amendment with respect to control plants, independently of calcium sulfate doses added ($P \leq 0.001$). Differently, in Bluegold TFA were enhanced (~30%) at G1 ($P = 0.014$; Figure 4.5C).

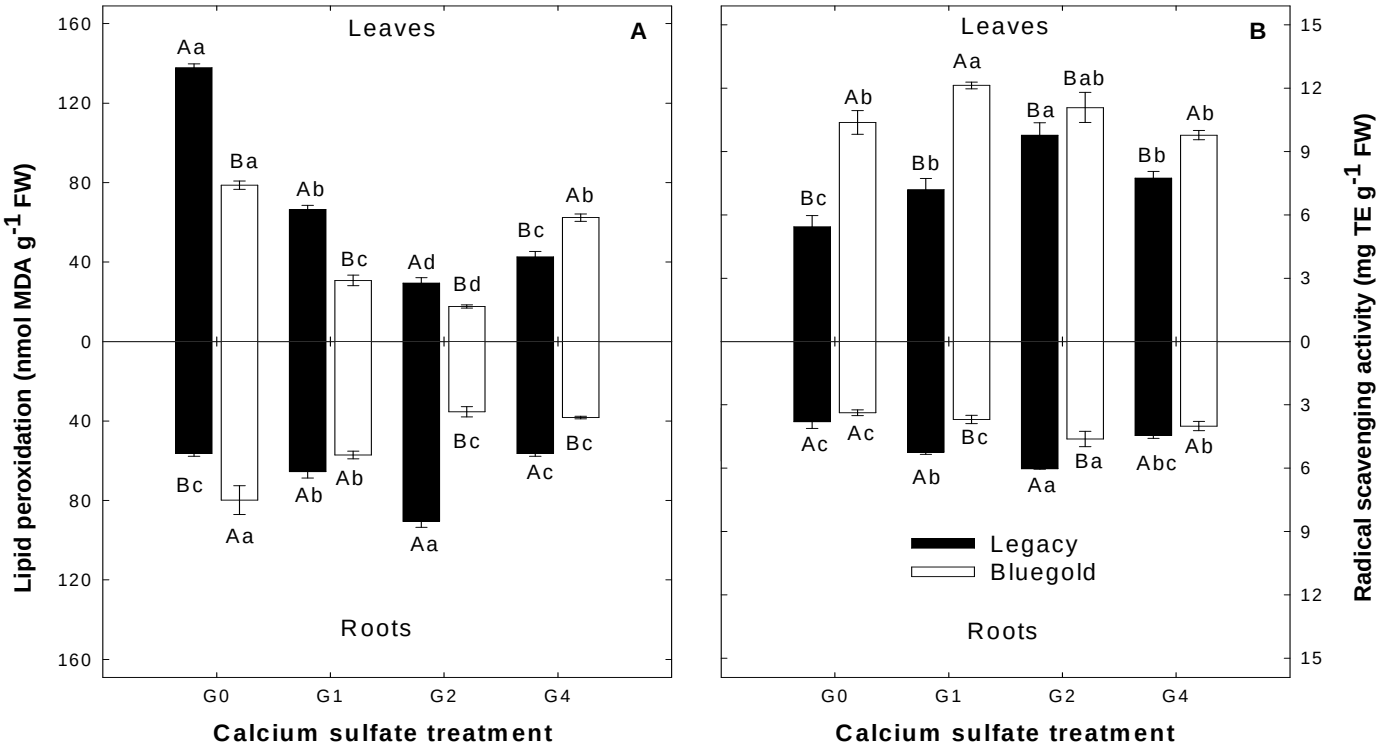

Figure 4.2. Lipid peroxidation (A) and radical scavenging activity (B), in leaves and roots of two highbush blueberry cultivars grown in an Al saturated Andisol amended with calcium sulfate for 60d. Values represent the mean of six replicates ±S.E. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same treatment. Different upper case letters show differences ($P \leq 0.05$) between cultivar for the same treatment.

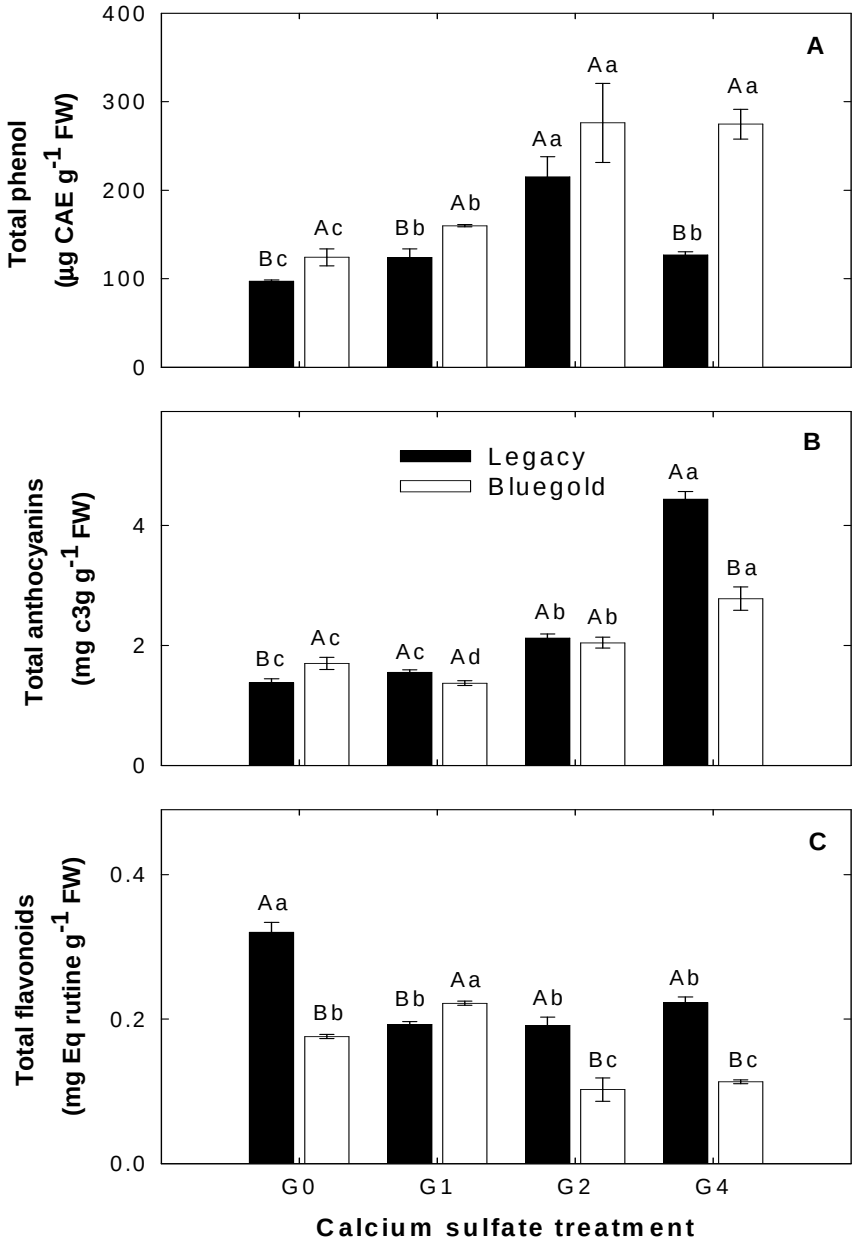

Figure 4.3. Total phenol (A), anthocyanin (B), and flavonoids (C) content in leaves of two highbush blueberry cultivars grown in an Al saturated Andisol amended with calcium sulfate for 60d. Values represent the mean of six replicates ±S.E. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same treatment. Different upper case letters show differences ($P \leq 0.05$) between cultivar for the same treatment.

4.3.5.4 Antioxidant enzyme activity

Whereas in Legacy, foliar SOD activity increased (41%) by G4 treatment ($P \leq 0.05$), Bluegold also did it at G1 compared to control plants ($P \leq 0.050$, Figure 4.6). In addition, leaves SOD activity of Bluegold was higher than Legacy (169%) in all the evaluated treatments ($P \leq 0.001$). While in roots, Legacy showed and arise of its SOD activity under G1 (63%) and G2 (200%) treatments, compared to control plants ($P \leq 0.006$), instead Bluegold only experimented changes in respect to the control plants at G1 ($P = 0.027$). Legacy showed a 64% higher SOD activity in roots than Bluegold in calcium sulfate doses G2 ($P \leq 0.001$). An enhanced CAT activity in leaves of Legacy (~543%) was found in treatment G4, in comparison to control plants ($P \leq 0.001$; Figure 4.6). For Bluegold, all calcium sulfate treatments increased significantly CAT according with the increase of calcium sulfate doses used. In roots, CAT activity after increased in both cultivars in respect to the plants non-subjected to soil calcium sulfate treatments (control) ($P \leq 0.050$, figure 4.6).

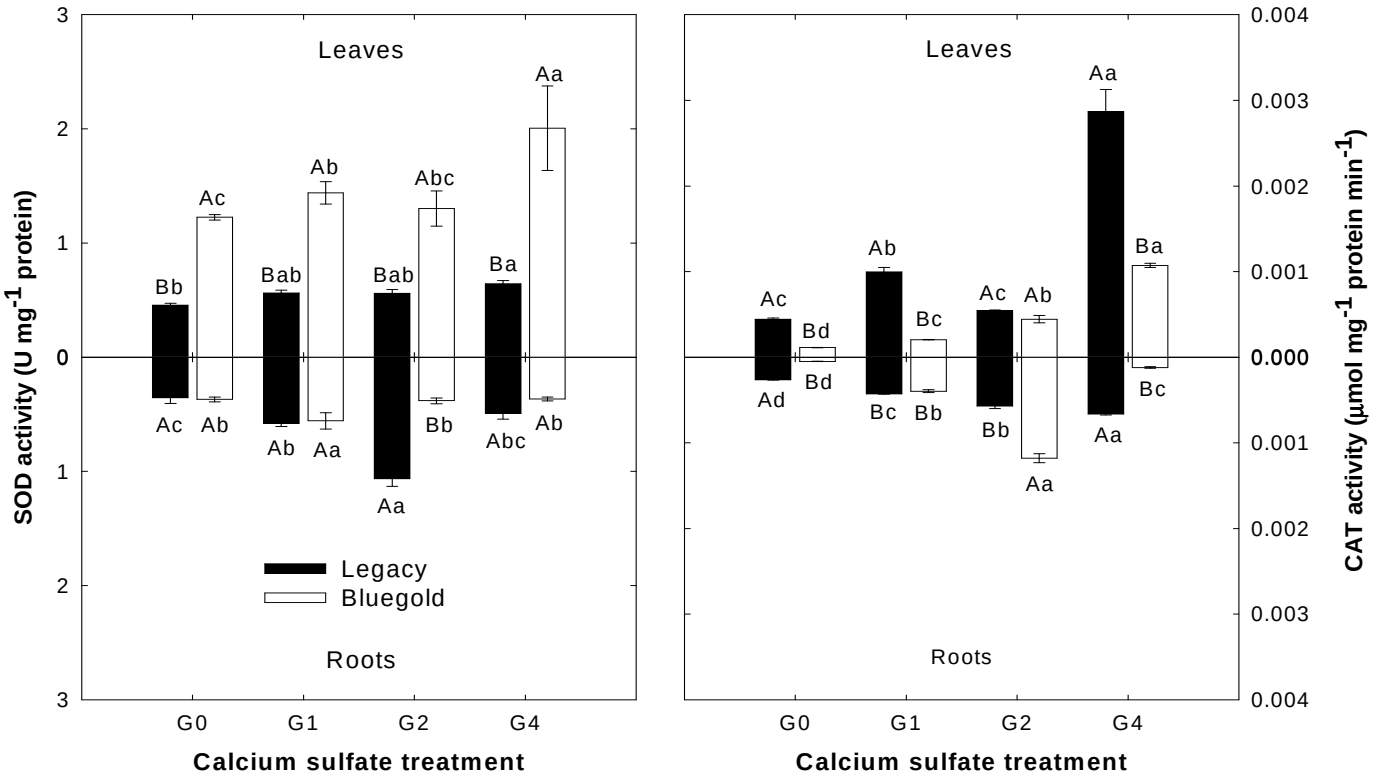

Figure 4.4. Superoxide dismutase (SOD) and catalase (CAT) activity in leaves and roots of two highbush blueberry cultivars grown in an Al saturated Andisol amended with calcium sulfate for 60d. Values represent the mean of six replicates ±S.E. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between treatments for the same treatment. Different upper case letters show differences ($P \leq 0.05$) between cultivar for the same treatment.

