Metabolomic analyses of highbush blueberry (*Vaccinium corymbosum* L.) cultivars revealed mechanisms of resistance to aluminum toxicity

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Highlights

- Ascorbic acid content is increased in roots of the Al-resistant cultivars under Al treatment.
- Aluminum treatment increased the exudation of oxalic acid in Al-resistant cultivars.
- The NADP-MDH activation state is increased for the effect of the aluminum in roots and leaves of Al-resistant cultivars.
- Aluminum affects the expression levels of enzymes of the TCA cycle in cultivars of highbush blueberry.

Abstract

Aluminum (Al) is an important factor that limits plant growth under acidic soil conditions. However, several plant species developed distinct mechanisms that limit the damage caused by high Al concentrations. In highbush blueberry (*Vaccinium corymbosum*), the Al resistance mechanisms are not fully understood. This study was designed to evaluate the effect of Al toxicity on roots and leaves of highbush blueberry genotypes with contrasting Al resistance [Star (Al-sensitive) and Camellia and Cargo (Al-resistant)] and identify the main molecular and physiological strategies underpinning adaptive Al stress responses in nutrient solution. After 48 h of Al treatment, the reduced form of ascorbate (ASC) was higher in roots, but unchanged in leaves of Cargo and Camellia genotypes compared to the control. We also observed decreased root exudation of oxalate in the Al-treated sensitive cultivar Star throughout the treatment period. However, in the resistant cultivar (Camellia), the exudation of oxalate increased 2.4- and 2.8-fold at 24 and 48 h, respectively. Al treatment differentially affected the enzyme activity and gene expression of the tricarboxylic acid (TCA) cycle enzymes. NAD-dependent malate dehydrogenase (NAD-MDH) expression in roots of cultivar Cargo was reduced at 24 h and increased at 48 h, whereas in leaves the expression was higher at 24 h and decreased at 48 h

compared to the control. Citrate synthase (CS) activity in Al-resistant Cargo roots diminished at 24 h, increasing afterwards, without variation in the *CS* gene expression, compared with the initial time point (t=0). In Al-resistant Camellia roots, the gene expression and the activity of CS decreased during Al exposure. NADP-dependent malate dehydrogenase (NADP-MDH) activity showed increased activity and gene expression at 24 h, in the leaves of cultivar Cargo, whereas in roots the gene expression decreased, but the activation state of NADP-MDH increased. The expression of genes encoding TCA cycle enzymes did not differ significantly in the Al-sensitive cultivar Star during Al exposure. In conclusion, the exudation of organic acid anions, particularly oxalate, plays an important role in Al resistance of highbush blueberry genotypes whilst elevated levels of ASC in roots, also contribute to the Al-resistance mechanisms exhibited by genotypes Camellia and Cargo.

Key words: TCA cycle, oxalate, ascorbate, organic acid anions.

1. Introduction

In acid soils, toxic aluminum (Al) is the main element that limits the productivity of most crops (Anoop et al., 2003; Ryan et al., 2011). Plants have developed two main types of mechanisms to lessen the damage caused by Al toxicity: (i) avoidance or exclusion mechanisms, whereby Al detoxification occurs in the rhizosphere through the exudation of organic acid anions that chelate toxic Al and make it harmless; and (ii) tolerance mechanisms, whereby Al in the cells is detoxified and sequestered into the vacuole with no damage to, or alteration of, biological processes (Yang et al., 2013; Zhang et al., 2019).

Al chelation in the rhizosphere is dependent on the exudation of organic acid anions through Al-activated anion channels in the plasma membrane (Kochian, 1995; Yang et al., 2013; Nunes-Nesi et al., 2014). The Al-exclusion mechanism is related to two families of plasma membrane-localized transporters, namely ALMT (aluminum activated malate transporter) and MATE (multidrug and toxic compound extrusion), that confer Al resistance to plants. The exclusion mechanism is an

important strategy of cultivated and wild plant species that grow in acid soils containing high concentrations of toxic Al (Barceló and Poschenrieder 2002; Yang et al., 2013). Woody plants species such as *Cryptomeria*, *Pinus*, *Populus,* and *Eucalyptus*, among others, also feature this mechanism (Brunner and Sperinsen 2013).

In response to Al stress, organic acid anions can be secreted in two distinct patterns. The pattern I suggests that exudation of organic acid anions does not require protein biosynthesis for the transport out of the cells, whereas the pattern II require *de novo* expression of transporter genes and protein formation (Ma et al., 2001; Zhang et al., 2019).

Several transporter types involved in uptake and subsequent root-to-shoot translocation of Al in plants have been related to the Al tolerance mechanisms. In *Oryza sativa*, the *OsNrat1* (Nramp aluminum transporter 1) gene encodes a plasma membrane protein that belongs to the Nramp (natural resistance-associated macrophage protein) family; it has been related specifically to the transport of trivalent Al ions. In addition, ALS1 is a tonoplast-localized ATP-binding cassette (ABC) transporter responsible for sequestrating Al into the root-cell vacuoles. These two proteins function cooperatively: NRAT1 mediates entry of Al into the cells, and the ALS1 sequesters Al into the vacuole (Zhang et al., 2019).

The mitochondrial tricarboxylic acid (TCA) cycle is a pathway containing eight enzymes whose functions are to oxidize acetyl-CoA into CO_2 and produce NADH, $FADH_2$, ATP, as well as the intermediates for important metabolic processes such as nitrogen assimilation, photosynthesis, regulation of enzymes, and photorespiration (Zhang and Fernie 2018). The activity of TCA cycle enzymes is also related to the Al-resistance mechanisms in plants regarding the biosynthesis of organic acid anions that chelate Al (Ma et al., 2001; Nunes-Nesi et al., 2014). Ligaba et al., (2004) showed that activities of CS, MDH and phospho*enol*pyruvate carboxylase (PEPC, EC 4.1.1.31) increased in *Brassica napus* roots under Al toxicity. These authors concluded that Al changed organic acid metabolism, inducing the accumulation and secretion of citrate and malate. In *Eucalyptus camaldulensis*, citrate content in roots was increased under Al stress due to the suppression of

citrate decomposition and the reduction in activity of aconitase (ACO, EC 4.2.1.3) and $NADP^+$-isocitrate dehydrogenase ($NADP^+$ ICDH, EC 1.1.1.42) (Ikka et al., 2013).

In *Paraserianthes falcataria*, Al enhanced mitochondrial *CS* gene expression and increased mitochondrial CS activity (Osawa and Kojima, 2006). In *Medicago sativa*, a Al-resistant genotype had higher levels of *MsCS* transcripts, CS activity and citrate exudation in roots compared with the Al-sensitive genotype (Sun et al., 2020). The overexpression of cytosolic MDH provoked a 4-fold increase in the concentration of organic acid anions in roots of *Medicago sativa*, producing higher rates of exudation under Al toxicity (Tesfaye et al., 2001).

Other metabolites, such as ascorbate (ASC), are important in responses to different environmental stresses (Bartoli et al., 2005; Plaxton and Podestá, 2006; Sweetlove et al., 2007; Nunes-Nesi et al., 2014). ASC is the most abundant soluble antioxidant molecule in plant cells, contributing to stress tolerance (Noctor and Foyer 1998; Smirnoff, 2000; Melino et al., 2009; Gallie, 2013). In chloroplasts, ASC is a key in maintaining redox status of organelles and as a coenzyme of the violaxanthin de-epoxidase (VDE) (Müller-Moulé et al., 2002; Horton and Ruban, 2005; Ivanov, 2014; Foyer, 2015). In Al-tolerant genotypes of *Secale cereale*, Souza et al., (2016) reported that ASC levels in leaves increased by 23% after 24 h of Al exposure, whereas in the Al-sensitive genotypes the ASC level did not change in the Al treatment. Increased levels of ASC in leaves provided protection to the photosynthetic apparatus under Al toxicity (Souza et al., 2016).

ASC is related to the oxalic acid biosynthesis in plant tissues (Loewus 1999; Kostman et al., 2001). The cleavage of ASC between carbon atoms 2 and 3 results in the formation of oxalic acid and L-threonic acid, which is the major pathway of oxalate production in plants (Melino et al., 2009; Cai et al., 2018). Oxalate subsequently plays an important role in detoxifying Al by forming a nontoxic Al-oxalate complex at a 1:3 ratio (log K = 12.4), which has a greater stability than the Al-citrate complex (log K = 8.1, 1:1 ratio) (Miyagi et al. 2013). *Fagopyrum esculentum* showed high resistance to Al toxicity related to oxalate secretion by roots (Ma et al., 1997; Zheng et al., 1998).

Highbush blueberry (*Vaccinium corymbosum*) is one of the main fruit crops in southern Chile (Millaleo et al., 2019), and is usually cultivated in volcanic ash-derived soils, with strong acidity and high availability of Al (Manquián-Cerda et al., 2018). It is therefore crucial to understand the Al-resistance mechanisms in diverse highbush blueberry genotypes and particularly to evaluate changes in the metabolic processes related to organic acid biosynthesis. Currently, the Al-resistance mechanisms in *V. corymbosum* are not fully understood. In our study, we used genotypes of *V. corymbosum* differing in Al resistance (Al-sensitive Star and Al-resistant Cargo and Camellia; Cárcamo et al., 2019) to evaluate their metabolic responses and identify the Al-resistance mechanisms in highbush blueberry.

2. Materials and methods

2.1. *Plant material and growth conditions*

Star (USOOPP10675P) (Al-sensitive) and Camellia (US 20070118942A1) and Cargo (US 2013023926OP1) (Al-resistant) genotypes of highbush blueberry (*Vaccinium corymbosum* L.) were used in this study. The Cargo cultivar is grown in the south, whereas Star and Camellia are cultivated in the central zone of Chile, due to differential low temperature requirements of each cultivar. One-year-old plants (40 cm in height) were conditioned in plastic pots containing 18 L of Hoagland solution (Hoagland and Arnon 1959) with the initial pH of 5.6 that was lowered daily for two weeks until achieving pH 4.5. The composition of this nutrient solution was 3.0 mM KNO_3, 2.0 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 0.1 mM KH_2PO_4, 1.0 mM NH_4NO_3, 20 µM Fe-EDTA, 25 µM H_3BO_3, 10 µM $MnSO_4$, 0.4 µM $CuSO_4$, 2.0 µM $ZnSO_4$, and 0.07 µM $(NH_4)_6Mo_7O_{24}$, and was renewed every 3 days. The growth chamber conditions were 16/8 h light/dark photoperiod, 22±2°C temperature, 70% relative air humidity, and light intensity around 300 µmol photons m^{-2} s^{-1}. The treatments were: control (without Al) and 200 µM Al as $AlCl_3$, both at pH 4.5 adjusted twice daily during the 48-h treatment. Physiological parameters were evaluated at 24 and 48 h of Al exposure; samples from fully expanded leaves and roots were harvested in the

middle of the light period, snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

2.2 Ascorbic acid and dehydroascorbate determination

Ascorbic acid content (ASC) was quantified by the protocol described by Kampfenkel et al., (1995) with minor modifications. About 50 mg of plant material was ground in liquid nitrogen, homogenized in 330 µL of 1 mM EDTA + 0.1 M HCl and centrifuged at 12,000 *g* at 4 ºC for 10 min. An aliquot of 20 µL was used to measure the absorbance at 520 nm in a microplate spectrophotometer (EPOCH, BioTek Instruments, Inc., headquartered in Winooski, VT, USA). The levels of ascorbic acid were determined from standard curve using the sodium ascorbate as standard. The content of dehydroascorbate (DHA) was determined by subtracting the measurements without N-ethylmaleimide (NEM)**.**

2.3 Metabolomics

Metabolites were analyzed using approximately 15 mg of dry root or leaf tissues. The extraction, derivatization, addition of standards, and sample injection into gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) were carried out as described by Lisec et al., 2006. Chromatograms were evaluated using TAGFINDER 4.0 software (Luedemann et al., 2008). The mass spectra were cross-referenced with those in the Golm Metabolome Database (Kopka et al., 2005). The amount of each metabolite was determined as the relative metabolite abundance, calculated by normalization of signal to that of Ribitol (internal standard) as described by Lisec et al., (2006). The data were calculated based on the dry weight for leaves and roots and reported following the recommended reporting format (Fernie et al., 2011).