4.4 DISCUSSION

The effects calcium sulfate addition to reduce Al content and nutrient improvement have been widely reported by several authors in different crops grown in acid soils with high Al^{3+} concentration (Alva *et al.*, 1991; Carvalho and van Raig, 1997; Mora *et al.*, 2002; Caires *et al.*, 2006). This amendment shoot and roots growth can be improve in crop plants subjected to abiotic environmental stresses (Korcak, 1988; Tuna *et al.*, 2007). In an experiment with tomato plants (*Lycopersicon esculentum* Mill.) under saline stress (NaCl), Tuna *et al.*, (2007) added calcium sulfate at 5 and 10 mM significantly improved growth plant and physiological variables affected by salt stress. In an Abiotic stress as toxic Al our results suggest an effect of this amendment on increase elongation of plants in both cultivars (Table 4.2), although in Al tolerant Legacy lower calcium sulfate doses than Bluegold were required for this purpose. The root:shoot ratio in Legacy was not changed with treatments, whereas in Bluegold this ratio was decreased, indicating that root growth was stimulated by amendment additione. The improvement in plant growth can be a response toia better nutrient balance in plants, because this calcareous amendment caused significant reductions of Al content in evaluated tissues (Andersson, 1993), especially in Al-tolerant Legacy (Table 4.2). Similar effects were observed by Reyes-Díaz *et al.* (2011) in Al-tolerant blueberry cultivars Brigitta and Legacy subjected to nutrient solution with Al toxicity (100µM) and recovered with calcium sulfate (5mM). Mathews and Joost (1989) and Carvalho and van Raig (1997) reported that the formation of lesser toxic and insoluble aluminum sulfate ($AlSO_4^+$) in soil after calcium sulfate application contributed to ameliorate harmful effect of Al^{3+}, by complexation with SO_4^{2-} groups in the outer sphere Al that could be excluded by roots (Alva *et al.*, 1991). In addition, Mora *et al.* (1999a) and Cichota *et al.* (2007) proposed also that displacement of Al^{3+} from cation exchange sites by Ca^{2+} is frequently in variable charge soil such as Andisol used in our experiment (Table 4.1).

On the other hand, improved foliar and root nutrient concentration, mainly Ca and in lesser extent K, and P, showed that effective role of calcium sulfate on Al ameliorating contributed to improved tissues nutrient level, although Mathews and

Joost (1989) reported that excessive calcium sulfate applications can create K and Mg deficiencies. Sanderson and Eaton (2004) in lowbush blueberry (*Vaccinium angustifolim* Ait.) reported significant increases in leaf nutrient contents as P, Ca, K, and S after gypsum addition for two cropping cycle. Calcium sulfate addition increased root S content only in Al sensitive Bluegold (G2), according to reviewed by Rennenberg (1984) who reported that S uptake is triggered by an increase of polyvalent cations as Ca^{2+} or Mg^{2+}, although in this cultivar no increases of these cation in roots were observed. Sanderson *et al*. (1995) reported critical levels of S for blueberries from 0.08 to 0.14%). However, no relationship between Ca and S were found. For Legacy, an increase of foliar Ca due to calcium sulfate addition resulted in an improved S (r=0.63), K (r=0.671), and P (r=0.615) contents, and no correlation between a raised Ca and Al were found (Table 4.4). Additionally, calcium sulafte application helped to reduce (~50%) foliar and root Na content in Legacy and Bluegold. Increased Na concentrations in roots are associated to inhibitions in uptake and transport of Ca and K, thus leading to nutritional imbalances in saline environments stresses (Tuna *et al.*, 2007). Our experiment showed that Na was negatively correlated with Ca content in Legacy roots (r=─0.85, $P \leq 0.001$), but no correlation with K content were found. However; Al content were positively correlated with Na content in Legacy leaves (r=0.74; P=0.004) and both leaves and roots of Bluegold (r=0.63; P=0.024 and r=0.67; P=0.015). Negative relationships between Ca and Mg have been reported for pastures by Ritchey *et al*. (1995) and Ritchey and Snuffer (2002), whereas in blueberries treated with gypsum, Mg content were not affected either leaves and roots (Korcak, 1988). Similar results in our blueberry plants were obtained, for Legacy where no correlation between them were observed, whereas Bluegold showed a significant interaction between Ca and Mg in roots (r=0.706; P=0.009).

The interaction between Ca and Al has been widely associated at physiological and biochemical disorders in cropped plants (Delhaize and Ryan, 1995; Kochian, 1995; Schaberg *et al.*, 2006; Meriño-Gergichevich *et al.*, 2010). After 60d, Ca/Al molar ratio (Ca/Al) in both cultivars was significantly raised, depending on calcium sulfate doses. In generally the highest Ca/Al were achieved at G4. In leaves

this ratio was higher in Bluegold, whereas in roots Legacy showed higher Ca/Al ($P \leq 0.050$, Table 4.4). It has reported that this ratio is increased after gypsum addition to soil (Ritchey and Snuffer, 2002; Weber-Blaschske and Rehfuess, 2002). However they do not mention to the differences between different cultivars from species. Calcium sulfate significantly increased Ca/Al molar ratio, although this ratio in leaves were higher in Al sensitive Bluegold than Al tolerant Legacy (Table 4.4). For leaves in woody species Cronan and Grigal (1995) reported that a Ca/Al ratio less than 12.5 would indicate 50% risk of Al toxicity and Ca/Al ratio lower than 6.5 a 75% risk. According to our experiment, lower foliar Ca/Al showed by Al tolerant cultivar according to these authors, would indicate a better efficiency of uptake and transport of Ca than Al sensitive Bluegold. In roots of Legacy and Bluegold molar ratio varied from 0.5 to 8.2 and 1.8 to 3.7 respectively, similar to those ratios obtained for oak (*Quercus robur*), pine (*Pinus radiata*) and eucalyptus (*Eucalyptus nitens*) plants grown in acidic soil (Álvarez *et al.*, 2005).

Aluminum has been shown to cause interference in water relations of plants (Kochian, 1995; Mossor Pietraszewska, 2001; Rout *et al.*, 2001) by alteration of cell wall porosity in root cells. In wheat, grown in soil columns at acid pH (4.70) and Al saturation (65%), Ψ_{leaf} and RWC were increased with gypsum addition (Zaifnejad *et al.*, 1996). In our highbush blueberry plants, calcium sulfate treatments leaded Ψ_{leaf} to more negative values, compared to the control plants, probably due to increased cation concentration both roots and leaves (Figure 4.1). According to Taiz and Zieger (1998) a better accumulation of solutes (mediated by an improved Ca/Al) by cells, is a process without an accompanying decrease in turgor, resulting in an osmotic adjustment. This osmotic adjustment is related to an inhibited Al accumulation in the root tips (Yang *et al.*, 2010). Leaf water potential was compared with results reported for blueberries by Hickleton *et al.* (2000), Glass *et al.* (2005) and Bryla and Strik (2007), who reported for blueberries stand up in soil Ψ_{leaf} until ─1.5 MPa for drought season during summer in North hemisphere. Legacy (Al tolerant) had a lesser negative Ψ_{leaf} than Bluegold in control plants, although in Legacy Ψ_{leaf} was negatively correlated with foliar contents of Al and Na ($r=-0.79$; $P=0.002$ and $r=-0.66$; $P=0.018$, respectively) and root Al content ($r=-0.73$; $P=0.006$). In tolerant cultivar,

RWC was significantly increased by gypsum in respect to the control plants, showing a better hydric performance than Bluegold. Jiang and Huang (2001) describe that external Ca application improved RWC in tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass (*Poa pratensis* L.) under heat stress for 30 days, although these application were not related to osmotic potential and osmotic balance. Also, Ca application reduced the loss of chlorophyll content under stress, by inhibition of photoxidation and/or better membrane integrity.

Reyes-Díaz *et al.* (2010) studied photochemical parameters responses in blueberry cultivars (Legacy and Bluegold) subjected to Al toxicity (25, 50, 100, and 200µM) for 21 days. Results showed that Bluegold was the most affected by toxic Al, especially at beginning of experiment, explained by a lower RSA in this Al sensitive cultivar. The fluorescence parameter ΦPSII and ETR, were increased in both cultivars, showing Legacy high values at 30 days, whereas Bluegold at 60 days, in amended plants. These results agree with reported by Reyes-Díaz *et al.* (2011), where these parameters responded to $CaSO_4$ treatments mainly in tolerant cultivars. These increased photochemical parameters were concomitant with a reduction of NPQ, mainly in tolerant cultivar at the end of experiment, due to NPQ gives information about heat dissipation when an excess of light energy in the PSII (Maxwell and Johnson, 2000). Literatures have reported negative interactions between NPQ and photosynthetic pigment content (Bilger and Björkman, 1990; Demmig-Adams and Adams, 1996). Nonetheless, Chen *et al.* (2005) suggested that NPQ was not a key factor on dissipation of excessive thermal energy in Al treated leaves of tangerine (*Citrus reshni* Hort. Ex Tanaka), likely due to Haliwell-Asada route and photorespiration involving in dissipating of excessive thermal energy. Fluoresecence parameters were favored by foliar Ca/Al 6 in Legacy and 14 in Bluegold.

Pereira *et al* (2006) found that Al caused a decrease in Chlorophyll synthesis by inhibition of enzymes responsible for monopyrrole porphobilonogen production, which is constituent of Chl and cytochromes. However, in our two cultivars, no statistically significant correlation between foliar Al or Ca and Chl *a* or *b*, Chl *a*+*b*, and Chl *a*/*b* were found. Peixoto *et al.* (2002) found adverse effects of Al on leaf photosynthetic pigments both in tolerant and sensitive sorghum cultivars [*Sorghum*

bicolor (L.) Moench]. Reyes-Díaz *et al.* (2009) in blueberry, reported a decreased pigment concentration correlated with Al content, mainly in sensitive cultivars. Calcium sulfate increased Chl *a*, not by addition of external Ca, but for one improvement mineral balance in leaves, mainly in tolerant cultivar. The Na content was negatively correlated in both cultivars with Chl *b* concentration (r=from ─0.64 to ─0.680; $P \leq 0.05$), whereas its reduction by cvalcium sulfate addition would be a key factor. Legacy showed negative correlations between carotenoids content and Al (r=─0.81, $P=0.001$), Na (r=─0.56; $P \leq 0.05$), in contrast an improved Ca/Al molar ratio by calcium sulfate addition had positive effect on accessory pigment (r=0.86, $P \leq 0.001$) and S content (r=0.578, $P=0.044$). A better carotenoids are significantly correlated with decreased NPQ (r=0.850). In barley (*Hordeum vulgare* L.) seedlings subjected to Al (0, 0.08, 0.4, 2, and 10 µM) for 30d, carotenoids concentration was increased in relationship to Al treatment applied, whereas chlorophylls a and b were significantly reduced in respect to control seedling (Abdalla, 2008).

The scientific evidences show that Al can decrease electron transfers between both photosystems (PSII and PSI), increasing production of ROS (Lidon *et al.*, 1999; Scandalios, 2002), thus inducing lipid peroxidation in chloroplasts (Lidon *et al.*, 1999; Rout *et al* 2001). In highbush blueberry, Al content and LP was correlated positively in Legacy leaves (r=0.84; $P \leq 0.001$). The reduction of LP was associated to an improved Ca/Al in leaves (r=─0.67; $P=0.018$) and roots (r=─0.62; $P=0.031$) of Legacy, however in Al sensitive cultivar no relationships were found. Reyes-Díaz *et al.* (2011) reported similar effects of gypsum on reduction of LP in tolerant cultivar Legacy subjected to Al toxicity in hydroponic culture. The optimal foliar Ca/Al to reduced LP was situated between 6 and 7 for Legacy and over 10 for Bluegold (r=0.95).