2.4 Analysis of root exudates in nutrient solution

Root exudates were determined according to Millaleo et al., (2019) with minor modifications. After 24 and 48 h of the Al treatment, plants were rinsed with deionized water, transferred to pots containing 1.4 L of constantly aerated deionized water for 2 h, followed by collecting samples (45 mL) and storing them at -20°C. In

order to quantify the concentration of organic acid anions, root exudate subsamples were concentrated by lyophilization, and the residue was re-dissolved in 300 to 500 µL of deionized sterile water. The filtered samples (0.22 µm, Nylon, Rephile, Boston, USA) were injected into a high-performance liquid chromatography (HPLC) system (Merck-Hitachi model L-4200, Burladingen, Germany) equipped with a UV–visible detector and a sphere-column heater (Phenomenex Termamodel TS-130) according to Meier et al., (2012). The organic acid anions (oxalate, malate, citrate, and succinate) were identified by comparing the retention times of the standards and by spiking the samples with the standards of each organic acid anion.

2.5 Extraction and assay of enzyme activities

The enzyme extract was prepared as described by Gibon et al., (2004) using 50 mg fresh leaf or root tissues. The total activities of citrate synthase (CS), NAD-dependent isocitrate dehydrogenase (NAD-IDH), NAD-dependent malate dehydrogenase (NAD-MDH), and NADP-dependent malate dehydrogenase (NADP-MDH) initial activity (without the substrate (V0)) and total activity (under substrate-saturating conditions), and the activation state (total activity/initial activity), were measured as described by Omena-García et al., (2017).

2.6 Extraction of RNA and synthesis of cDNA

Total RNA was extracted from 150 mg of frozen blueberry roots or leaves, using the protocol of Inostroza-Blancheteau et al., (2011). RNAse-free DNAase I (Invitrogen, ThermoScientific, Wilmington, VA, USA, Cat. No. EN0521) was used to remove contaminating genomic DNA. The RNA concentration was measured spectrophotometrically using a NanoDrop TM 1000 (ThermoScientific) and a Quantus™ Fluorometer and QuantiFluor RNA (Promega, Madison, WIS, USA, Cat. No. E6150 and E3310). The purity of the total RNA was assessed using the A260/A280 and A260/A230 ratios. The first-strand cDNA was synthesized from 1.0 µg total RNA using a cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA, Cat. No.4368814).

2.7 Quantitative real-time PCR analysis

Reverse transcription-quantitative polymerase chain reaction (RT-PCR) analysis of *VcMDH-NAD*, *VcMDH-NADP*, *VcCS*, and *VcIDH-NADP* expression was carried out using a Step One Applied Biosystems (Applied Biosystems). The reaction mixture (15 µL) contained 7.5 µL of GoTaq® qPCR master mix 2X (Promega, Cat. No. A6001), 0.3 µL of each forward and reverse primers (10 µM each) (for the sequences see Table 1), and 3 µL of cDNA template. DNA amplification was conducted using the following thermocycling program: 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 40 s for *VcNAD-MDH,* whereas 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 40 s was used for *VcNADP-MDH* and *VcCS*; both programs were followed by 71 cycles of increasing the temperature from 57 to 95°C in increments of 0.5°C per 30-s cycle to obtain a melting curve. The gene encoding metallothionein in blueberry (EST Accession CF811253) was used as a reference gene as described previously (Naik et al., 2007).

2.8 Experimental design and statistical analyses

The experiment was performed in a repeated measures design with three genotypes, three replicates and three measurement times for the physiological and metabolic analyses. Due to the lack of difference in the parameters measured at the three time points (0, 24 and 48 h) in the treatment without Al, we considered the data at the initial time point (t=0 h) as the control for all sampling times.

When the data passed the Kolmogorov-Smirnov test for the normality and homogeneity of variances, we performed two-way repeated measures analysis of variance (where factors were genotypes and sampling times) and used the Tukey's test for mean comparisons. If data did not pass the Kolmogorov-Smirnov test, we used ANOVA on ranks repeated measures analysis.

In order to identify the variables that explained the differences among the genotypes differing in Al resistance, the principal components analysis (PCA) was done. We also included the data on Al concentration in highbush blueberry roots reported by Cárcamo et al., (2019) together with the variables measured in this study. For PCA,

the data of all the variable were normalized [log(2)] to minimize the effect of different units of measurement in the variance of each component. All analyses were performed by XLSTAT-LifeScience v.2020.

3. Results

3.1 *Ascorbic acid*

To explore the changes in the oxidized and reduced forms of ASC induced by Al toxicity, we analyzed ASC levels in the highbush blueberry cultivars differing in Al resistance. Regarding ASC levels in roots, a significant interaction between cultivar and the duration of Al exposure was observed ($p<0.001$) (Supplementary Table 1). The initial ASC levels in roots were 4-fold higher in Cargo and 2-fold higher in Camellia compared with the Al-sensitive Star. However, compared with t=0, the Star cultivar showed a 1.9-fold increase after 24 h and Camellia a 2.6-fold increase after 48 h of Al exposure, whereas cultivar Cargo showed decreased values (by 42%) after 24 h of Al exposure.

Dehydroascorbate (DHA) levels in roots decreased by 25, 34 and 23% after 48 h of the Al treatment in roots of Star, Camellia and Cargo, respectively. The ASC/DHA ratio in roots (being an indication of tissue oxidative stress) showed a significant interaction between cultivar and the duration of Al exposure ($p<0.001$) (Supplementary Table 1), with the initial time (t=0) and the final time (t=48) ASC/DHA ratio in roots being 1.9-fold and 4.5-fold higher in Camellia, but 3.9-fold and 3.4 higher in Cargo, respectively, compared to the Al-sensitive Star. Compared with t=0, the ASC/DHA ratio in roots of Camellia increased 3.9-fold at 48 h, whereas in Cargo roots there was a 33% decrease at 24 h, followed by a 1.4-fold increase at 48 h of Al exposure.

For the leaf ASC level, the only significant factor was the genotype ($p<0.001$), with Star and Cargo displaying 3.4- to 2.5-fold higher ASC levels than Camellia at all durations of the Al treatment (Table 2). The DHA level in leaves was influenced by the significant interaction cultivar x duration of Al exposure ($p<0.009$) (Supplementary Table 2). For all the Al exposure times (t=0, 24 and 48), Camellia

showed 2.1-fold higher DHA levels than Cargo. The leaves of Camellia had 14% decreased DHA levels at t=24, whereas Star showed significantly (by 36-39%) decreased DHA levels throughout the experiment.

The ASC/DHA ratio in leaves was influenced by the significant interaction between cultivar and duration of Al exposure ($p<0.014$) (Supplementary Table 2). Compared with Camellia, the ASC/DHA ratio increased 5.9-fold in Cargo and 4.3 in Star at t=0, 5-fold in Cargo and 6.1-fold in Star at t=24, and 6.6-fold in both Cargo and Star at t=48. In Star leaves, the ASC/DHA ratio increased around 1.6-fold after either 24 or 48 h of the Al treatment (Table 2).

3.2 Metabolite profiling

To investigate alterations in the major primary pathways in response to Al in contrasting genotypes, we performed metabolomic analysis. Metabolic profiles of *V. corymbosum* genotypes included 30 compounds in roots and 34 compounds in leaves under Al treatment (Supplementary Data 1 and 2).

Organic acid levels in roots decreased significantly during Al exposure. The glycolic acid level diminished by 27% in Star after 48 h, 29% in Camellia after 24 h and 22% in Cargo after 48 h of the Al treatment. Glyceric acid levels in roots diminished by 41% in Camellia and 45% in Cargo after 48 h. The Al treatment was associated with malic acid levels in roots decreasing by 90% in each Star and Cargo after 48 h. Root levels of citric acid were decreased on increasing the duration of the Al treatment (79-92% after 24 h, and 86-91% after 48 h, depending on the genotype). The Al treatment decreased the root levels of succinic and threonic acids only in the Star cultivar (by 54 and 79%, respectively) following 48 h of Al exposure (Fig. 2).

3.3 Root exudation of organic acid anions

We determined the main organic acid anions (oxalate, malate, citrate, and succinate) exuded by roots as an important physiological mechanism to reduce toxic Al. We found only oxalate in root exudates (Fig. 2). The exudation of this organic acid anion was influenced by the significant interaction cultivar x duration of Al exposure ($p<0.001$) (Supplementary Table 1). The differences in the oxalate

exudation among cultivars were higher (4.4-fold in Star and 4.7-fold in Cargo compared to Camellia) at t=0, whereas at t=24 and t=48 increases were, respectively, 1.6-fold and 2.8-fold in Camellia and 1.9-fold and 3.6-fold in Cargo, respectively in comparison with the Al-sensitive cultivar. Oxalate exudation was higher in Cargo than Star and Camellia after 24 and 48 h of Al exposure. In Camellia, we observed an increase of 2.4- and 2.8-fold after 24 and 48 h, respectively, of the Al treatment, whereas in Star and Cargo oxalate exudation diminished by, 64% and 35% at 24 h, and by 76% and 18% at the end of the experiment, respectively (Fig. 2).

3.4 Enzyme activities

ANOVA analysis of NAD-MDH activity revealed a significant interaction between the cultivars and the duration of Al exposure ($p<0.001$ in roots and $p<0.013$ in leaves) (Supplementary Table 1 and 2). The NAD-MDH activity in roots at t=0 and t=24 h was 1.8- to 1.9-fold higher in Camellia and 1.2- to 1.8-fold in Cargo compared with the Al-sensitive Star. The NAD-MDH activity in roots of Star and Camellia cutlivars showed a similar pattern during Al exposure, a decrease of 30-26% at 24 h, and an increase around 2.9- and 1.6-fold at 48 h compared with t=0 (Fig. 3a). However, there was a continuous decrease (by about 50%) throughout the Al treatment (Fig. 3a). In leaves, the NAD-MDH activity was higher (1.4- to 5.6-fold) in the Al-tolerant cultivars compared with the Al-sensitive cultivar at all times (Fig. 3b).

The NADP-MDH initial activity in roots was influenced by the significant interaction between cultivar and duration of Al exposure ($p<0.006$). At t=24 h compared with t=0, the initial NADP-MDH activity was 4.7-fold higher in Star and 5.4-fold in Cargo compared with the roots of Camellia (Supplementary Table 1). However, compared with t=0, the NADP-MDH initial activity increased significantly (3.4-fold) in roots of the Star cultivar following 48 h of Al treatment, whereas the Camellia cultivar displayed a decrease of 69% at 24 h, and then a 3.6-fold increase at 48 h (Fig. 3C). In Star leaves, NADP-MDH initial activity decreased significantly (by 77%) after 48-h Al exposure compared with the control (Fig. 3D).

The total NADP-MDH activity was influenced by the significant interaction between cultivars and length of Al exposure ($p<0.003$ in roots and $p<0.001$ in leaves) (Supplementary Table 1 and 2). The NADP-MDH total activity was lower in Camellia in roots at t=24 h and t=48 h by 78% and 82%, respectively and in Cargo by 53% and 67%, respectively compared with Star. Moreover compared to the untreated control, the total NADP-MDH activity increased significantly in Star roots (3.8- and 3-fold at t=24 and t=48 h, respectively), whereas in Cargo roots, increased significantly (1.9-fold) only at t=24 (Fig. 3E). Compared with t=0, Camellia leaves showed a significant increase (8.9-fold) in total NADP-MDH activity at t=24 h, whereas in Cargo there was a decrease (43%) after 48 h of the Al treatment (Fig. 3F).

In leaves the activation state of NADP-MDH was influenced by the significant interaction between cultivar and duration of Al exposure ($p<0.002$). In the untreated controls Cargo had lower (by 75%) NADP-MDH activation state than Camellia and Star, but at t=24 it was 2.5-fold higher in Cargo compared with Camellia and Star (Supplementary Table 2). The NADP-MDH activation state increased significant only in Cargo (6.9- and 4.2-fold at t=24 h and t=48 h, respectively) compared with the untreated control (Fig. 3H). In roots at t=24 h, the activation state of NADP-MDH was decreased (by 84%) in Camellia, whereas it increased 3-fold in Cargo at t=48 h compared with the control (Fig. 3G).

The interaction between cultivar and duration of Al exposure significantly influenced CS activity in roots and leaves ($p<0.001$). In roots, the CS activity increased 2.6-fold in Camellia and 9.8-fold in Cargo compared with Star during the 48-h exposure to Al (Fig. 4A). In Cargo roots, the CS activity showed a significant decrease around 45% at t=24 h, but a 1.2-fold increase in the CS activity at t=48 compared with the control (Fig. 4A). In leaves, the CS activity was higher in the Al-tolerant cultivars than the Al-sensitive cultivar Star at all time points (Supplementary Table 2). However, in Star and Camellia cultivars, the CS activity in leaves was reduced significantly (by 42% and 23%) at t=24 h, whereas in Star there was a decrease of around 71% at 48 h of Al exposure (Fig. 4B).

Similar to the other enzymes, the interaction was significant for the NAD-IDH activity in roots ($p<0.001$) and leaves ($p<0.005$). In roots, the NAD-IDH activity was 3.8-fold higher in Camellia and 2.7-fold higher in Cargo compared with Star at t=24h. However, the NAD-IDH activity in roots showed a significant decrease in all highbush blueberry genotypes between the untreated control and t=48 h and between t=0 and t=24 h in Star and Cargo only (Fig. 4C). In leaves, the NAD-IDH activity was 5.8-, 1.4- and 3.9-fold higher in Cargo than in Al-sensitive Star at t=0, t=24 h and t=48 h, respectively (Supplementary Table 2). After 48-h exposure to Al, the NAD-IDH activity was significantly (by 1.7-fold) higher in Cargo leaves than those of the other cultivars (Fig. 4D).

3.5 Expression of genes coding for TCA cycle enzymes

Given that Al toxicity can affect gene expression, we next investigated the transcript levels of TCA cycle enzymes, *NAD-MDH*, *NADP-MDH* and *CS* in roots and leaves of all *V. corymbosum* cultivars by quantitative real-time (qRT)-PCR. The changes in the expression of *VcNAD-MDH* gene in roots and leaves were not significant (Fig. 5A, B). However, the *VcNADP-MDH* expression in roots was influenced significantly by the interaction ($p<0.007$). At t=24, the *VcNADP-MDH* expression in roots was 54% lower in Camellia than Star, but there was no difference at the other two durations of Al exposure (Supplementary Table 1). In leaves at t=24 h, the *VcNADP-MDH* expression increased 1.5- and 2.3-fold in Star and Cargo, respectively, compared with the untreated control (Fig. 5D), but then diminished by 36% only in Star after 48 h of exposure to Al (Fig. 5D). The relative expression of *VcCS* was influenced by the significant interaction between genotype and the duration of Al exposure in roots ($p<0.006$) and leaves ($p<0.009$) (Supplementary Table 1 and 2). The *VcCS* expression in roots was 90% lower in Camellia, at t=24 h and t=48 h compared with the untreated control, compared with Al-sensitive Star (Fig. 5E), whereas in leaves the *VcCS* expression was 2.1- and 2.9-fold higher in Cargo than in the Al-sensitive Star at t=24 h and t=48 h, respectively (Fig. 5F).

3.6 Multivariate analyses

The data obtained were averaged and normalized, and the Al concentration in roots and shoots (reported by Cárcamo et al., 2019) was included in a principal component analysis (PCA). For roots, the first component (PC1), which explained 41.4% of total variance, comprised all the organic acids, amino acids (excluding asparagine), and ASC, the ASC/DHA ratio, the NAD-MDH activity, and the NADP-MDH activation state (Fig. 6A). The second principal component (PC2) explained 21.1% of total variance and grouped the variables oxalic acid, total ASC, asparagine, *VcNADP-MDH* expression, and initial NADP-MDH activity and CS activity (Fig. 6A).

In leaves of all genotypes, the first component (PC1) explained 43.1% of total variance, and the most important variables were concentrations of Al, ASC, DHA, total ASC, organic acids (except threonate, malate and glycolate) and amino acids (excluding proline and serine), ASC/DHA ratio, and NAD-IDH activity (Fig. 7A). The PC2 explained 26.4% of total variance; PC2 comprised threonate, malate and glycolate, proline and serine amino acids, and NAD-MDH and CS activities as the principal variables (Fig. 7A).

In the PCA score plots, we observed a separation among the three cultivars. In roots, there were two groups, one including the Al-resistant Camellia and Cargo, and the other containing the Al-sensitive Star cultivar (Fig. 6B). In leaves, each cultivar was separated into independent group (Fig. 7B).

4. Discussion

4.1 Genetic variation in Al tolerance of blueberry cultivars

Differences in Al resistances not only exist among different species, but also among local populations of the same species, with high Al concentration in acid soils being a significant selection pressure for population divergence driving the natural process of adaptation (Brunner and Sperisen 2013). Our data revealed significant differences in Al resistance among the three genotypes of *V. corymbosum*, associated with changes in tissue Al concentration, organic acid synthesis, TCA cycle enzymes activity and the redox status in roots (Fig. 6A). In leaves, the main differences among the genotypes were in TCA cycle enzymes expression and activity and ASC levels (Fig 7A,B). The genotypic differences could be associated

with the different pedigrees, with the cultivars Camellia and Star being derived from a cross between different species (*V. corymbosum* x *V. darrowi*) and the cultivar Cargo from a cross between the *V. corymbosum* genotypes Bluegold x Ozarkblue. Santos et al., (2019) also found wide inter- and intra-species variability in Al resistance, and Reyes-Díaz et al., (2009) confirmed that genotypes of *V. corymbosum* differ in Al resistance.

4.2 Redox regulation of metabolism in genotypes differing in Al resistance

It is been documented that Al triggers ROS production, affecting internal homeostasis in several plant species (de Sousa et al., 2016). To cope with oxidative damage induced by ROS, plants developed both enzymatic and non-enzymatic antioxidant defense mechanisms (Sharma et al., 2012). Our results showed significantly higher root level of ASC and a higher ASC/DHA ratio in the Al-resistant cultivars Camellia and Cargo compared with Al-sensitive Star after 48 h of Al exposure (Table 2). These results suggest increased ASC levels may represent an adaptation to Al stress. In addition, these findings may be related to an effectiveness of the ASC-GSH regenerating enzyme system maintaining the ASC and DHA pools, which can contribute to controlling oxidative stress caused by metals in plants (Paradiso et al., 2008; Anjum et al., 2011). Similarly, Fotopoulos et al., (2010) indicated that ASC/DHA ratio can be considered a marker of oxidative stress, which is in accordance with our results whereby ASC/DHA ratio was positively and significantly correlated with Al concentration in roots and leaves of Al-sensitive Star (Supplementary Figure 1A and 2A). By contrast, Al-resistant Cargo did not show any significant difference in the ASC and DHA levels and ASC/DHA ratio in leaves during Al exposure (Table 2). Hence, our results on Al-resistant genotypes are in agreement with the report by de Sousa et al. (2016) that ASC levels in Al-tolerant genotypes of *Secale cereale* did not vary significantly under Al exposure. Consistent with its Al sensitivity, the Star cultivar had higher Al levels in roots and impaired photosynthesis and gas exchange (Cárcamo et al. 2019) which represent indicators of more severe oxidative stress (as shown by increased ASC/DHA ratio; Table 2) (see also Szarka et al., 2013).

Malate dehydrogenases (NAD-MDH and NADP-MDH) are oxidoreductases that catalyze the reversible reactions of malate and oxaloacetate, using NAD^+ and $NADP^+$ as coenzymes (Scheibe 2004; Li et al., 2016). NADP-dependent malate dehydrogenase is a key enzyme controlling malate levels as well as those of reducing equivalents; as such the activation state of NADP-MDH is utilized as a signal of the redox status due to its rapid adjustment to changing metabolic conditions (Scheibe and Stitt 1988; Hebbelmann et al., 2012). In the present study, NAD-MDH and NADP-MDH showed higher activity and a positive correlation between Al concentration and NADP-MDH activation state in leaves of the Cargo (Al-resistant) cultivar (Supplementary Figure 2C). These findings complemented the report by Cárcamo et al., (2019), who showed that this cultivar exhibited higher photosynthetic pigments level, stable photosynthesis, stomatal conductance and fluorescence parameters, and increased malate levels in leaves under Al exposure in comparison with the Al-sensitive cultivar Star. Hence, the MDH isoforms in highbush blueberry may represent a strategic system for balancing the redox status in plant cells and chloroplasts, contributing to the Al resistance mechanisms. These findings are consistent with the MDH isoforms representing a compensatory mechanism activated under stress (Tomaz et al., 2010), allowing the export of reducing equivalents from the cells (Hebbelmann et al., 2012).