The increase of antioxidant activity in plants amended with calcium sulfate was positively affected in leaves and roots of Legacy and Bluegold, in comparison to the control plants, although Bluegold showed a higher RSA than Al tolerant cultivar ($P \leq 0.050$, Figure 4.2B). Calcium may be involved in plant tolerance to different stresses by antioxidant metabolism regulation (Jiang and Huang, 2001), this finding is in agreement with our results that showed an improved Ca^{2+} content have a

significant influence on RSA. On the other hand, Al contents in Legacy were negatively correlated with RSA in leaves (r=─0.68; *P*=0.014) and roots (r=─0.72; *P*=0.008), indicating that a reduction of this metal in tolerant cultivar tissues should increased RSA. A number of studies have reported that in parallel to increased ROS levels, Al stress also could be an induces an increase in the antioxidant activity to overcome deteriorating effects of these toxic species, increasing the tolerance to Al stress (Guo *et al.*, 2006; Khan *et al.*, 2007).

Antioxidant activity is associated to several secondary metabolites to overcome stresses caused by environmental factors such as Al^{3+}. In this way, highbush blueberries are considered a rich source of antioxidant compounds such as phenols, anthocyanins and flavonoids, with nutritional properties for human health (Prior *et al.*, 1998; Kalt *et al.*, 2001; Mittler *et al.*, 2004; Dragović-Uzelac *et al.*, 2010). However, our results showed that Al content in sensitive cultivar negatively affected TPC (r=─0.64; *P*=0.024), whereas in Legacy TAN were reduced under greater Al content (r=─0.74; P=0.005), and TFA was positively correlated with Al content (r=0.64; *P*=0.023). Foliar Ca content had positive influence on TAN in Bluegold (r=0.61; *P*=0.032). Little information has been reported about the real effects from calcium sulfate in activity of these molecules. Thus we suggest that gypsum increased TPC and TAN, because to ameliorative role of this calcareous amendment on Al concentration in soil solution (Meriño-Gergichevich *et al.*, 2010, Reyes-Díaz *et al.*, 2011). In relation to the same line, antioxidant enzymes set contributed to defensive system as first barrier against harmful effects of Al^{3+} in plant metabolism. In our experiment, SOD and CAT showed lower activity in Al stressed plants in both organs of two cultivars, and then increased by calcium sulfate addition (Figure 4.4). Bluegold in leaves had a higher SOD activity than Legacy, because in Bluegold a greater Ca contents were found after application (r=0.69; *P*=0.012). Calcium is a regulator of several enzymes, playing a role as intra- and extracellular messenger (Sanders *et al.*, 2002; Silva *et al.*, 2005). Gong *et al.* (1997) on maize (*Zea mays* L.), and Jiang and Huang (2001) in tall fescue and Kentucky bluegrass, have reported that external Ca addition increased ascorbate peroxidase (APX, EC 1.11.1.11), CAT, glutahione reductase (GR, EC 1.6.4.2), and SOD. For blueberry

cultivars, Reyes-Díaz *et al.* (2011) reported that calcium addition toxic Al caused an increment of SOD activity nutrient solution in respect to stressed plants (100 µM). In agreement with our findings, Al content had a detrimental effect on SOD and CAT activity, showing Legacy in leaves and roots significant negatively correlations between Al and SOD (r=─0.61 and r=─0.59 respectively), and CAT in roots (r=─0.94). For Bluegold, radical SOD and Al were (r=0.61; *P*=0.032). So, calcium sulfate addition reduced Al content and restored balance between ROS and antioxidant enzymes, with special focus in Al tolerant cultivars (Legacy), where LP and SOD was correlated in leaves band roots (r=0.60; *P*=0.036 and r=0.83; *P*≤0.001 respectively). It could be assumed that in Legacy these antioxidant enzymes not represent the most important mechanism, but to alleviate environmental impacts like Al stress. The molar ratio between Ca and Al ranges 6 in Legacy and 14 for Bluegold.

4.5 CONCLUSION

According to the results presented in this study, calcium sulfate addition was a good alternative to enhance the inhibited growth of shoot and roots in blueberry plants by aluminum. These positive effects on plants growth certainly were produced by a better nutritional content and reduced aluminum content in tissues, caused by amendment. An improved Ca/Al molar ratio was observed in both cultivars, although is necessary more studies to evaluate the role of this ratio in blueberry plant performance. Contradictory results about hydric relation were observed with gypsum application, whereas leaf water potential showed more negative values, probably induced by higher nutrient in xylematic flux, relative water content was increased, especially in tolerant cultivar. Increased fluorescence parameters (ΦPSII and ETR) in Legacy, was related to a reduced non photochemical quenching after gypsum addition. Thus, more light energy was drive to photochemical reactions. Also, amended plants showed a higher antioxidant capacity mainly in tolerant cultivar. Al toxicity did not increased antioxidant compounds in leaves and roots of blueberry and was negatively correlated, except flavonoids in Legacy. Finally, from agronomical manure point of view, blueberries Al tolerant cultivar would require lower amendment doses than those sensitive ones, however gypsum also ameliorate harmful effect of Al on Bluegold at higher doses.

Acknowledgements

This study was supported by FONDECYT Nº 11080231 and Comisión Nacional de Ciencia y Tecnología de Chile (CONICYT) for doctoral thesis support AT-24100159. The authors thanks to Erik von Baer and Marcelo Labra from Semillas Baer, Cajón, Temuco, Región de La Araucanía, Chile and Commercial farm Berries San Luis, Lautaro, Región de La Araucanía, Chile. C. Meriño-Gergichevich thanks to MECESUP FRO 0601 Maintenance Grant and Congress Attendance Scholarship CONICYT, Ximena Garrido Ramírez, Jessica Yañez Delgado, and Mariela Mora Garrido for technical assistance.

4.6. REFERENCES

Abdalla, M. M. 2008. Physiological aspects of aluminium toxicity on some metabolic and hormonal contents of *Hordeum vulgare* seedlings. Aust. J. Basic Appl. Sci. 2, 549 ─ 560.

Aebi, H. E .1983. Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Verlag Chemie, Weinhern, pp 273 ─ 286.

Alva, A. K., Kerven, G. L., Edwards, D. G., Asher, C. J. 1991. Reduction in toxic aluminum to plants by sulfate complexation. Soil Sci. 152, 351 ─ 359.

Alvarez, E., Fernández-Marcos, M. L., Monterroso, C., Fernández-Sanjurjo, M. J. 2005. Application of aluminium toxicity indices to soils under various forest species. Forest Ecol. Manag. 211, 227 ─ 239.

Andersson, M. E. 1993. Aluminium toxicity as a factor limiting the distribution of *Allium ursinum* (L.). Ann. Bot. 72, 607 ─ 611.

Angeletti, P., Castagnasso, H., Miceli, E., Terminiello, L., Concellón, A., Chaves, A., Vicente A. R. 2010. Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*) varieties. Postharvest Biol. Tech. 58, 98 ─103.

Barceló, J., Poschenrieder, C. 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environ. Ex. Bot. 48, 75 ─ 92.

Bilger, W., Björkman, O. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurement of light-induced absorbance changes, fluorescence and photosynthesis in *Hedera canariensis*. Photosynth. Res. 25, 173 ─ 175.

Blatt, C. R., McRae, K. H. 1997. Marketable yield response of two lowbush blueberries to preplant applications of gypsum, calcitic and dolomitic limestones. J. Small Fruit Viticulture 5, 13 ─ 26.

Bolan, N. S., Syers, J. K., Sumner, M. E. 1993. Calcium-induced sulfate adsorption by soils. Soil Sci. Soc. Am. J. 57, 691 ─ 696.

Björkman, O., Demmig, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170, 489 ─ 504.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248 ─ 254.

Bryla, D. R., Strik, B. C. 2007. Effects of cultivar and plant spacing on the seasonal water requirements of highbush blueberry. J. Amer. Soc. Hort. Sci. 132, 270 ─ 277.

Caires, E. F., Churka, S., Garbuio, F. J., Ferrari, R. A., Morgano, M. A. 2006. Soybean yield and quality as a function of lime and gypsum applications. Sci. Agric. 63, 370 ─ 379.

Campbell, C. G., Garrido, F., Illera, V., García-González, M., T. 2006. Transport of Cd,
Cu and Pb in an acid soil amended with phosphogypsum, sugar foam and phosphoric rock. Appl. Geochem. 21, 1030 ─ 1043.

Carvalho, M. C. S., van Raij, B. 1997. Calcium sulphate, phosphogypsum and calcium carbonate in the amelioration of acid subsoils for root growth. Plant Soil 192, 37 ─ 48.

Chen, L. S., Qi, Y. P., Liu, X. H. 2005. Effects of aluminum on light energy utilization and photoprotective systems in citrus leaves. Ann. Bot. Lon. 96, 35 ─ 41.

Cheng, S., Willmann, M. R., Chen, H., Sheen, J. 2002. Calcium signaling through protein kinases. The arabidopsis calcium-dependent protein kinase gene family. J. Plant Physiol. 129, 469 ─ 485.

Cichota, R., Voegeler, I., Bolan N. S., Clothier, B. E. 2007. Simultaneous adsorption of calcium and sulfate and its effect on their movement. Soil Sci. Soc. Am. J. 71, 703 ─ 710.

CIREN. 2002. Estudio agroecológico. Descripciones de suelos, materiales y símbolos. IX Región. Centro de Información de Recursos Naturales. Publicación 122, 343p.

Close, D. C., Beadle, C. L., Brown, P. H., Holz, G. K. 2000. Cold-induced photoinhibition affects establishment of *Eucalyptus nitens* (Deane and Maiden) and *Eucalyptus globulus* Labill. Trees – Struct. Funct. 15, 32 ─ 41.

Delhaize, E., Ryan, P. R. 1995. Aluminum toxicity and tolerance in plants. J. Plant Physiol. 107, 315 ─ 321.

Demmig-Adams, B., Adams III, W. W. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis.Trends Plant Sci. 1, 21 ─ 26.

Dragović-Uzelac, **V., Savić, Z., Brala, A., Levaj, B., Bursać-Kovačević, D., Biško, A. 2010.** Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the northwest Croatia. Food Technol. Biotechnol. 48, 214 ─ 221.

Du, Z., Bramlage, W. J. 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food Chem. 40, 1566 ─ 1570.

Foy, C. D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. In F Adams, Ed, Soil Acidity and Liming. American Society of Agronomy, Madison, Wi, 57 ─ 97.

Garrido, F., Illera, V., Vizcaíno, C., García-González, M. T. 2003. Evaluation of industrial by-products as soil acidity amendments: chemical and mineralogical implications. Eur. J. Soil Sci. 54, 411 ─ 422.

Glass, V. M., Percival, D. C., Proctor, J. T. A. 2005. Tolerance of lowbush blueberries (*Vaccinium angustifolium* Ait.) to drought stress. II. Leaf gas exchange, stem water potential and dry matter partitioning. Can. J. Plant Sci. 85: 919 ─ 927.

Gong, M., Chen, S. N., Song, Y. Q., Li, Z. G. 1997. Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. Aus. J. Plant Physiol. 24, 371 ─ 379.

Gough, R. E. 1997. Blueberries-north and south. J. Small Fruit Viticulture 4, 71 ─ 106.

Guerrero, J. A., Ciampi P. L., Castilla, C. A., Medel S. F., Schalchli S. H., Hormazabal, U. E., Bensch, E., Alberdi, M. 2010. Antioxidant capacity, anthocyanins, and total phenols of wild and cultivated berries in Chile. Chil. J. Agr. Res. 70, 537 ─ 544.

Guerrero, J. A. 2006. Capacidad antioxidante, fenoles y antocianinas totales e inhibición de *Botrytis cinerea* Pers. Ex Fr. por extractos crudos de fruta de cultivares de arándano alto (*Vaccinium corymbosum* L.) según localidad de la zona Sur de Chile. PhD Thesis. Universidad Austral de Chile, Valdivia, Chile.

Guo, T., Chen, Y., Zhang, Y., Jin. Y. 2006. Alleviation of Al toxicity in barley by addition of calcium. Agr. Sci. China 5, 828 ─ 833.

Heath, R. L., Packer, L. 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189 ─ 198.

Hepler, P. K. 2005. Calcium: a central regulator of plant growth and development. Plant Cell 17, 2142 ─ 2155.

Hicklenton, P. R., Reekie, J. Y., Gordon, R. J. 2000. Physiological and morphological traits of lowbush blueberry (*Vaccinium angustifolium* Ait.) plants in relation to post-transplant conditions and water availability. Can. J. Plant Sci. 80, 861 ─ 867.

Hsiao, T. C. 1990. Measurements of plant water status, p. 243–279. In: B.A. Steward and D.R. Nielsen (eds.). Irrigation of agriculture crops. Agron. Monogr. No. 30. ASA-CSSA-SSSA Publ., Madison, Wis.

Illera, V., Garrido, F., Vizcayno, C., García-González, M. T. 2004. Field application of industrial by-products as Al-toxicity amendments: chemical and mineralogical implications. Eur. J. Soil Sci. 55, 681 ─ 692.

INE. 1998. VI Censo Nacional Agropecuario 1997. INE, Santiago, Chile, 214 p.

Inostroza-Blancheteau, C., Reyes-Díaz, M., Aquea, F., Nunes-Nesi, A., Alberdi, M., Arce-Johnson, P. 2011. Biochemical and molecular changes in response to aluminium-stress in highbush blueberry (*Vaccinium corymbosum* L.). Plant Physiol. Bioch. 49, 1005 ─ 1012.

Jiang, Y., Huang, B. 2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J. Exp. Bot. 52, 341 ─ 349.

Kalt, W., Ryan, D. A. J., Duy, J. C., Prior, R. L., Ehlenfeldt, M. K., Vander Kloet, S. P. 2001. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). Agric. Food Chem. 49, 4761 ─ 4767.

Khan, N. A., Samiullah, S., Singh, S., Nazar, R. 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J Agron. Crop Sci. 193, 435 ─ 444.

Kochian, L. V., Pineros, M. A., Hoekenga, O. A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274, 175 ─ 195.

Kochian, L. V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 237 ─ 260.

Korcak, R. F. 1988. Fluidized bed material applied at disposal levels: effects on an apple orchard. J. Am. Soc. Hort. Sci. 113, 189 ─ 193.

Li, X. M., Li, X. L., Zhou, A. G. 2007. Evaluation of antioxidant activity of the polysaccharides extracted from *Licium barbarum* fruits in vitro. Eur. Polym. J. 43, 448 ─ 497.

Lichtenthaler, H., Wellburn, A. R. 1983. Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 603, 591 ─ 592.

Lidon, F. C, Barreiro, M. J., Ramalho, J. C., Lauriano, J. A. 1999. Effects of aluminum toxicity on nutrient accumulation in maize shoots: implications on photosynthesis. J. Plant Nutr. 22, 397 ─ 416.

Lyrene, P. M., Munoz, C. 1997. Blueberry production in Chile. J. Small Fruit Viticulture 5, 1 ─ 20.

Ma, B., Wan, J., Shen, Z. 2007. H_2O_2 production and antioxidant responses in seeds and early seedlings of two different rice varieties exposed to aluminum. J. Plant Growth Regul. 52, 91 ─ 100.

Mathews, B. W., Joost, R. E. 1989. Use of sulfate salts to reduce subsoil aluminum toxicity: a review. J. Haw. Pac. Agri. 2, 24 ─ 30.

Maxwell, K., Johnson, G. N. 2000. Chorophyll fluorescens- a practical guide. J. Exp. Bot. 345, 659 ─ 668.

Meriño-Gergichevich, C., Alberdi, M., Ivanov, A. G., Reyes-Díaz, M. 2010. Al^{3+}-Ca^{2+} interaction in plants growing in acid soils: Al-phytotoxicity response to calcareous amendments. J. Soil. Sci. Plant Nutr. 10, 217 ─ 243.

Mittler, R., Vanderauwera, S., Gallery, M., van Breusegem, F. 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9, 490 ─ 498.

Mora, M. L, Alfaro, M., Williams, P., Stehr, W., Demanet, R. 2004. Effect of fertiliser input on soil acidification in relation to growth and chemical composition of a pasture, and animal production. R.C. Suelo Nutr. Veg. 4, 29 ─ 40.

Mora, M. L., Cartes, P., Demanet, R., Cornforth, I. S. 2002. Effects of lime and gypsum on pasture growth and composition on an acid Andisol in Chile, South America. Commun. Soil Sci. Plant Anal. 33, 2069 ─ 2081.

Mora, M. L., Schnettler, B., Demanet, R. 1999a. Effect of liming and gypsum on soil chemistry, yield, and mineral composition of ryegrass grown in an acidic Andisol. Commun. Soil Sci. Plant Anal. 30, 1251 ─ 1266.

Mora, M. L., Baeza, G., Pizarro, C., Demanet, R. 1999b. Effect of calcitic and dolomitic lime on physicochemical properties of a Chilean Andisol. Commun. Soil Sci. Plant Anal. 30, 427 ─ -439.

Mossor-Pietraszewska, T. 2001. Effect of aluminium on plant growth and metabolism. Acta Biochim. Pol. 3, 673 ─ 686.

Nanzyo, M., Shoji, S., Dahlgren, R. A. 1993. Physical characteristics of volcanic ash soils. In volcanic ash soils – genesis, properties and utilization. Eds. S Shoji, M Nanzyo and R A Dahlgren, pp. 189 – 207. Elsevier, Amsterdam.

Oberley, L. W., Spitz, D. R. 1986. Nitroblue tetrazolium. In Handbook of methods for oxygen radical research. Edited by Grenwald, R. A. pp. 217 ─ 220. CRC Press, Boca Raton, Florida.

ODEPA (Chile). 2011. Boletín de frutales: arándanos. Sección mercados y rubros. [on line]. Odepa. http://www.odepa.gob.cl [request: october, 15 ─ 2011].

Peixoto, H. P., da Matta, F. M., da Matta, J. C. 2002. Responses of the photosynthetic apparatus to aluminum stress in two sorghum cultivars. J. Plant Nutr. 25, 821 ─ 832.

Pereira, L. B, Tabaldi, L. A, Goncalves, J. F., Juckeoski, G. O., Pauletto, M. M., Weis, S. N., Nicoloso, F. T., Bocher, D., Rocha, J. B. T., Schetinger, M. R. C. 2006. Effect of aluminum on σ-aminolevulinic acid dehydratase (ALA-D) and the development of cucumber (*Cucumis sativus*). Environ. Exp. Bot. 57, 106 ─ 115.

Poschenrieder, C., Gunsé, B., Corrales, I., Barceló, J. 2008. A glance into aluminum toxicity and resistance in plants. Sci Total Environ. 400, 356 ─ 368.

Prior, R. L., Cao, G., Martin, A., Sofić, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, M. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 46, 2686 ─ 2693.

Rengel, Z., Robinson, D. L. 1989. Aluminum effects on growth and macronutrient uptake by annual ryegrass. Agron J. 81, 208 ─ 215.

Reyes-Díaz, M., Meriño-Gergichevich, C., Alarcón, E., Alberdi, M., Horst, W. 2011. Calcium sulfate ameliorates the effect of aluminum toxicity differentially in genotypes of highbush blueberry (*Vaccinium corymbosum* L.). J. Soil Sci. Plant Nutr. 11, 59 ─ 78.

Reyes-Díaz, M., Inostroza-Blancheteau, C., Millaleo, R., Cruces, E., Wulff-Zottele, C., Alberdi, M., Mora, M. L. 2010. Long-term aluminum exposure effects on physiological and biochemical features of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 135, 212 ─ 222.

Reyes-Díaz, M., Alberdi, M., Mora, M. L. 2009. Short-term aluminum stress differentially affects the photochemical efficiency of photosystem II in highbush blueberry genotypes. J. Amer. Soc. Hort. Sci. 134, 14 ─ 21.

Ritchey, K. D., Snuffer, J. D. 2002. Limestone, gypsum, and magnesium oxide influence restoration of an abandoned Appalachian pasture. Agron. J. 94, 830 ─ 839.

Ritchey, K. D., Feldhake, C. M., Clark, R. B., de Sousa, D. M. G. 1995. Improved water and nutrient uptake from subsurface layers of gypsum-amended soils. p. 157 ─ 181. In D.L. Karlen *et al.* (ed.) agricultural utilization of urban and industrial by-products. Spec. Publ. 58. asa, cssa, and sssa, madison, wi.

Rout, G. R., Samantara, S., Das, **P. 2001.** Aluminium toxicity in plants: a review. Agronomie 21, 3 ─ 21.

Sadzawka, A., Grez, R., Carrasco, M., Mora, M. 2007. Métodos de análisis de tejidos vegetales. 53p. Santiago, Chile, Comisión de Normalización y Acreditación Sociedad Chilena de la Ciencia del Suelo.

Sanders, D., Pelloux, J., Brownlee, C., Harper, J. F. 2002. Calcium at the crossroad of signaling. Plant Cell 14, 401 ─ 407.

Sanderson, K. R., Eaton, L. J. 2004. Gypsum- An alternative to chemical fertilizers in lowbush blueberry production. J. Small Fruits Rev. 3, 57 ─ 71.

Scandalios, J. G. 2002. The rise of ROS. Trends Biochem. Sci. 27, 483 ─ 486.

Schaberg, P. G., Tilley, J. W., Hawley, G. J., Dehayes, D. H., Bailey, S. W. 2006. Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. Forest Ecol. Manag. 223, 159 ─ 169.

Shao, H. B., Chu, L. Y., Shao, M. A., Jaleel, C. A., Mi, H. M. 2008. Higher plant antioxidants and redox signaling under environmental stresses. C. R. Biologies 331, 433 ─ 441.

Silva, I. R., Ferrufino, A., Sanzonowicz, C., Smyth, T. J., Israel, D. W., Carter jr, T. E. 2005. Interactions between magnesium, calcium, and aluminum on soybean root elongation. R. Bras. Ci. Solo. 29, 747 ─ 754.

Slinkard, K., Singleton, V. L. 1997. Phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticulture 28, 49 ─ 55.

Smart, R. E., Bingham, G. E. 1974. Rapid Estimates of Relative Water Content. Plant Physiol. 53, 258 ─ 260.

Sumner, M. E. 1993. Gypsum and acid soils: the world scene. Adv. Agron. 51, 1 ─ 32.

Taiz, L., Zeiger, E. 1998. Plant Physiology 2º Edition. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts. 792p.

Takahashi, T., Ikeda, Y., Nakamura, H., Nanzyo, M. 2006. Efficiency of gypsum application to acid Andosols estimated using aluminum release rates and plant root growth. J. Soil Sci. Plant Nutr. 52, 584 ─ 592.

Tisdale, S. L., Nelson, W. L., Beaton, J. D. 1985. Soil fertility and fertilizers. Mac Millan
Publishing Company, New York, Fourth Edition, chapter 8, pp 329 ─ 339.

Toma, M., Saigusa, M., Gafoku, N., Sumner, M. E. 2005. Effects of gypsum on amelioration of subsoil acidity in Andisols. J. Integr. Field Sci. 2, 69 ─ 71.

Trehane, J. 2004. Blueberries, cranberries and other *Vaccinium*. Cambridge: Timber Press. 256p.

Tuna, A. L., Kaya, C., Ashraf, M., Altunlu, H., Yokas, I., Yagmur, B. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ. Exp. Bo.t 59, 173 ─ 178.

Wang, J. P., Harsh, R., Guo-Ping, Z., Neville, M., Zhou, M. X. 2006. Aluminium tolerance in barley (*Hordeum vulgare* L.): physiological mechanisms, genetics and screening methods. J. Zhejiang Univ. Sci. B 7, 769 ─ 787.

Watanabe, T., Osaki, M. 2002. Mechanisms of adaptation to high aluminum condition in native plant species growing in acid soils: a review. Commun. Soil Sci. Plant Anal. 33, 1247 ─ 1260.

Weber-Blaschke, G., Rehfuess, K. E. 2002. Correction of Al toxicity with European ash (*Fraxinus excelsior* L.) growing on acid soils by fertilization with Ca and Mg carbonate and sulfate in pot experiments. Forest Ecol. Manag. 167, 173 ─ 183.

Yamamoto, Y., Kobayashi, Y., Devi, S. R., Rikiishi, S., Matsumoto, H. 2003. Oxidative stress triggered by aluminum in plant roots. Plant Soil 255, 239 ─ 243.