4.3 Metabolite changes associated with Al exposure in genotypes of blueberry with contrasting Al resistance

The energy in the cell is accumulated in proton gradients and pairs of redox compounds ($NADPH/NADP^+$, $NADH/NAD^+$, ASC/DHA). In addition to these redox pairs, organic acid biosynthesis is particularly important in plant metabolism as well as in the mechanisms of Al resistance (Panda et al., 2007; Igamberdiev and Bykova 2018). In the present study, *V. corymbosum* cultivars displayed significantly decreased organic acids levels in roots during Al exposure, especially in the Al-sensitive cultivar Star (Fig. 1). Similarly, in Al-sensitive cultivars of *Phaseolus vulgaris* root Al exposure decreased organic acid levels the (Lee and Foy 1986). In addition, we observed also diminished organic acid concentrations in root of the Al-resistant Cargo and Camellia cultivars, which could have been related to increased

exudation of organic acid anions (Lee and Foy 1986) as we observed in Al-resistant Camellia regarding oxalate exudation (Fig. 2).

Amino acids serve as the precursors of many metabolites, with numerous functions related to growth and responses to various stresses (Su et al., 2019). Using metabolomics in the present study, we observed that amino acid levels were unaltered in highbush blueberry roots and leaves under short-term Al exposure (Fig. 1), similarly to the data obtained by the direct quantification methods in the same genotypes (Cárcamo et al., 2019). Our findings are also in keeping with the observation that in *Matricaria chamomilla* shoots, the concentrations of free amino acids were largely unaffected by the Al treatment (Kováčik et al., 2010).

4.4 Mechanisms of Al resistance in the cultivars of highbush blueberry

Roots are the main Al target, being the site of perception of, and response to, Al toxicity (Zhang et al., 2019). Plants have developed two types of mechanisms of resistance to Al based on internal detoxification or avoidance of the external Al, both based mainly on organic acid anions (Santos et al., 2019). Our results showed enhanced oxalate exudation by roots of Al-resistant cultivar Camellia after Al exposure (Fig. 2) and that could be associated with low Al concentration in Camellia roots as found by Cárcamo et al., (2019). Kochian et al., (2004) also showed a strong correlation between Al resistance and Al-activated organic acid anion exudation in numerous plants species. Enhanced oxalate exudation from roots (Klug and Horst 2010) and internal oxalate complexation of Al in roots and leaves were important for Al resistance in *Fagopyrum esculentum* (Ma et al., 1997; Wang et al., 2015). Similarly, oxalate exudation from roots was found to contribute to Al resistance in other species such as tea (*Camellia sinensis*) (Morita et al., 2011; Prasad and Singh 2017). In the present study, oxalate was the main organic acid anion detected in the root exudates, being present in a higher levels in the Al-resistant genotypes than the Al-sensitive cultivar Star during Al exposure (Fig. 2); similar results were also reported in other woody plant species such as *E. camaldulensis*, *Pinus sylvestris*, *Picea abies* and *Camellia sinensis* (Ahonen-Jonnarth et al., 2000; Nguyen et al., 2003; Heim et al., 2001; Meyer et al., 2010; Morita et al., 2011).

The Al-resistant Camellia cultivar displayed increasing root ASC levels across the duration of Al exposure (by contrast to a decline in the levels of this metabolite in the Al-sensitive Star) (Table 2), and additionally exhibited an increased oxalate exudation (compared with a decrease in Star) with increased duration of Al exposure (Fig. 2). The other Al-resistant cultivar Cargo also had a high ASC levels and high oxalate exudation after 48-h Al exposure, suggesting that ASC is a key precursor for oxalic acid synthesis in the Al-resistant *V. corymbosum* cultivars. Similarly in rice, the levels of ASC increased together with an increase in the levels of oxalate under Al toxicity (Guo et al., 2005).

Numerous studies have examined the biological roles of Al-stress-induced genes and their relationship with biosynthesis of organic acids. For example, Abd El-Moneim et al., (2015) suggested that overexpression of *MDH* genes could improve Al tolerance in rye. Our analyses indicated that the gene expression of TCA cycle enzymes *VcCS*, *VcNAD-MDH* and *VcNADP-MDH* tended to increase in leaves of cultivar Cargo during Al exposure (Fig. 5). Also, in the previous studies of this cultivar, it showed a slight increase in the malate levels after 24 h of Al exposure (Cárcamo et al., 2019). Similarly, Tesfaye et al., (2001) reported that the overexpression of nodule-enhanced MDH in alfalfa resulted in a small (but significant) increase in the malate levels in tissues of transgenic lines. Hence, Al can enhance the expression of TCA cycle enzymes related genes in at least some Al-resistant cultivars of *V. corymbosum*.

Our results suggest that the difference in the response of the Al-resistant cultivars Cargo and Camellia could be related to the patterns of organic acid anion exudation described by Ma et al., (2001). Upon exposure to Al, a significant increase in the exudation of oxalate by Camellia roots would suggest that exudation of organic acid anions does not require protein biosynthesis for the transport out of the cells (Pattern I), whereas the changes (after 24-h exposure) in the expression of the CS and NADP-MDH genes (Fig. 4) and NADP-MDH enzyme activity (Fig. 3) and an increase in oxalate exudation after 48-h exposure to Al (Fig. 2) in Cargo cultivar would require *de novo* expression of transporter genes and protein formation (Pattern II).

In conclusion, Al resistance in the *V. corymbosum* cultivars Camellia and Cargo appears to be dependent on oxalate exudation (Al-exclusion mechanism), supported

by an increase in ASC levels and increased activity of enzymes CS and NADP-MDH. Nevertheless, as the main changes was observed at 48 h, it is suggested that longer-term experiments to confirm possible patterns among the evaluated tolerant genotypes are still necessary.

Author Contributions

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

Conflict of Interest statement

The authors declare no conflict of interest.

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Figure captions

A

	Star			Camellia			Cargo		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Glycolic acid	Aa	Aa	Ab	Ba	Cb	Bab	Ba	Bb	Bab
Glyceric acid	Aa	Aa	Aa	Ba	Bb	Ba	Ba	Bb	Bb
Malic acid	Aa	Aab	Ab	Ba	ABa	Aa	ABa	Bb	Ab
Citric acid	Aa	Aab	Ab	Ba	Bb	Abab	ABa	Bb	Bb
Succinic acid	Aa	Aa	Ab	Ba	Ba	Aa	ABa	Ba	Aa
Threonic acid	Aa	Aab	Ab	Ba	Ba	Aa	ABa	Ba	Aa

B

Glycolic acid	Aa	Aa	Aa	Ba	Ba	Ba	Ba	Ba	Ba
Glyceric acid	ABa	ABa	Aa	Aa	Aa	Aa	Ba	Ba	Aa
Malonic acid	Ca	Ca	Ca	Aa	Aa	Aa	Ba	Ba	Ba
Malic acid	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
Succinic acid	ABa	Aa	Aa	Aa	Aa	Aa	Ba	Aa	Ba
Threonic acid	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
Quinic acid	Ba	Aa	ABa	Aa	Aa	Aa	Ba	Aa	Ba

-7.4	Negative
-5.6	
-3.7	
-1.9	
0	
0.5	Positive
0.9	
1.4	
1.9	

Figure 1. Heat map representing the changes in relative organic acids contents in roots **(A)** and leaves **(B)** of highbush blueberry (*V. corymbosum*) after exposure to 200 µM Al for 0, 24 and 48 h in Al-sensitive Star and Al-resistant Camellia and Cargo cultivars. The full data sets from these metabolic profiling studies are available in

Table S1. The color code of the heat map is given as the log_2 of the fold-change in the scale legend. The values are the averages of three biological replicates. The 0 h point corresponds to the average between the start of the experiment and the respective controls for each time (24 and 48 h) because no significant differences were found. Uppercase letters show significant differences (P<0.05) among the genotypes, and lowercase letters denote significant differences (P<0.05) among the Al-exposure durations according to the Tukey test.

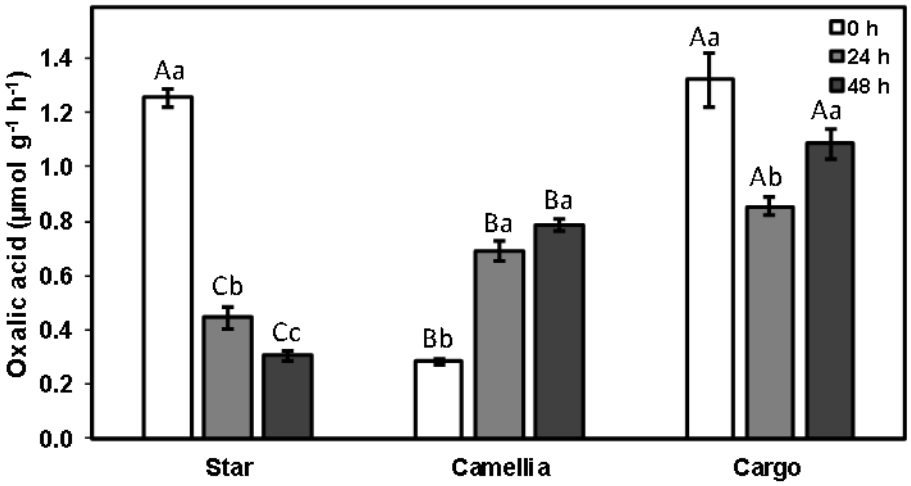


Figure 2. Oxalic acid concentration in root exudates of Star, Camellia and Cargo cultivars of *V. corymbosum* exposed to the Al treatment (200 µM Al) for 0, 24 and 48 h. The values are the averages of three independent biological replicates (± standard error). Uppercase letters indicate significant differences (P<0.05) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences (P<0.05) among the Al-exposure durations for a given cultivar according to the Tukey test.

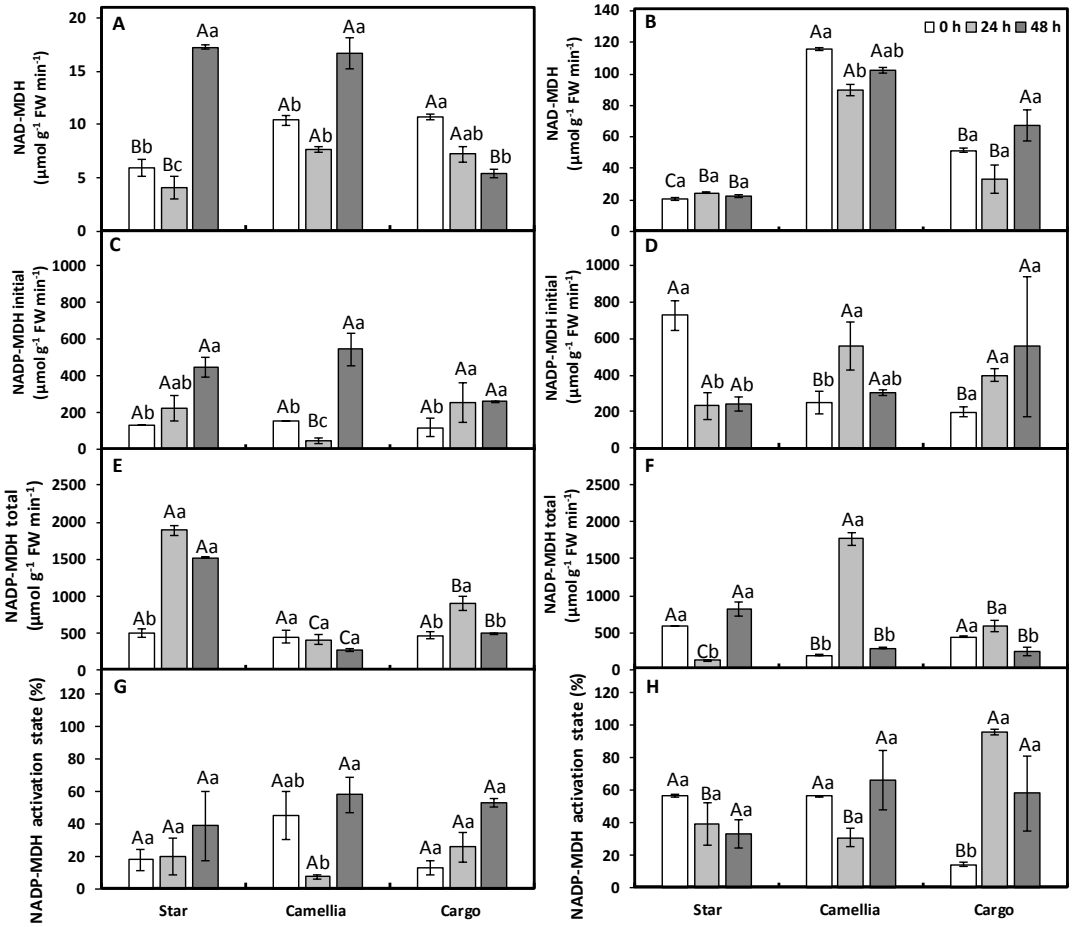
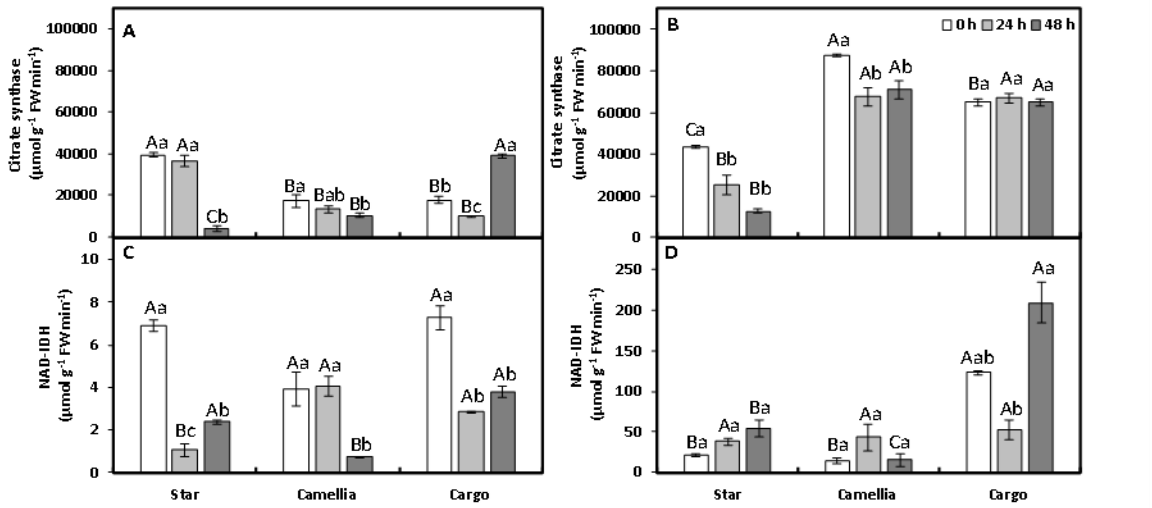


Figure 3. Malate dehydrogenase isoforms in roots (left graphs) and leaves (right graphs) of *V. corymbosum* cultivars Star, Camellia and Cargo exposed to the Al treatment (200 µM Al) for 0, 24 and 48 h. **A)**, and **B);** NAD-dependent malate dehydrogenase, **c)** and **d)** NADP-dependent malate dehydrogenase (NADP-MDH) initial activity, **d)** and **f)**; NADP-MDH total activity and, **g)** and **h)**; NADP-MDH activation ratio. The values are the averages of three independent biological replicates (±standard error). Uppercase letters indicate significant differences ($P<0.05$) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences ($P<0.05$) among the Al-exposure durations for a given cultivar according to the Tukey test.



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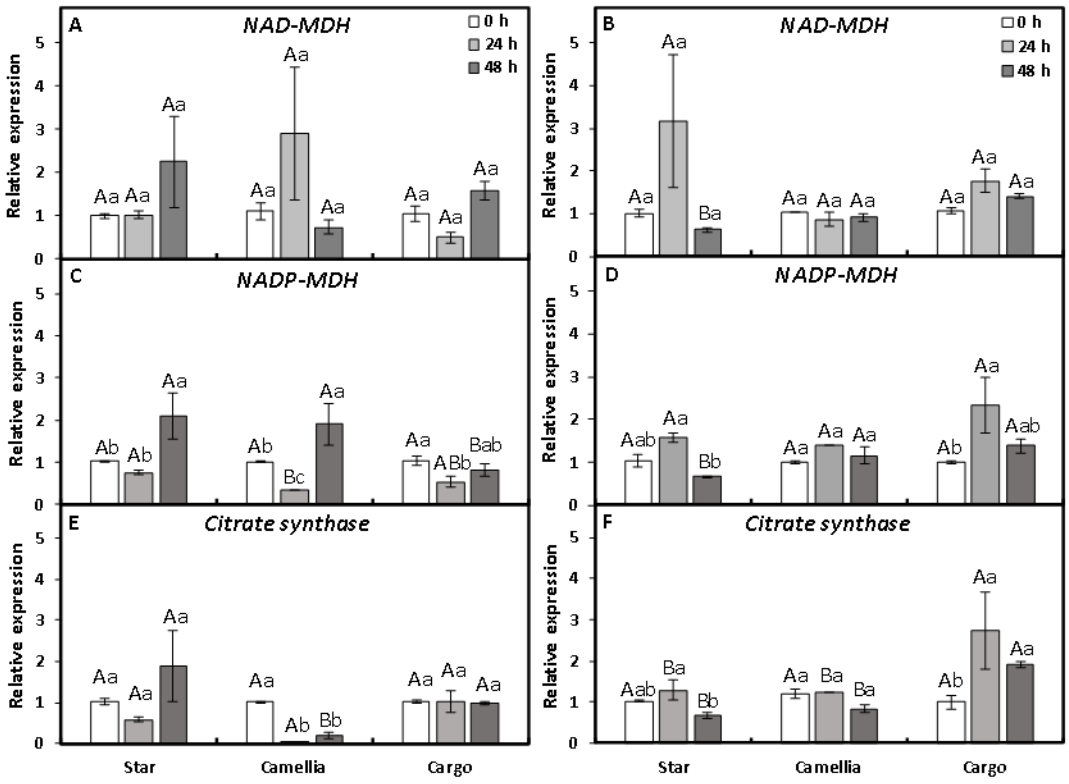
938 **Figure 4.** Citrate synthase (CS) and NAD-dependent isocitrate dehydrogenase
939 (NAD-IDH) activity in roots (A and C, respectively) and leaves (B and D, respectively)
940 of *V. corymbosum* cultivars Star, Camellia and Cargo exposed to the Al treatment
941 (200 μM Al) for 0, 24 and 48 h. The values are the averages of three independent
942 biological replicates (± standard error). Uppercase letters show significant
943 differences (P<0.05) among the cultivars at a given Al-exposure duration, and
944 lowercase letters show significant differences (P<0.05) among the Al-exposure
945 durations for a given cultivar according to the Tukey test.



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947

948 **Figure 5**: Expression changes of genes coding for TCA cycle enzymes in roots (A,
949 C and E) and leaves (B, D and F) of *V. corymbosum* cultivars Star, Camellia and
950 Cargo exposed to the Al treatment (200 µM Al) for 0, 24 and 48 h. A) and B) NAD-
951 MDH, NAD-dependent malate dehydrogenase, C) and D) NADP-MDH, NADP-
952 dependent malate dehydrogenase, and E) and F) citrate synthase. The values are
953 the averages of three independent biological replicates (± standard error).
954 Uppercase letters indicate significant differences ($P<0.05$) among the cultivars at a
955 given Al-exposure duration, and lowercase letters show significant differences
956 ($P<0.05$) among the Al-exposure durations for a given cultivar according to the
957 Tukey test.

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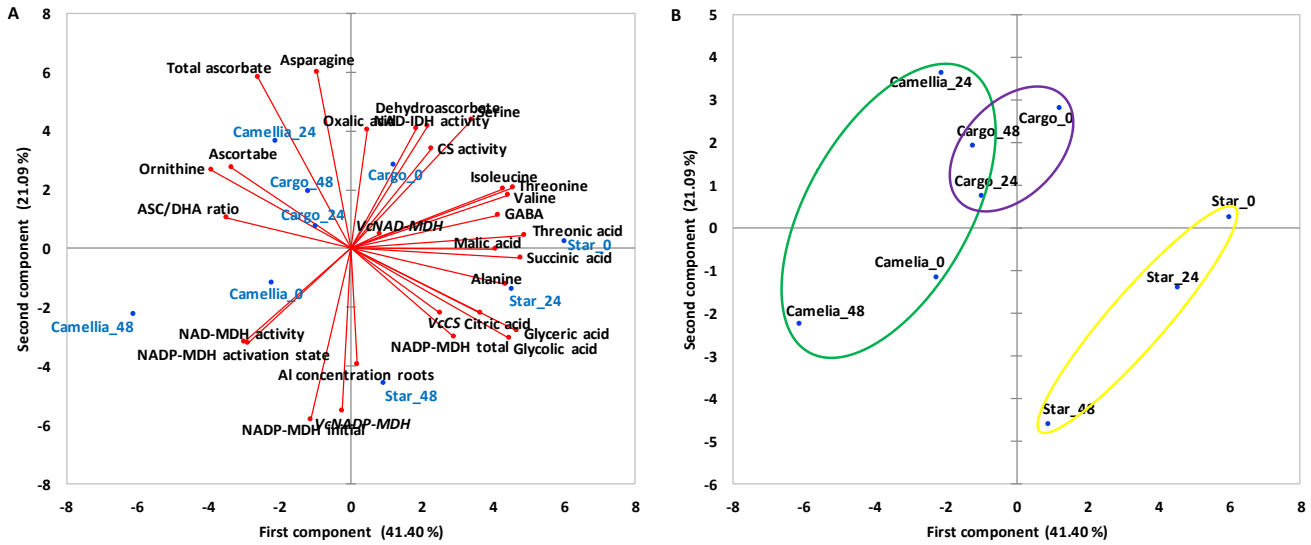

Figure 6. Principal component analysis (PCA) of enzyme activities and metabolomics data in roots of *V. corymbosum* cultivars exposed to the Al treatment (200 µM Al)**.** The PCA was performed based on the correlation matrix. Numbers in parentheses give the percent of the total variation explained by the PC1 and PC2. Figure **A)** show the biplot and **B)** scores plot obtained from resulting distribution for all cultivars. Color circles represent the clusters formed by each cultivar. Abbreviations: NAD-dependent malate dehydrogenase activity (NAD-MDH activity), NADP-dependent malate dehydrogenase activation state (NADP-MDH activation state), NADP-dependent malate dehydrogenase total activity (NADP-MDH total), NADP-dependent malate dehydrogenase initial activity (NADP-MDH initial), NAD-dependent isocitrate dehydrogenase activity (NAD-IDH activity), citrate synthase activity (CS activity), NAD-dependent malate dehydrogenase gene expression (*VcNAD-MDH*), NADP-dependent malate dehydrogenase gene expression (*VcNADP-MDH*), citrate synthase gene expression (*VcCS*) and γ-aminobutyric acid (GABA).