Yamamoto, Y., Kobayashi, Y., Devi, S. R., Rikiishi, S., Matsumoto, H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. J. Plant Physiol. 128, 63 ─ 72.

Yang, Z.-B., Eticha, D., Rao, I. M., Horst, W. J. 2010. Alteration of cell-porosity is involved in osmotic stress-induced enhancement of aluminium resistance in common bean (*Phasseolus vulgaris* L.). J. Exp. Bot. 61, 3245 − 3258.

Zaifnejad, M., Clark, R. B., Ritchey, K. D., Baligar, V. C., Parrish, D. J. 1996. Growth, photosynthesis, and water relations of wheat grown on acid soil amended with coal combustion by-products. Crop sci. 36, 968 ─ 974.

Zhang, W. H., Rengel, Z. 1999. Aluminium induces an increase in cytoplasmic calcium in intact wheat root apical cells. Aust. J. Plant Physiol. 26, 401 ─ 409.

Zhuang, X. P., Lu, Y. Y., Yang, G. S. 1992. Extraction and determination of flavonoids in ginko, Chin. Herb. Med. 23, 122 ─ 124.

Chapter 5. *General discussion*

5.1 GENERAL DISCUSSION

Nowadays, the great development of blueberry orchards in Chile have been promoted due to high priced of their small fruits. Espinoza *et al*. (2009) has reported over 10,000 ha cropped, with an average production of 55,000 t (ODEPA, 2011). Because to its benefical nutritional properties for human health by the high level of antioxidants, such as anthocyanin, flavonoid, and polyphenols compared to the other vegetable species (Howard *et al.*, 2003 Brambilla *et al*., 2008), which have important metabolic functions such as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and/or metal ion chelators (Sinelli *et al*., 2008). In this way, Yamamoto *et al.* (2002) and Ma *et al.* (2007) reported that antioxidant activity is associated to overcome stresses caused by environmental factors, which can trigger an overproduction of ROS in cellular organelles. However, their antioxidant features will depend on several environmental factors such as cultivar, growing season, growing location and the soil features (Kalt *et al.*, 2001; Howard *et al.*, 2003; Dragović-Uzelac *et al*., 2010; Ribera *et al*., 2010).

In Southern Chile, between Regions of Bío-Bío and Los Lagos (34º and 44º S), acid Andisols comprises about 2,360,000 ha (INE, 1998; Besoain, 1999), and their chemical and physical characteristics have enabled highbush blueberry development (Sanderson *et al*., 1995; Trehane, 2004). Thus, Besoain *et al*. (1999) and Mora *et al*. (2004) have reported that typically Andisols contain exchangeable Al^{3+} concentration of >1.0 cmol+/kg, which would causes toxicity in highbush blueberry, decreasing growth of root and shoots, nutrient uptake, and photosynthesis process mainly in sensitive cultivars (Blatt and McRae, 1997; Reyes-Díaz *et al*., 2009; 2010; 2011). To avoid Al^{3+} injuries, an agronomical practice carried out to reduce Al^{3+} release is Ca application. The Ca amelioration result in direct physiologcal effects, and reductions in the activity of Al^{3+} via increases of ionic strength (Lewis, 1990). Stout and Priddy (1996) reported yield increases in alfalfa (~20%) concomitant with an increase of ~45% in root Ca/Al, when plants were treated with $CaSO_4$ amendment compared to the non-treated ones. It is remarkable that this amendment can reduce Al toxicity in acid soils without altering the soil pH necessary for the good growth and development

of some crops well adapted to acidity, such as commercial berries (Gough, 1997; Mora *et al.*, 2002; Takahashi *et al.*, 2006a, b). For Ulrich *et al*. (1978) and Cronan and Grigal (1995) Ca/Al in soil solution could be use as an indicator of potential stress by Al^{3+}. Moreover, is reasonable to think that a decrease Ca/Al in tissues can be associated to decrease Ca and increased Al, influencing morphology and physiological functions in plants. Vanguelova *et al*. (2007) reported relationships between Al^{3+} and imbalanced nutrient content in plant tissues, especially Ca^{2+} and Mg^{2+}. However, the information about the effects of this interaction in physiological and biochemical features is scarcy.

The relation between Ca and Al presented in Chapter two point out to understand the relationship between them and their effects on physiological and biochemical features in cultivated plants grown in acid soils. It has been some 70 years Al is considered as major limitation to plant growth and crop production, causing injuries in roots of plants (Von Uexküll and Mutert, 1995; Lewis, 1990). Although, the Al-toxicity effects are clearly identified, symplastic and apoplastic targets for Al allocation are discussed (Marienfeld *et al*., 2000; Eticha *et al*., 2005). The proposed three mechanisms by Delhaize and Ryan (1995), to explain Al binding in roots and the interaction with Ca, suggest at symplasm level an inhibition of Ca^{2+}transport and disruption of Ca^{2+} homeostasis in cytoplasm by Al^{3+}, and third mechanism via apoplasm would be related to Ca^{2+} displacement by Al^{3+} from pectin bounds in cell wall.

Although, the first symptoms of Al^{3+} damages in plant roots have been well established, on upper parts such as stems, leaves and/or fruits it is still matter of discussion. Recently, negative effects of Al^{3+} on photosynthesis and photochemical parameters have been studied where this cation appears to preferentially impair thylakoids and photosynthetic electron transport chain (Chen *et al*., 2010; Reyes-Díaz *et al*., 2011). According to Chen *et al*. (2005), a reduction of CO_2 assimilation rate in Al stressed tangerine (*Citrus reshni* Hort. ex Tanaka) seedlings was mediated by an decreased ETR, arise closure of PSII reaction centers and photorespiration, possibly by an over production of ROS in chloroplast, inducing a lipid peroxidation (Lidon *et al*., 1999).

On the other hand, some reports regarding to interaction between Ca^{2+} and reduction in the toxic Al^{3+} activity in tissues, have demonstrated that Ca^{2+} addition would decreases enzymatic antioxidant activity of SOD and CAT enzymes (Ogawa *et al*., 2000; Jiang and Zhang, 2003). Nevertheless, other authors suggested that the antioxidant activity could be favored by Ca^{2+} addition. Because, is considered as a secondary messenger in the oxidative stress responses of plants by regulation of enzymatic scavengers (Price *et al*., 1994; Knight *et al*., 1996). Therefore, Ca^{2+} may be involved in plant tolerance to different stress by regulation of antioxidant metabolism (Jiang and Huang, 2001; Cheng *et al*., 2002). Signaling events associated to ROS sensing comprised Ca^{2+} and Ca^{2+}/binding proteins such as calmodulin and the activation of G/proteins (Mittler *et al*., 2004; Foyer and Noctor, 2005). Jiang and Zhang (2003) studied the role of Ca^{2+} in signal transduction raised between this divalent cation and ROS originated from plasmamembrane NADPH oxidase in abscisic acid (ABA) induced/antioxidant defence, and they concluded that cross/talk Ca^{2+} and ROS originated from cell membrane/bound/NADPH is involved in the ABA signal transduction leading to the induction of antioxidant enzyme as SOD, CAT, ascorbate peroxidase (APX) and gluthatione reductase (GR) activities. The knowledge of the non-enzymatic antioxidant defense against Al stress is less recognized than the enzymatic ones. Further studies are needed to better understand the mechanisms involved in the Ca-Al relation that affect physiological and biochemical processes such as photosynthesis, cellular respiration, antioxidant activities, signal transduction and cellular homeostasis in plants growing in acid soils like Andisol.

In order to determine the calcium salts (calcium sulfate) effects on Ca/Al relation in highbush blueberry, Chapter three ascertain physiological and biochemical aspects. Where two blueberry cultivars Legacy and Bluegold were grown for 15 days in a nutrient solution containing increased $CaSO_4$ concentrations (2.5, 5, and 10 mM) and Al (100 and 200 μM). In both cultivars, Ca content and Ca/Al were increased up to ~100% and 180%, respectively, by adding $CaSO_4$ concomitant with a reduction in foliar Al in both Legacy and Bluegold ($r=-0.80$; $P\leq0.001$ and $r=-0.74$; $P\leq0.001$, respectively). An improved Ca/Al had a positive effect on photochemical parameters

in both cultivars ($P \leq 0.05$), as well as in the reduction of oxidative stress expressed as LP. Also TPC and SOD, particularly in Legacy were significantly increased. In sugar maple, St. Clair *et al*. (2005) foliar Ca/Al was positively correlated with some photosynthetic prameters such as CO_2 exchange rate and Chl a+b content, but antioxidants enzyme activity were negatively affected by this increased molar ratio.

In highbush blueberry, $CaSO_4$ was an effective amendment to improve the Ca content concomitant with reduced Al contents, showing an important contribution to Ca balance in Bluegold (considered as Al-sensitive). The enhanced photochemical parameters (ΦPSII and ETR) in both cultivars, probably due to a decrease in LP caused by Al toxicity, although no strong interaction between radical scavenging activity, the Ca/Al and LP were found. With respect to antioxidant compounds, only in Legacy (as Al-tolerant) a direct relationship between TPC, SOD and Ca/Al was observed ($P \leq 0.05$). However, more studies about the effects of adding $CaSO_4$ to Al-saturated soil on these features were necessaries.

In Chapter four, the aim of study was to known wether calcium sulfate amendment counteract the deleterious effects of toxic Al on physiological and biochemical processes in same evaluated highbush blueberry cultivars grown in an Al-saturated Andisol from Gorbea Series (Table 4.1). According to results, $CaSO_4$ addition represented a good alternative to enhance the inhibited growth of shoot and roots by Al. These effects on plant growth certainly were produced by a better nutritional content and reduced Al in tissues mediated by an improved Ca/Al in both cultivars. Contradictory results about hydric relations in plants under $CaSO_4$ application compared to those subjected in Al-saturated soil were observed, whereas Ψ_{leaf} showed more negative values, probably induced by higher nutrient content in xylematic flux and osmotic adjustment, the relative water content (RWC) was increased, especially in Legacy. In wheat plants grown in soil columns at acid pH (4.70) and Al saturation (65%), Ψ_{leaf} and RWC were increased with gypsum addition (Zaifnejad *et al*., 1996). Comparable to our study, Hickleton *et al*. (2000), Glass *et al*. (2005) and Bryla and Strik (2007), reported Ψ_{leaf} until -1.5 MPa for stressed highbush blueberry plants under drought season in the Northern hemisphere. Legacy had a lesser negative Ψ_{leaf} than Bluegold in control plants, although Ψ_{leaf} was negatively

correlated with foliar and root contents of Al (r=–0.79; *P*=0.002) and (r=–0.73; *P*=0.006) respectively. Thereby, Jiang and Huang (2001) described that external Ca application improved RWC in tall fescue (*Festuca arundinacea* L.) and Kentucky bluegrass (*Poa pratensis* L.) under heat stress for 30 days, although these application were not related to osmotic potential and osmotic balance. In addition, the authors reported that Ca addition reduced the loss of chlorophyll content under stress, by inhibition of photoxidation and/or better membrane integrity.

In our experiment, increased fluorescence parameters (ΦPSII and ETR) in Legacy, were related to a reduced non-photochemical quenching (NPQ) after $CaSO_4$ addition, which suggest that the absorbed energy from actinic light (300 μmol CO_2 m^{-2} s^{-1}) was driven to the CO_2 assimilation process. These results are in agreement with reported by Reyes-Díaz *et al*. (2011), where ΦPSII and ETR positively responded to $CaSO_4$ treatments mainly in tolerant cultivar. The interactions between NPQ and photosynthetic pigment content have been widely reported (Bilger and Björkman, 1990; Demmig-Adams and Adams, 1996). Nonetheless, Chen *et al*. (2005) suggested that NPQ was not a key factor on dissipation of excessive thermal energy in Al treated leaves of tangerine (*Citrus reshni* Hort. Ex Tanaka), probably due to Haliwell-Asada route and photorespiration involved in dissipating excessive thermal energy. Scientific evidences showed that Al^{3+} can decrease electron transfers between both photosystems (PSII and PSI), increasing production of ROS (Lidon *et al*., 1999; Scandalios, 2002), thus inducing LP in chloroplasts (Lidon *et al*., 1999; Rout *et al* 2001). In our study the correlation in Legacy leaves between LP and Al content (r=0.84; $P \leq 0.001$) was decreased by an improved Ca content in leaves of (r=–0.66; *P*=0.018) and roots (r=–0.62; *P*=0.031) of Legacy.

In Legacy leaves, the negative correlation between Al content and RSA (r=–0.68; *P*=0.014) and roots (r=–0.72; *P*=0.008) not showed relation with number of studies that have reported that in parallel to increased ROS, Al stress also induces an increase in the antioxidant activity to overcome deteriorating effects of toxic species, increasing the plant tolerance to Al stress (Guo *et al*., 2006; Khan *et al*., 2007). Similarly, antioxidant activity is associated with several secondary metabolites to overcome stresses caused by environmental factors such as Al^{3+}. Interestingly, Al

toxicity did not increased antioxidant compounds in leaves and roots of studied highbush blueberry and was negatively correlated, except TFA in Legacy. Finally, from agronomical point of view, blueberries Al tolerant cultivar would require lower $CaSO_4$ amendment doses than those sensitive ones. However, $CaSO_4$ also ameliorate harmful effect of Al on Bluegold at higher doses than Legacy. Our results showed that Al content in the sensitive cultivar Bluegold negatively affected TPC (r=–0.64; *P*=0.024), whereas in Legacy TAN was reduced under greater Al content (r=–0.74; *P*=0.005), and TFA was positively correlated with Al content (r=0.64; *P*=0.023), and foliar Ca content had positive influence on TAN in Bluegold (r=0.61; *P*=0.032). Little information has been reported about the real effects of $CaSO_4$ on the activity of these compounds, thus we suggest that this amendment increased TPC and TAN in order to ameliorate Al concentration in soil solution. Antioxidant enzymes set contributed to defensive system as the first barrier against harmful effects of Al^{3+} in plant metabolism. In our experiment, SOD and CAT showed lower activity in Al stressed plants in both organs of two cultivars, and then increased by $CaSO_4$ addition (Figure 4.6). Bluegold exhibited a higher SOD activity than Legacy, because in Bluegold greater Ca contents were found after $CaSO_4$ application (r=0.69; *P*=0.012), being a crucial intra- and extracellular regulator of several enzymes. Gong *et al*. (1997) on maize (*Zea mays*), and Jiang and Huang (2001) on tall fescue and Kentucky bluegrass have reported that external Ca addition increased ascorbate peroxidase (APX, EC 1.11.1.11), CAT, glutahione reductase (GR, EC 1.6.4.2), and SOD activities. For highbush blueberry cultivars, $CaSO_4$ addition to ameliorate the toxic Al on nutrient solution (100 µM) caused an increment of SOD activity in respect to control and stressed plants, mainly in Al sensitive cultivar Bluegold (Reyes-Díaz *et al*., 2011). According to our findings, Al content had a detrimental effect on SOD and CAT activity, showing in Legacy leaves and roots significant negative correlations between Al and SOD (r=–0.61 and r=–0.60 respectively), and CAT in roots (r=–0.94), whereas in Bluegold radical SOD and Al content was also negatively correlated (r=–0.61; *P*=0.032). Thus, $CaSO_4$ addition reduced Al content and restored balance between ROS and antioxidant enzyme, with special focus on Al tolerant cultivars (Legacy), where the reduction of LP and increased SOD were

correlated in leaves and roots (r=0.60; P=0.036 and r=0.83; $P \leq 0.001$ respectively). It could be assumed that these enzymes represent an important mechanism in Legacy to alleviate environmental impacts such as Al stress.

5.1.1 Aluminum concentration in acids environment and the antagonism of calcium

In consideration to all reviewed information and performed experiment about the role of Ca/Al in a fruit species like highbush blueberry was possible to design a model related to interaction among physiological and biochemical features a this ratio.

In the acidification of soils, due to natural or anthropogenic causes, Al is released into the soil solution and becomes the single most important factor limiting crop production in these soils. Calcium as base cation can ameliorate the toxicity provocated by high Al concentration in soil. However, the place where is developed and mechanism of this interaction is currently discussed. In chapter two it was studied different three possibilities for the Ca and Al interaction in cell such as via apoplast, symplast and cytosolic. Aluminum accumulation in cell wall, where it strongly binds to the negatively charged binding sites provide by pectins. Also, this cation shows high affinity for oxygen donor ligands (e.g. carboxylate or phosphate groups) from plasmamembrane, where its main target are membrane phospholipids causing breakdown and peroxidation. However, the interaction at cytosolic level is considered by authors as principal cellular compartment where is carried out the interaction of Al and Ca.

5.1.2 Cytosolic Ca and stimuli perception

Cytosolic Ca ($[Ca^{2+}]_{cyt}$) is regulated by the coordination of passive fluxes (Ca^{2+} channels) and active transport (Ca^{2+}-ATPases Ca^{2+}-antiporters) across the plasma membrane and/or endomembranes, and the buffering capacity of the cytosol. Transient changes in $[Ca^{2+}]_{cyt}$ have been reported in response to various signals, including abiotic stress. It well known that Ca participates in signal transduction as second messenger. This downstream signaling events associated with ROS sensing

involve Ca^{2+} and Ca^{2+}-binding proteins, such as calmodulin (CaM), the activation of G-proteins, and the activation of phospholipid signaling, which results in the accumulation of phosphatidic acid. It is possible that the localization of ROS signals in specific cellular sites is similar to that of Ca^{2+} signals in response to external stimuli. The development of intercellular ROS sensors analogous to the protein sensors for Ca^{2+} would help considerably in studying the spatial and temporal nature of ROS signaling in plants.

The information encoded in transient $[Ca^{2+}]_{cyt}$ changes is decoded by a group of Ca^{2+}-binding proteins giving a cascade of downstream effects, including altered protein phosphorylation and gene expression patterns. In plants the many Ca^{2+}-binding proteins fall into two classifications: 1) Stimuli sensors such as n (CaM), CaM-related proteins and calcineurin B-like proteins (CBL) function through bimolecular interactions. They undergo a conformational change induced by Ca^{2+} before interacting with and changing the activity or structure of the target proteins. 2) Sensor responders such as the Ca^{2+}-dependent protein kinases (CDPK) function at first through intramolecular interactions and undergo a Ca^{2+}-induced conformational change that alters the activity of proteins or their structure.

5.1.3 Aluminum triggers changes in cytosolic Ca

Aluminum causes increases in $[Ca^{2+}]_{cyt}$ potentially disrupting numerous biochemical and physiological processes, including those relative to growth of roots, shoots, water absorption and nutrient uptake, oxidative stress, enzymes activity, among others. Aluminum cause depolarization and hyperpolarization of plasmamembranes, Ca^{2+} is released from the exchange sites thus increasing the free Ca^{2+} concentration in the apoplast, increasing influx of $Ca^{2+}$from apoplast to cytosol, also inhibites the regulation of Ca^{2+} flux from cellular organelles (vacuoles, endoplasmic reticulum or plastids) to cytosol, which leads to an excessive increase in the cytosolic Ca^{2+} activity and triggering callose synthesis, disruption in perception of signal, transduction and defensive responses to environmental stimuli. callose synthesis.

5.1.4 Regulation of structural and cytosolic Ca by restored Ca/Al molar ratio

The stoichiometric atomic ratio (molCa/molAl) facilitates the comparison with the Ca/Al in soil solution and above-ground plant ratios. Concentrations of Al and Ca in the fine root are determined by soil solution concentrations of Al and Ca, soil acidity, soil exchangeable Al and Ca concentrations, root uptake capabilities, and translocation to above-ground plant parts. Additional factors that may be species or genotypically specific include plant tolerances to Al, internal detoxification mechanisms, and exclusion of Al from the symplast. Thus, it is reasonable that a decrease in root Ca/Al ratio, due to decreased Ca and increased Al, will rapidly affected root physiological functions and root morphology as a consequence of unbalanced nutrient uptake and plant nutrient status.

The first stage in the regulation of Ca level in cell will be at cell surface (both apoplasm and symplasm) by displacement of toxic Al^{3+}, afterward in the second stage continues with the restoration of Ca on cell surfaces, regulating negativity of cell wall and plasmamembranes. This mechanism will restore Ca concentration in cytosol, providing the potential for the ready import of Ca^{2+} into the cytosol where it acts as a second messenger (100 -200 nM).

Chapter 6. *General conclusions*

6.1 GENERAL CONCLUSIONS

The addition of calium sulfate was an effective treatment to increase Ca/Al molar ratio in Al-toxicity. This molar ratio in nutrient or soil solution, under controlled conditions, was directly related to increase Ca/Al in tissues of fruit crop as highbush blueberry, concomitant with a reduction Al and enhanced Ca contents in leaves and roots, especially in leaves. However, two evaluated cultivars exhibited different foliar Ca/Al values, whereas Al-tolerant Legacy ranged from 4 to 7, in Bluegold was situated between 10 and 16, indicating different genotypic responses and mechanism involved to Al toxicity.

The morphological parameters were recovery in two cultivars, particularly Bluegold, with increased Ca/Al molar ratio in plants, due to a reduction of Al^{3+} concentration. Moreover, higher performances of physiological features as photochemical efficiency (ΦPSII and ETR) and net photosynthesis were observed in both cultivars, when foliar ratios were improved. The major photosynthetic yield could be related to a reduced oxidative stress triggered by Al^{3+} in endomembranes and proteins of chloroplasts. In relation to hydric relation, leaf water potential showed more negative values with increased Ca/Al molar ratio suggesting a higher osmotic adjustment but no loss in cell turgor.

Increased radical scavenging activity was directly related to reduction in lipid peroxidation by regulation in foliar Ca/Al molar ratio in both cultivars. The non-enzymatic antioxidant total phenol and anthocyanin contents were enhanced according to increased Ca/Al molar ratio in leaves. According to enzymatic antioxidant, foliar Ca/Al raised superoxide dismutase in leaves of Al-sensitive Bluegold, and catalase activity in both cultivars with results. These antioxidant features showed the highest performance in leaves when Ca/Al were 6 in Legacy and 14 in Bluegold.

The determination of adequates Ca/Al molar ratio ranges to improve physiological and biochemical performance, according with genotypic characteristic can be an important usefulness contribution to regulation of Ca addition to soils with high concentration of Al^{3+}. In addition, benefits to farmer, different cultivars would

require different calcium sulfate doses to improve growth and nutritional status, being Ca/Al molar ratio a potential parameter to evaluate the fruit plant condition in orchard.

6.2 THESIS OUTLOOKS

In the current scenario for optimizing agricultural production in the growing global demand for food, several studies have been conducted under molecular, biochemical and physiological criteria to find the best alternatives for crops that will not always be established in areas with optimal environmental conditions. Added to this, it is important to address local issues related to the use of available natural resources and to better understand the interactions between them. Further studies are needed to better understand the mechanisms involved in the Ca-Al interactions that affect both physiological and biochemical processes as photosynthesis, respiration, antioxidant activities, signal transduction and cellular homeostasis in plants growing in acid soils like Andisols.

Our research provides valuable information about the use of calcium amendment to improve the nutrient levels and reduce the risk factors related to highbush blueberry cultivation in soil conditions from Southern Chile. The application of calcium sulfate improved minerals, especially Ca in the soil solution and in the plant by reducing the negative impact caused by high concentrations of Al^{3+}. Plants treated with calcium sulfate showed an increased in their physiological and biochemical characteristics. Among the latter, mention the increase in the content of some compounds with antioxidant power that can be transferred to the edible fruits and highly desired in the marketplace. Turning to these fruits in functional food and nutraceutical with excellent properties for health and diet of people.

6.3 REFERENCES

Arbona, V., Flors, V., Josep Jacas, J., García-Agustín, P., Gómez-Cadenas, A. 2003. Enzymatic and non-enzymatic antioxidant responses of carrizo citrange, a salt-sensitive citrus rootstock, to different levels of salinity. Plant Cell Physiol. 44, 388 ─ 394.

Besoain, M. E. 1999. Los suelos volcánicos y la fertilización fosfatada. p. 13-21. In: E. Besoain M., C. Rojas W. y A. Montenegro B. (eds.). Las rocas fosfóricas y sus posibilidades de uso agrícola en Chile. Colección Libros INIA Nº2, INIA, Santiago, Chile, 328p.