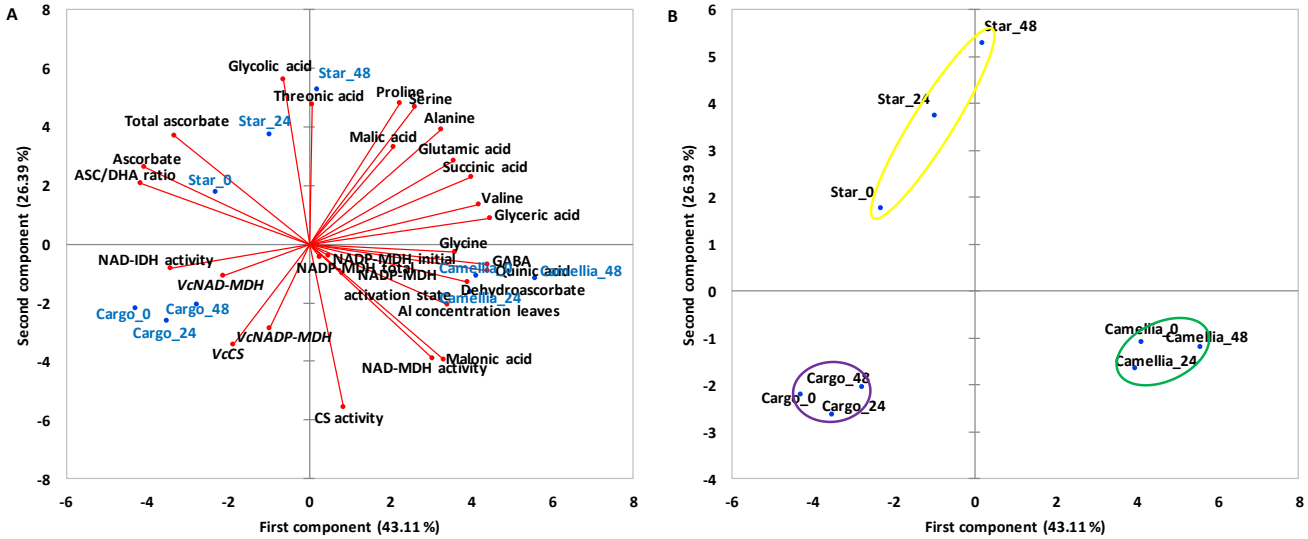

Figure 7. Principal component analysis of enzyme activities and metabolomics data in leaves of *V. corymbosum* cultivars exposed to the Al treatment (200 µM Al). The principal component analysis was performed based on the correlation matrix. Numbers in parentheses give the percent of the total variation explained by the PC1 and PC2. Figure **A)** show the biplot and **B)** scores obtained from resulting distribution for all cultivars. Color circles represent the clusters formed by each cultivar distance. For the list of abbreviations, see Figure 6.

Table 1. The sequences of primers used in this study. TCA cycle (forward [F] and reverse [R] primers). NAD-dependent malate dehydrogenase (NAD-MDH), NADP-dependent malate dehydrogenase (NADP-MDH), citrate synthase (CS), and housekeeping metallothionein (MET) genes.

Primers	Sequence (5'→3')	Reference
NAD-MDH-F	ACG ATC TGT TCA ACA TCA ATG C	This work
NAD-MDH-R	GCT CCC AGT ACT TTG ATT TTG G	
NADP-MDH-F	ACG ATC TGT TCA ACA TCA ATG C	This work
NADP-MDH-R	TCC CAG CAT AGA AGG TCT TAG C	
CS-F	GTA GAC ACG GTG CCC AAA TC	This work
CS-R	TCA TGG TGG AGC AAA TGA AG	
MET-F	ACC CTG ACA TGA GCT TCT CG	Naik et al., 2007
MET-R	ACC CAA ATC TCT GCT TGC TG	

Table 2. Ascorbate (ASC) and dehydroascorbate (DHA) levels and ASC/DHA ratio in roots and leaves of *V. corymbosum* cutivars Star, Camellia and Cargo exposed to the Al treatment (200 µM Al) for 0, 24 and 48 h. The values are the averages of three independent biological replicates (± standard error). Uppercase letters denote significant differences (P<0.05) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences (P<0.05) among the Al-exposure durations for a given cultivar according to the Tukey test.

Roots

Leaves

ASC (mol g^{-1}DW)

	0 h			24 h			48 h			0 h			24 h			48 h		
Star	278	± 43	Cb	554	± 165	Aa	367	± 25	Bab	9130	± 299	Aa	11039	± 224	Aa	9602	± 1204	Aa
Camellia	552	± 8	Bb	626	± 50	Ab	1189	± 141	Aa	3238	± 70	Ba	3206	± 270	Ca	3256	± 122	Ba
Cargo	1116	± 9	Aa	648	± 20	Ab	1233	± 70	Aa	8796	± 125	Aa	7964	± 396	Ba	9066	± 714	Aa

DHA (mol g^{-1}DW)

	0 h			24 h			48 h			0 h			24 h			48 h		
Star	511	± 2	Aa	419	± 28	Aab	384	± 26	Ab	3325	± 144	Ba	2143	± 252	Bb	2037	± 35	Bb
Camellia	501	± 4	Aa	542	± 55	Aa	331	± 56	Ab	4575	± 52	Aa	3941	± 167	Ab	4567	± 89	Aa
Cargo	499	± 4	Aa	427	± 35	Aa	383	± 20	Aa	2188	± 63	Ca	1961	± 85	Ba	1932	± 57	Ba

ASC /DHA ratio

	0 h			24 h			48 h			0 h			24 h			48 h		
Star	0.58	± 0.02	Cb	1.39	± 0.5	Aa	0.96	± 0.1	Bab	3.03	± 0.2	Bb	4.94	± 0.3	Aa	4.70	± 0.5	Aa
Camellia	1.11	± 0.04	Bb	1.17	± 0.1	Ab	4.35	± 1	Aa	0.71	± 0.0	Ca	0.81	± 0.0	Ba	0.71	± 0.0	Ba
Cargo	2.28	± 0.04	Aab	1.54	± 0.1	Ab	3.26	± 0.3	Aa	4.19	± 0.1	Aa	4.06	± 0.1	Aa	4.68	± 0.2	Aa

Supplementary Material

	Star									Camellia									Cargo								
Metabolites [DW (g)]	0 h			24 h			48 h			0 h			24 h			48 h			0 h			24 h			48 h		
Alanine	1,2002548	0,2792963	ABa	1,46961426	0,16039725	Aa	1,43092198	0,37139114	Aa	1,25143352	0,20619368	Aa	0,90001004	0,19449995	ABa	1,44082256	0,10351571	Aa	0,48368829	0,05020805	Ba	0,51666528	0,0610318	Aa	0,59450237	0,1796567	Ba
Valine	0,81036043	0,27677545	Ba	1,25775243	0,44426204	Aa	1,17252472	0,64547683	Aa	1,7995819	0,19397277	Aa	1,52725203	0,02295504	Aa	1,18588552	0,13468081	Aa	0,33525787	0,03028846	Ba	0,34898919	0,05948537	Ba	0,55398252	0,13411672	Aa
Proline	1,15214181	0,29453803	Aa	2,80908106	1,30176779	Aa	2,07091174	0,59060845	Aa	1,29916454	0,16850925	Aa	0,87802723	0,12311378	Aa	0,54124026	0,05917005	Ba	0,2061597	0,01557659	Ba	0,19265368	0,00454288	Ba	0,24386787	0,01373333	Ba
Serine	1,71691613	0,64075714	ABa	1,97494715	0,64699683	Aa	2,06223951	0,76818221	Aa	1,0081231	0,12308311	Aa	1,02165862	0,04997475	Aa	0,74896489	0,16645502	ABa	0,22736689	0,04221153	Ba	0,24204806	0,04816855	Ba	0,28430157	0,00740712	Ba
GABA	0,5766368	0,23541759	Ba	0,90284872	0,41350845	ABa	0,42334254	0,10813077	Ba	2,15023348	0,70523631	Aa	2,11796575	0,53973404	Aa	1,79426372	0,7381928	Aa	0,21923524	0,03122037	Ba	0,31417843	0,08466914	Ba	0,41406874	0,14239134	Ba
Glutamic acid	0,93132348	0,16349832	Aa	1,48337317	0,6854102	Aa	1,38522428	0,36639158	Aa	1,03249389	0,09341131	Aa	1,14111312	0,36634822	Aa	1,21701802	0,12528957	Aa	0,77611257	0,07826932	Aa	0,59285967	0,12029731	Aa	0,67765971	0,10447493	Aa
Glycolic acid	3,38110403	0,77693816	Aa	2,98765468	2,05174311	Aa	3,63070895	1,03037839	Aa	0,00932116	0,00219036	Ba	0,02001404	0,01162561	Ba	0,01006412	0,00158692	Ba	0,00584813	0,00098819	Ba	0,0085043	0,00102098	Ba	0,00676016	0,00109778	Ba
Glyceric acid	0,99470653	0,21632219	Aba	0,98611219	0,14334458	ABa	1,06131801	0,22002314	Aa	1,22449873	0,10657238	Aa	1,27675597	0,09017858	Aa	1,47851255	0,21994243	Aa	0,6012087	0,06307318	Ba	0,71484593	0,09933678	Ba	0,83986294	0,05032301	Aa
Malonic acid	0,19117105	0,04766829	Ba	0,1747099	0,04550324	Ba	0,20988252	0,04215016	Ba	2,28586996	0,39186106	Ba	1,93739407	0,28180532	Ba	2,29592653	0,41488265	Aa	0,58361441	0,08664849	Ba	0,52068037	0,0275418	Ba	0,47048613	0,03989773	Ba
Malic acid	0,90010507	0,08216389	Ca	1,24711389	0,17128404	Ca	1,28060015	0,27306433	Ca	1,02524575	0,27423036	Aa	1,08277277	0,05289553	Aa	1,12464325	0,15895148	Aa	0,67370163	0,08801371	Aa	0,99101916	0,13067717	Aa	1,04244757	0,28725897	Aa
Succinic acid	1,04823238	0,17272534	ABa	1,03001504	0,06671973	Aa	1,18177094	0,10440492	Aa	1,18096331	0,07815656	Aa	1,07461323	0,08364546	Aa	1,33462348	0,05107683	Aa	0,67601511	0,02378231	Ba	0,86526966	0,03186469	Aa	0,71936349	0,05970798	Ba
Threonic acid	1,03403329	0,12825094	Aa	1,17362998	0,0936196	Aa	1,16188024	0,08132988	Aa	1,03223125	0,12933331	Aa	0,95325739	0,11641402	Aa	0,90296893	0,04836716	Aa	0,86018544	0,05170556	Aa	0,94705829	0,04054704	Aa	1,01964963	0,13429803	Aa
Quinic acid	0,80258081	0,08050567	Ba	0,95082543	0,08385197	Aa	0,99261578	0,23403718	ABa	1,20675678	0,03662987	Aa	1,18868925	0,1029975	Aa	1,31207791	0,03744127	Aa	0,81032536	0,02767314	Ba	0,88303022	0,03604956	Aa	0,8993613	0,04700223	Ba
Phosphoric acid	0,67293977	0,18535903	Ba	0,90297954	0,11530716	Ba	0,77021504	0,19871873	Ba	1,71399376	0,31509356	Ba	1,62615981	0,21641179	Ba	1,94569625	0,34317468	Ba	0,46503287	0,05235885	Aa	0,52411587	0,06464401	Aa	0,41788062	0,01877438	Aa
Lyxose	0,27605852	0,03998001	Ca	0,40444383	0,08253103	Ca	0,33833202	0,04685927	Ca	0,99110768	0,19120597	Ba	0,8883335	0,0925238	Ba	0,90306297	0,0586223	Ba	1,76978017	0,17276531	Aa	1,75799884	0,19260268	Aab	1,39262228	0,12665572	Ab
Rhamnose	0,50451432	0,05438053	Ca	0,69636647	0,06508771	Ca	0,65632854	0,13324947	Ba	1,32619831	0,0874636	Aa	1,39806442	0,07385577	Aa	1,54714877	0,08270719	Aa	0,94735443	0,06580916	Ba	1,05903573	0,07010833	Ba	0,92176006	0,05253686	Ba
Sorbose	0,79111392	0,07180399	Ba	0,94620734	0,10158168	Aa	0,99051862	0,12516333	Aa	1,13077693	0,02909867	Aa	1,11834384	0,08706138	Aa	1,24309366	0,03906821	Aa	0,86339636	0,03325887	ABa	0,88416701	#¡DIV/0!	Aa	0,8909223	#¡DIV/0!	Aa
Glucose	0,94356683	0,09682592	Aa	1,13014295	0,17005932	Aa	1,12359499	0,12953006	Aa	1,06781624	0,05037958	Aa	1,06283446	0,0899957	Aa	1,18534733	0,03027889	Aa	0,82044349	0,03784774	Aa	0,87410937	0,02750943	Aa	0,94150673	0,02159149	Aa
Glucose, 2-amino-2-deoxy-,	2,31168163	0,44664744	Aa	1,97422375	0,19026385	Aa	1,88035442	0,18723487	Aa	1,29970996	0,73940015	Aa	0,40920869	0,26029083	Bab	0,21442975	0,01732295	Bb	0,14252019	0,02728188	Ba	0,14534825	0,00951272	Ba	0,3058388	0,13153266	Ba
Glucoheptose	1,06738083	0,10306178	Aa	1,62872968	0,41934102	Aa	1,18831203	0,16413485	Aa	0,87988819	0,11920139	Aa	1,37383412	0,22943046	Aba	1,03161805	0,10798436	Aa	0,65689969	0,0766167	Aa	0,79046591	0,14961625	Ba	0,80096305	0,1149768	Aa
Sucrose	0,3051785	0,02599533	Aa	0,39728715	0,07713458	Aa	0,34177866	0,04775871	Ba	0,53171519	0,09029438	Aa	0,57445996	0,14026273	Aa	0,29492698	0,00900711	Ba	1,52539858	0,27499443	Aa	2,07940072	0,48323106	Aa	3,35595483	1,38713504	Aa
Lactulose	0,68255779	0,07559826	Ba	0,80462581	0,0661149	Ba	0,64522395	0,03620359	Ba	0,69369497	0,1117898	Ba	0,58363668	0,05157434	Ba	0,74460438	0,12998042	Ba	1,54650493	0,12636346	Aa	1,6519817	0,12550788	Aa	1,61859801	0,04741426	Aa
Maltose	0,59128343	0,0615274	Ca	0,66534719	0,02936358	Ca	0,63246209	0,0394161	Ca	0,9575851	0,07241706	Ba	1,01305866	0,04966968	Ba	1,0166374	0,07979157	Ba	1,27225831	0,06177928	Aa	1,46531938	0,05678447	Aa	1,42868274	0,08557367	Aa
Turanose	0,67163787	0,0990323	Ba	1,0738182	0,25832205	ABa	1,05419341	0,26325032	Ba	1,33182925	0,17163943	Aa	1,5100866	0,11511228	Aa	1,68887803	0,15125547	Aa	0,58979678	0,04794053	Ba	0,69534587	0,06086939	Ba	0,68169603	0,08753258	Ba
Gentiobiose	0,54663893	0,04686811	Ba	0,698343	0,05693108	Ba	0,59826495	0,05338848	Ba	0,75284908	0,04309083	Ba	1,10086517	0,24667477	Ba	0,77711778	0,04356457	Ba	1,4216705	0,07363037	Aa	1,61553379	0,16885512	Aa	1,61643793	0,17386625	Aa
Raffinose	1,07020873	0,09587132	Aa	1,74294525	0,63719444	Aa	1,35273336	0,39074719	ABa	0,38721839	0,08914802	Aa	0,26850305	0,04238899	Ba	0,29681971	0,03227924	Aa	1,23893653	0,20049323	Aa	1,37253957	0,3216969	Aa	1,59713468	0,2748263	Ba
Melezitose	0,6966946	0,12632798	ABb	1,77999287	0,47794768	Aa	1,05051153	0,20495218	Ab	0,39546447	0,05348909	Ba	0,4770952	0,0616195	Ba	0,31978153	0,06881372	Ba	1,41516433	0,07754887	Aa	1,55344709	0,03533995	Aa	1,70342318	0,05506282	Aa
Gulonic acid, 2-oxo-	1,66942572	0,2180446	Aa	1,55653609	0,19724988	Aa	1,47718443	0,23338909	Aa	1,56387019	0,43201078	Aa	0,89123873	0,16433642	ABb	0,66219316	0,03913375	ABb	0,37745216	0,03848065	Ba	0,38780503	0,08187519	Ba	0,40260177	0,09088902	Ba
Galactonic acid	1,198182	0,19079329	Aa	1,00217203	0,03870719	Aa	1,02268283	0,13921074	ABa	1,12808549	0,04161918	Aa	1,09183981	0,03686095	Aa	1,14222303	0,03142625	Aa	0,78909945	0,0436523	Aa	0,80230849	0,09383172	Aa	0,77410059	0,02642909	Ba
Inositol, myo-	0,78159616	0,08240247	Ba	0,90701844	0,10689396	Ba	0,96711145	0,14226269	Ba	1,23548474	0,0406364	Aa	1,23845713	0,11099054	Aa	1,34693788	0,06389983	Aa	0,81554826	0,03889823	Ba	0,90785592	0,05541963	Ba	0,89455959	0,0573774	Ba
Gulonic acid-1,4-lactone	1,01987362	0,08961724	Aa	1,07775683	0,11512505	Ba	1,07457378	0,07758986	Aa	1,24376263	0,08089065	Aa	1,43856499	0,07500604	Aa	1,30416569	0,13924856	Aa	0,58889477	0,05304881	Ba	0,6981985	0,08355903	Ca	0,70830269	0,06738782	Ba
Glucosone, 3-deoxy-	1,55601321	0,1220668	Aa	1,45737733	0,30316356	Aa	1,53036385	0,19474579	Aa	0,92932934	0,08674032	Ba	0,85247438	0,06361836	Ba	0,89709374	0,05136619	Ba	0,58023609	0,03922458	Ba	0,61022499	0,0546255	Ba	0,70664618	0,01925442	Ba
Glycerol	1,58886771	0,26711628	Aa	1,33203217	0,1627321	Aa	1,28502219	0,17065776	Aa	0,99845638	0,18711225	ABa	0,76985414	0,17049517	Aa	1,24119686	0,28084287	Aa	0,59718369	0,11259211	Ba	0,61813894	0,13719006	Aa	0,58102938	0,14369358	Aa
Galactinol	1,54543123	0,10360352	Aa	1,72462603	0,45890836	Aa	1,43157016	0,21560146	Aa	0,92212924	0,10179905	ABa	0,79407598	0,15010507	Ba	0,92391653	0,13876546	Aa	0,5535256	0,06144513	Ba	0,48378056	0,04908487	Ba	0,78166899	0,04751737	Aa

Supplementary Data 1: Metabolites in leaves. Values used to graph the determinations made in leaves. The values are the averages of three independent biological replicates (± standard error). Uppercase letters denote significant differences (P<0.05) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences (P<0.05) among the Al-exposure durations for a given cultivar according to the Tukey test.