Bilger, W., Björkman, O. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurement of light-induced absorbance changes, fluorescence and photosynthesis in Hedera canariensis. Photosynth. Res. 25, 173–175.

Blatt, C. R., McRae, K. H. 1997. Marketable yield response of two lowbush blueberries to preplant applications of gypsum, calcitic and dolomitic limestones. J. Small Fruit Viticulture 5, 13 ─ 26.

Blokhina, O., Virolainen, E., Fagerstedt, K. V. 2003. Antioxidants, oxidative damage, and oxygen deprivation stress: A review. Ann. Bot.-Lon. 91, 179 ─ 194.

Brambilla, A., Lo Scalzo, R., Bertolo, G., Torreggiani, D. 2008. Steam-blanched highbush blueberry (*Vaccinium corymbosum* L.) juice phenolic profile and antioxidant capacity in relation to cultivar selection. J. Agr Food Chem. 56, 2643 ─ 2648.

Broquen, P., Lobartini, J. C., Candan, F., Falbo, G. 2005. Allophane, aluminum, and organic matter accumulation across a bioclimatic sequence of volcanic ash soils of Argentina. Geoderma 129, 167 ─ 177.

Brunner, I., Brodbeck, S., Walthert, L. 2002. Fine root chemistry, starch concentration, and "vitality" of subalpine conifer forests in relation to soil pH. Forest Ecol. Manag. 165, 75 ─ 84.

Bryla, D. R., Strik, B. C. 2007. Effects of cultivar and plant spacing on the seasonal water requirements of highbush blueberry. J. Amer.Soc.Hort.Sci. 132, 270–277.

Chen, L., Qi, Y., Jiang, H., Yang, L., Yang, G. 2010. Photosynthesis and photoprotective systems of plants in response to aluminum toxicity. Afr. J. Biotechnol. 9, 9237 ─ 9247.

Chen, L. S., Qi, Y. P., Liu, X. H. 2005. Effects of aluminum on light energy utilization and photoprotective systems in citrus leaves. Ann. Bot. Lon. 96, 35 ─ 41.

Cheng, S., Willmann, M. R., Chen, H., Sheen, J. 2002. Calcium signaling through protein kinases. The arabidopsis calcium-dependent protein kinase gene family. Plant Physiol. 129, 469 ─ 485.

Cronan, C. S., Grigal, D. F. 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. J. Environ. Qual. 24, 209 ─ 226.

Delhaize, E., Ryan, P. R. 1995. Aluminum toxicity and tolerance in plants. J. Plant Physiol. 107, 315 ─ 321.

Demmig-Adams, B., Adams III, W. W. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis.Trends Plant Sci. 1, 21–26.

Dragović-Uzelac, **V., Savić, Z., Brala, A., Levaj, B., Bursać-Kovačević, D., Biško, A. 2010.** Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the northwest Croatia. Food Technol. Biotechnol. 48, 214 ─ 221.

Espinoza, J. G., Briceño, E. X., Chávez, E. R., Úrbez-Torres, J. R., Latorre, B. A. 2009. *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. Plant Dis. 93, 1187 ─ 1194.

Eticha, D., Stass, A., Horst, W. J. 2005. Cell-wall pectin and its dgree of methylation in the maize root-apex: significance for genotypic differences in aluminium resistance. Plant Cell Environ. 28, 1410 ─ 1420.

Foyer, C. H., Noctor, G. 2005. Oxidant and antioxidant signalling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 28, 1056 ─ 1071.

Glass, V. M., Percival, D. C., Proctor, J. T. A. 2005. Tolerance of lowbush blueberries (*Vaccinium angustifolium* Ait.) to drought stress. II. Leaf gas exchange, stem water potential and dry matter partitioning. Can. J. Plant Sci. 85: 919–927.

Gong, M., Chen, S. N., Song, Y. Q., Li, Z. G. 1997. Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. Aus. J. Plant Physiol. 24, 371 - 379.

Gough, R. E. 1997. Blueberries-north and south. J. Small Fruit Viticulture 4, 71 ─ 106.

Guo, T., Chen, Y., Zhang, Y., Jin. Y. 2006. Alleviation of Al toxicity in barley by addition of calcium. Agric. Sci. China 5, 828 – 833.

Hepler, P. K. 2005. Calcium: A central regulator of plant growth and development. Plant Cell 17, 2142 ─ 2155.

Hicklenton, P. R., Reekie, J. Y., Gordon, R. J. 2000. Physiological and morphological traits of lowbush blueberry (*Vaccinium angustifolium* Ait.) plants in relation to post-transplant conditions and water availability. Can. J. Plant Sci. 80, 861–867.

Horst, W. J., Wang, Y., Eticha, D. 2010. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Ann. Bot. 106, 185 ─ 197.

Howard, L. R., Clark, J. R., Brownmiller, C. 2003. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. J. Sci. Food Agric. 83, 1238 ─ 1247.

Huang, D., Ou, B., Prior, R. L. 2005. The Chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, 1841 ─ 1856.

INE. 1998. VI Censo Nacional Agropecuario 1997. INE, Santiago, Chile, 214 p.

Jiang, Y., Huang, B. 2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J. Exp. Bot. 52, 341 ─ 349.

Jiang, M., Zhang, J. 2003. Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in absicic acid-induced antioxidant defence in leaves of maize seedlings. Plant Cell Environ. 26, 929 ─ 939.

Jones, D. L., Blancaflor, E. B., Kochian, L. V., Gilroy, S. 2006. Spatial coordination of aluminium uptake, production of reactive species, callose production and wall rigidification in maize roots. Plant Cell Environ. 29, 1309 ─ 1318.

Kalt, W., Ryan, D. A. J., Duy, J. C., Prior, R. L., Ehlenfeldt, M. K., Vander Kloet, S. P. 2001. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). Agric. Food Chem. 49, 4761 ─ 4767.

Khan, N. A., Samiullah, S., Singh, .S, Nazar, R. 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J. Agron. Crop Sci. 193, 435 — 444.

Kinraide, T. H. 1998. Three mechanisms for the calcium alleviation of mineral toxicities. J. Plant Physiol. 118, 513 ─ 520.

Kinraide, T. B., Ryan, P. R., Kochian, L. V. 1994. Al^{3+} – Ca^{2+} interactions in aluminum rhizotoxicity. Planta 192, 98 ─ 103.

Knight, H., Trewavas, A. J., knight, M. 1996. Cold calcium signaling in *Arabidopsis* involves two cellular pools and change in calcium signature after acclimation. Plant Cell 8, 489 ─ 503.

Kochian, L. V., Pineros, M. A., Hoekenga, O. A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil 274, 175 ─ 195.

Klug, B., Specht, A., Horst, W. J. 2011. Aluminium localization in root tips of the aluminium-accumulating plant species buckwheat (*Fagopyrum esculentum* Moench). J. Exp. Bot. 62, 5453 ─ 5462.

Klug, B., Horst, W. J. 2010. Spatial characteristics of aluminium uptake and translocation in roots of buckwheat (*Fagopirum esculentum*). Physiol. Plantarum 139, 181 ─ 191.

Langer, H., Cea, M., Curaqueo, G., Borie, F. 2009. Influence of aluminum on the growth and organic acid exudation in alfalfa cultivars grown in nutrient solution. J. Plant Nutr. 32, 618 ─ 628.

Lecourieux, D., Ranjeva, R., Pugin, A. 2006. Calcium in plant defence-signalling pathways. New Phytol. 171, 249 ─ 269.

Lewis, T. E. 1990. Environmental chemistry and toxicology of aluminum. 2º ed. Lewis Publishers, Inc. 344p.

Lidon, F.C., Barreiro, M. J., Ramalho, J.C., Lauriano, J.A. 1999. Effects of aluminum toxicity on nutrient accumulation in maize shoots implications on photosynthesis. J. Plant Nutr. 22, 397 ─ 416.

Ma, B., Wan, J., Shen, Z. 2007. H_2O_2 production and antioxidant responses in seeds and early seedlings of two different rice varieties exposed to aluminum. J. Plant Growth Regul. 52, 91 ─ 100.

Marienfeld, S., Schmol, N., Klein, M., Schröder, W. H., Kuhn, A. J., Horst, W. J. 2000. localisation of aluminium in root tips of *Zea mays* and *Vicia faba*. J. Plant Physiol 156, 666 ─ 671.

Mittler R., Vanderauwera, S., Gallery, M., van Breusegem, F. 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9, 490 ─ 498.

Miqyass, M., van Gorkom, H. J., Yocum, C. F. 2007. The PSII calcium site revisited. Photosynth. Res. 92, 275 ─ 287.

Mora, M. L, Alfaro, M., Williams, P., Stehr, W., Demanet, R. 2004. Effect of fertiliser input on soil acidification in relation to growth and chemical composition of a pasture, and animal production. R.C. Suelo Nutr. Veg. 4, 29 ─ 40.

Mora, M. L., Cartes, P., Demanet, R., Cornforth, I. S. 2002. Effects of lime and gypsum on pasture growth and composition on an acid Andisol in Chile, South America. Commun. Soil Sci. Plant Anal. 33, 2069 ─ 2081.

Mora, M. L., Schnettler, B., Demanet, R. 1999. Effect of liming and gypsum on soil chemistry, yield, and mineral composition of ryegrass grown in an acidic Andisol. Commun. Soil Sci. Plant Anal. 30, 1251 ─ 1266.

Mossor-Pietraszewska, T. 2001. Effect of aluminium on plant growth and metabolism. Acta Biochim. Pol. 3, 673 ─ 686.

ODEPA (Chile). 2011. Boletín de frutales: arándanos. Sección mercados y rubros. [on line]. Odepa. http://www.odepa.gob.cl [request: october, 15 ─ 2011].

Ogawa, T., Matsumoto, C., Takenaka, C., Tezuka, T. 2000. Effect of Ca on Al-induced activation of antioxidant enzymes in the needles of hinoki cypress (*Chamaecyparis obtusa*). J. For. Res. 5, 81 ─ 85.

Peixoto, H. P., da Matta, F. M., da Matta, J. C. 2002. Responses of the photosynthetic apparatus to aluminum stress in two sorghum cultivars. J. Plant Nutr. 25, 821 ─ 832.

Pintro, **J., Calba, H., Fallavier, P., Barloy, J. 1998.** Effects of different calcium and sulfate concentrations in nutrient solutions on ionic strength values, aluminum activity, and root growth of maize plants. J. Plant Nutr. 21, 2381 ─ 2387.

Price, A. H., Taylor, A., Ripley, S. J., Cuin, T. T., Ashenden, T. 1994. Oxidative signals in tobacco increase cytosolic calcium. Plant Cell 6, 1301 ─ 1310.

Prior, R. L., Wu, X., Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53, 4290 ─ 4302.

Reyes-Díaz, M., Meriño-Gergichevich, C., Alarcón, E., Alberdi, M., Horst, W. 2011. Calcium sulfate ameliorates the effect of aluminum toxicity differentially in genotypes of highbush blueberry (*Vaccinium corymbosum* L.). J. Soil Sci. Plant Nutr. 11, 59 ─ 78.

Reyes-Díaz, M., Inostroza-Blancheteau, C., Millaleo, R., Cruces, E., Wulff-Zottele, C., Alberdi, M., Mora, M. L. 2010. Long-term aluminum exposure

effects on physiological and biochemical features of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 135, 212 ─ 222.

Reyes-Díaz, M., Alberdi, M., Mora, M. L. 2009. Short-term aluminum stress differentially affects the photochemical efficiency of photosystem II in highbush blueberry genotypes. J. Amer. Soc. Hort. Sci. 134, 14 ─ 21.

Ribera, A., Reyes-Díaz, M., Alberdi, M., Zuñiga, G. E., Mora, M. L. 2010. Antioxidant compounds in skin and pulp of fruits change among genotypes and maturity stages in highbush blueberry (*Vaccinium corymbosum* L.) grown in Southern Chile. J. Soil Sci. Plant Nutr. 10, 509 ─ 536.

Ritchey, K. D., Snuffer, J. D. 2002. Limestone, gypsum, and magnesium oxide influence restoration of an abandoned Appalachian pasture. Agron. J. 94, 830 ─ 839.

Rout, G. R., Samantara, S., Das, **P. 2001.** Aluminium toxicity in plants: a review. Agronomie 21, 3 ─ 21.

Ryan, P. R., Delhaize, E. 2010. The convergent evolution of aluminium resistance in plants exploits a convenient currency. Funct. Plant Biol. 37, 275 ─ 284.

Ryan, P. R., Reid, R. J., Smith, F. A. 1997. Direct evaluation of the Ca^{2+}-displacement hypothesis for Al toxicity. J. Plant Physiol. 113, 1351 ─ 1357.

Ryan, P. R., Kochian, L. V. 1993. Interaction between aluminum toxicity and calcium uptake at the root apex in near-isogenic lines of wheat (*Triticum aestivum* L.) differing in aluminum tolerance. Plant Physiol. 102, 975 ─ 982.

Sanderson, K. R., Carter, M. R., lvany, J. A. 1995. Effects of gypsum on yield and nutrient status of native lowbush blueberry. Can. J. plant Sci. 26, 361 ─ 366.

Scandalios, J. G. 2002. The rise of ROS. Trends Biochem Sci 27, 483 – 486.

Schaberg, P. G., Tilley, J. W., Hawley, G. J., Dehayes, D. H., Bailey, S. W. 2006. Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. Forest Ecol. Manag. 223, 159 ─ 169.

Seling, S., Wissemeier, A. H., Cambier, P., Van Cutsem. 2000. Calcium deficiency in potato (*Solanum tuberosum* ssp. *tuberosum*) leaves and its effects on the pectic composition of the apoplastic fluid. Physiol. Plantarum 109, 44 ─ 50.

Sharma, P., Dubey, R. S. 2007. Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. Plant Cell Rep. 26, 2027 ─ 2038.

Sinelli, N., Spinardi, A., Di Egidio, V., Mignani, I., Casiraghi, E. 2008. Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. Postharvest Biol. Tec. 50, 31 ─ 36.

Sivaguru, M., Horst, W. J., Eticha, D., Matsumoto, H., 2006. Control of response to aluminium stress. In: Nick P. ed. *Plant microtubules: potential for biotechnology*: Springer Verlag, 103 ─ 120.

St. Clair, S. B., Carlson, J. E., Lynch, J. P. 2005. Evidence for oxidative stress in sugar maple stands growing on acidic, nutrient imbalanced forest soils. Oecologia 145, 258 ─ 269.

Stout, W. L., Priddy, W. E. 1996. Use of flue gas desulfurization (FGD) by-product gypsum on alfalfa. Commun. Soil Sci. Plant Anal. 27, 2419 ─ 2432.

Takahashi, T., Ikeda, Y., Nakamura, H., Nanzyo, M. 2006a. Efficiency of gypsum application to acid Andosols estimated using aluminum release rates and plant root growth. J. Soil Sci. Plant Nutr. 52, 584 ─ 592.

Takahashi, T., Ikeda, T., Fujita, K., Nanzyo, M. 2006b. Effect of liming on organically complexed aluminum of nonallophanic Andosols from northeastern Japan. Geoderma 130, 26 ─ 34.

Trehane, J. 2004. Blueberries, cranberries and other *Vaccinium*. Cambridge: Timber Press. 256p.

Toma, M., Saigusa, M., Gafoku, N., Sumner, M. E. 2005. Effects of gypsum on amelioration of subsoil acidity in Andisols. J. Integr. Field Sci. 2, 69 ─ 71.

Ulrich, B., Mayer, R., Khanna, P. 1978. Deposition von Luftver-unreinigungen und ihre Auswirkungen in Waldokösystemen in Solling. Schr. Forstl. Fak. Univ. Gött Vers Anst 58, 1 ─ 29.

Vanguelova, E. I., Hirano, Y., Eldhuset, T. D., Sas-Paszt, L., Bakker, M. R., Püttsepp, Ü., Brunner, I., Lõhmus, K., Godbold, D. 2007. Tree fine root Ca/Al molar ratio – Indicator of Al and acidity stress. Plant Biosystems 141, 460 ─ 480.

Velioglu, Y. S., Mazza, L., Gao, L., Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113 ─ 4117.

Von Uexküll, H. R., Mutert, E. 1995. Global extent, development and economic impact of acid soils. Plant Soil 171, 1 ─ 15.

Wang, L. J., Li, S. H. 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants. Plant Sci. 170, 685 ─ 694.

Yamamoto, Y., Kobayashi, Y., Devi, S. R., Rikiishi, S., Matsumoto, H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. J. Plant Physiol. 128, 63 ─ 72.

Yocum, C. F. 2008. The calcium and chloride requirements of the O_2 evolving complex. Coordin. Chem. Rev. 252, 296 ─ 305.

Zhang, X. B., Liu, P., Yang, Y. S., Gen-Di, X. 2007. Effect of Al in soil on photosynthesis and related morphological and physiological characteristics of two soybean genotypes. Bot. Stu. 48, 435 ─ 444.

APPENDICES

Table 1. The calculated chemical speciation of nutrient solution. The chemical speciation in each solution was calculated using the computer speciation program GEOCHEM-PC version 2.0 (Parker *et al.* 1995).

.

	$CaSO_4$ (mM)								
	0			2.5		5		10	
	Al^{3+} (μM)								
	0	100	200	100	200	100	200	100	200
Ca (%)									
Free	93.35	92.75	94.32	85.58	85.79	80.58	80.77	74.32	74.47
With SO_4	6.48	5.79	5.61	14.32	14.13	19.33	19.15	25.61	25.46
SO_4 (%)									
Free	85.02	80.04	78.12	75.70	74.50	72.71	71.86	68.51	67.96
With Ca^{2+}	12.90	11.68	11.18	18.40	18.15	22.54	22.33	27.92	27.76
With Al^{3+}	-	2.18	4.83	1.35	2.86	1.01	2.10	0.70	1.42
Al^{3+} (%)									
Free	-	33.04	38.68	24.98	27.08	21.79	22.99	18.70	19.24
With SO_4	-	20.93	23.38	42.64	45.42	52.57	54.75	62.87	62.07
With OH	-	6.74	7.88	4.49	4.87	3.58	3.76	2.70	2.77

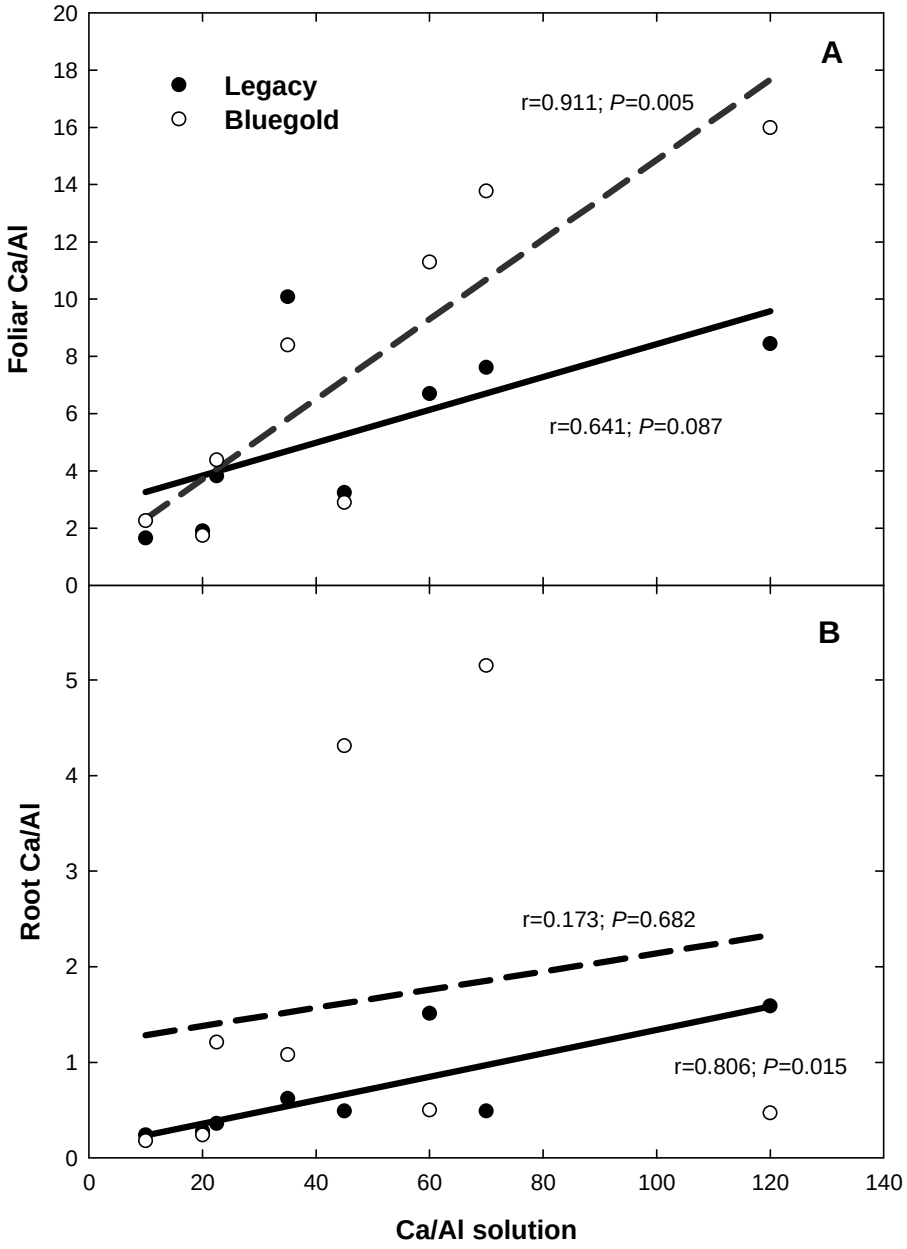

Figure 1. Relationship between nutrient solution and plant tissues Ca/Al molar ratio of two blueberry cultivars under Al toxicity amended with $CaSO_4$. Values represent mean of four replicates. Regression lines are presented to assist with the visual interpretation

Table 2. Effect of gypsum (kg ha^{-1}) application on mineral element of acid soil used in experiment after 60d. Values represent the average of three replicates ± S.E. Different lower case letters indicate statistically significant differences ($P \leq 0.05$) between gypsum treatments for the same cultivar.

	Legacy soil				Bluegold soil			
	$CaSO_4$ treatment (kg ha^{-1})							
Parameter measured*	0	1000	2000	4000	0	1000	2000	4000
Olsen-P (ppm)	26.6b	27.6ab	28.6a	26.3b	25.0b	14.3c	24.3b	27.3a
K (ppm)	164.2b	174.6ab	183.7a	183.7a	239.8a	199.4b	209.8b	209.8b
pH-H_2O	4.57c	4.77b	4.87a	4.89a	4.53b	4.78a	4.76a	4.75a
MO	13.0a	13.5a	13.2a	13.0a	10.0a	10.0a	10.0a	10.0a
K (cmol+/kg)	0.42b	0.44ab	0.47a	0.47a	0.61a	0.51b	0.53b	0.53b
Na (cmol+/kg)	0.08a	0.08a	0.10a	0.12a	0.10a	0.07ab	0.05b	0.05b
Ca (cmol+/kg)	0.27d	0.70c	1.40b	2.57a	0.35d	0.73c	1.43b	2.22a
Mg (cmol+/kg)	0.46b	0.54ab	0.57a	0.58a	0.58a	0.61a	0.62a	0.54a
Al (cmol+/kg)	0.95a	0.88a	0.78b	0.70b	0.93a	0.74b	0.64bc	0.58c
S bases (cmol+/kg)	1.28d	1.78c	2.55b	3.75a	1.66c	1.92c	2.64b	3.34a
CEC(cmol+/kg)	2.23d	2.66c	3.34b	4.45a	2.59b	2.06c	3.29b	4.20a
Al saturation (%)	42.6a	33.1b	23.5c	15.7d	35.9a	6.7c	19.6b	20.3b
S (ppm)	40.6d	60.6c	82.6b	106.6a	55.3c	53.0c	105.0b	137.0a
Al ext. (ppm)	1091a	1088a	1040ab	990b	1014a	1002a	869b	636c