Metabolites [DW (g)]	0 h			24 h			48 h			0 h			24 h			48 h			0 h			24 h			48 h		
Alanine	1,69715317	0,26976318	Aa	1,66009256	0,38251944	Aa	1,39157959	0,10764071	Aa	0,36480347	0,05183031	Ba	0,4184184	0,02100621	Ba	0,41140159	0,03615071	Ba	1,03231157	0,18078234	Aa	0,79608591	0,21889924	Ba	1,13388552	0,35332225	Aa
Valine	1,60912974	0,27996164	Aa	1,32641803	0,1573754	Aa	0,89586003	0,26260423	Aa	0,54280555	0,07784967	Ba	0,70273749	0,06907094	Ba	0,47820509	0,07192648	Ba	1,06164621	0,19374841	Aa	0,97653111	0,1362486	ABa	1,19308528	0,11511601	Aa
Isoleucine	1,53793308	0,26814444	Aa	1,1386665	0,00632639	Aa	0,97996689	0,3464201	ABa	0,63397582	0,07590465	Ba	0,79205349	0,13492882	Aa	0,51471856	0,06325342	Ba	0,99644449	0,16347227	ABa	1,00828361	0,15008178	Aa	1,22960416	0,09445474	Aa
Serine	1,21056004	0,22722408	ABa	1,17283095	0,24759545	Aa	0,79840899	0,31752487	Aa	0,42434893	0,0829968	Ba	1,51137048	0,88508211	Aa	0,41476183	0,11173517	Aa	1,40810633	0,41852131	Aa	0,87690407	0,2047456	Aa	1,13969307	0,33305875	Aa
Threonine	1,5491849	0,26156809	Aa	1,57535437	0,36284586	Aa	0,8527049	0,16433536	Aa	0,50475925	0,07992792	Ba	0,90372819	0,24979274	Aa	0,44525533	0,09357122	Ba	1,09959164	0,237162	Aa	0,87685441	0,04289191	Aa	1,03903123	0,16896908	Aa
Ornithine	0,63801997	0,20699731	Aa	0,95727947	0,63248556	Aa	0,71019926	0,46648538	Aa	0,88636554	0,16974298	Aa	1,52714256	0,97728871	Aa	1,52588463	0,26418104	Aa	1,02828599	0,13973461	Aa	0,68121111	0,24046601	Aa	1,37227997	0,27966689	Aa
Asparagine	0,84403939	0,24752152	Aa	0,51548892	0,25582887	Aa	0,45572568	0,16174453	Aa	0,75012442	0,18960677	Aa	1,31133583	0,5392748	Aa	0,60627598	0,22050394	Aa	1,66395881	0,43698877	Aa	1,26591379	0,28321803	Aa	1,32901456	0,109616	Aa
GABA	1,32232629	0,26660854	ABa	1,5764577	0,34546091	Aa	0,79815534	0,04135474	ABa	0,58474488	0,06069912	Ba	0,56968149	0,11313443	Ba	0,5327638	0,1331092	Ba	1,31476215	0,2236177	Aa	0,82101415	0,17240317	ABa	1,25826088	0,3191901	Aa
Glycolic acid	1,77253903	0,10731344	Aa	2,15104057	0,0939361	Aa	1,2939142	0,0257983	Ab	0,65620872	0,05078393	Ba	0,46648362	0,0351911	Cb	0,63574899	0,05840326	Bab	0,85086519	0,10318353	Ba	0,61357302	0,09217673	Bb	0,66369391	0,00685475	Bab
Glyceric acid	2,09230763	0,21376271	Aa	2,15791312	0,41033222	Aa	1,25119469	0,06549861	Aa	0,5718461	0,05942648	Ba	0,33750584	0,08044278	Bb	0,36304199	0,04978364	Bab	0,83379456	0,16547506	Ba	0,45965236	0,10886083	Bb	0,43479542	0,06589186	Bb
Malic acid	3,23413676	0,70308444	Aa	1,25240719	0,81796233	Aab	0,31823907	0,08872373	Ab	0,46762685	0,09958472	Ba	0,21872751	0,07782826	ABa	0,20272749	0,11618507	Aa	1,17634831	0,20028715	ABa	0,13873654	0,01313627	Bb	0,11293835	0,05816178	Ab
Citric acid	3,54482246	1,20274218	Aa	0,73491763	0,28828812	Aab	0,49631711	0,117776	Ab	0,60135877	0,20352352	Ba	0,07928307	0,02488965	Bb	0,27563915	0,10617585	Abab	0,97924696	0,42698609	ABa	0,07926473	0,01398534	Bb	0,08372192	0,03475754	Bb
Succinic acid	1,72496964	0,35496876	Aa	1,52109877	0,1698548	Aa	0,79597988	0,12378274	Ab	0,81386552	0,07049167	Ba	0,67555664	0,07702154	Ba	0,53512268	0,07545776	Aa	1,05207142	0,22592415	ABa	0,70776285	0,06904121	Ba	0,58266602	0,0384168	Aa
Threonic acid	2,82080697	0,67112719	Aa	1,66371101	0,71656479	Aab	0,59260264	0,2787483	Ab	0,38120203	0,06557035	Ba	0,30534795	0,06099301	Ba	0,20223043	0,05196032	Aa	1,0840919	0,15644928	ABa	0,31748929	0,0328236	Ba	0,34641688	0,0904073	Aa
Phosphoric acid	1,97058559	0,43056595	Aa	0,76031424	0,59440506	Ab	0,1318347	0,01413589	Ab	1,4836441	0,26466857	ABa	0,27263407	0,05196747	Ab	0,32068358	0,12998727	Ab	1,5916117	0,42367827	Ba	0,21756866	0,01527315	Ab	0,20528198	0,06116327	Ab
Lyxose	0,24335179	0,02761705	Ba	0,32702445	0,0225904	Ba	0,28055573	0,01955879	Ba	1,50074841	0,11741524	ABa	1,80273895	0,45672528	Aba	1,41768127	0,43277501	Aa	0,9092654	0,26790029	Aa	1,32890052	0,15613616	Aa	1,53636787	0,54187676	Aa
Rhamnose	0,25231555	0,05452365	Ba	0,3147853	0,03825711	Ba	0,23258714	0,02927751	Ca	1,90654938	0,22958513	Ba	1,54019331	0,21184116	Aa	1,70751387	0,13285169	Ba	0,796943	0,14567135	Aa	1,2054351	0,06545403	Aa	1,08786943	0,26000907	Aa
Fucose	0,5709158	0,05487066	Ba	0,63105823	0,01127678	Ba	0,45161756	0,0355808	Ba	1,32841497	0,17414144	ABa	1,46292809	0,19730343	Aa	1,33866122	0,19439578	Aa	1,05975436	0,1093841	Aa	1,08701442	0,04269909	Aa	1,11055023	0,148812	Aa
Fructose	0,74334284	0,14487072	ABa	1,19987637	0,12069064	Aa	0,88483013	0,16449937	Aa	0,59421536	0,09585639	Aa	1,09417495	0,10546852	Aa	0,95554392	0,27300394	Aa	1,38816419	0,18858879	Ba	1,27919582	0,08536516	Aa	1,13493404	0,19254486	Aa
Sorbose	0,65898132	0,14633517	Aba	1,21453453	0,15267897	Aa	0,85466982	0,17020272	Aa	0,58276536	0,09547796	Aa	1,1250489	0,12089409	Aa	1,01728434	0,29506928	Aa	1,40903029	0,20371349	Ba	1,31284665	0,11715489	Aa	1,17406182	0,23141132	Aa
Glucose	0,24110475	0,08332541	Ba	0,52947852	0,04877164	Ba	0,51201587	0,09350765	Ba	0,86023894	0,09610445	ABa	1,41904069	0,11286822	Aa	1,30428046	0,42585277	Aa	1,37941643	0,29601079	Aa	1,74777278	0,19263516	Aa	1,52589144	0,33317707	Aa
Glucose, 2-amino-2-deoxy-	1,9059339	0,2243183	Aa	2,22734827	0,4172988	Aa	1,12200961	0,21375736	Ab	0,33577142	0,06756317	Ba	0,46221341	0,14679325	Ba	0,47420225	0,13742532	ABa	1,12204112	0,30682419	Ba	0,53175164	0,07615118	Ba	0,45498194	0,0500468	Ba
Sucrose	0,72016629	0,13780004	Aa	0,97493395	0,1789171	Aa	0,71130344	0,07380176	Aa	0,64023652	0,07615933	Aa	1,07369845	0,42639333	Aa	1,18278676	0,36917525	Aa	1,12182037	0,19818938	Aa	1,38679089	0,27657467	Aa	1,70604014	0,57336309	Aa
Trehalose, alpha,alpha'-	1,28839265	0,25035486	Aa	1,94277694	0,9131828	Aa	2,64491547	1,57030026	Aa	0,41426136	0,01289816	Aa	0,28618158	0,01162102	Aa	0,58116	0,07537084	Aa	0,9109643	0,19057534	Aa	0,59505652	0,07501448	Aa	0,72267286	0,02949382	Aa
Turanose	0,32506012	0,08030026	Ba	0,40305945	0,02033815	Ba	0,40026442	0,08460816	Ba	0,96647162	0,14443093	ABb	1,82150099	0,31870362	Aa	1,30096966	0,27375208	Aab	1,15640851	0,20137111	Ab	1,83712781	0,10895087	Aa	1,34119719	0,20589826	Aab
Inositol, myo-	0,67270146	0,11069077	Ba	0,79024436	0,23351317	Bab	0,43380746	0,05967678	Bb	0,49148619	0,07647867	Ba	0,61534258	0,07407135	Ba	0,58842209	0,06495133	Ba	1,89992509	0,31799921	Aa	1,85651395	0,20293729	Aa	1,58744408	0,33646849	Aa
Gluconic acid-1,5-lactone	3,41718459	0,58310006	Aa	1,26355108	0,49549499	Ab	0,4115208	0,1178515	Ac	0,34696273	0,04341088	Ba	0,23102896	0,02127381	Ba	0,2125682	0,03357806	Aa	0,85580822	0,23964514	Ba	0,34076164	0,02941372	Ba	0,30065823	0,03293098	Aa
Galactonic acid	3,59251751	0,59207695	Aa	1,30003705	0,55503869	Ab	0,35062217	0,11243609	Ac	0,29455359	0,04302693	Ba	0,13411332	0,03100982	Ba	0,13340467	0,01738125	Aa	0,91352542	0,25443102	Ba	0,26388173	0,05772248	Bab	0,21674804	0,02167665	Ab
Urea	1,33581142	0,28538269	Aa	1,18503186	0,39470077	Aa	0,94008381	0,09630679	Aa	0,54473461	0,06291821	Aa	1,15722399	0,09855278	Aa	0,904807	0,22278782	Aa	0,98667457	0,19249171	Aa	0,99245461	0,27184393	Aa	1,08595754	0,12699739	Aa
Glycerol	1,30013487	0,16703978	Aa	1,29056843	0,1645714	Aab	0,84512086	0,12808954	Ab	0,9509641	0,15827779	Aa	0,81090495	0,12017673	Ba	0,86554863	0,21418331	Aa	1,00453049	0,11414903	Aa	0,69098274	0,04032507	Ba	0,98561548	0,11184666	Aa

Supplementary Data 2: Metabolites in roots. Values used to graph the determinations made in leaves. The values are the averages of three independent biological replicates (± standard error). Uppercase letters denote significant differences (P<0.05) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences (P<0.05) among the Al-exposure durations for a given cultivar according to the Tukey test.

Supplementary Table 1: Data of the determinations evaluated in roots that show interaction between cultivars and length of Al exposure for each cultivar. The values are the averages of three independent biological replicates (± standard error). Uppercase letters denote significant differences (P<0.05) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences (P<0.05) among the Al-exposure durations for a given cultivar according to the Tukey test.

	Star			Camellia			Cargo			Cultivar:Time interacction
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	ANOVA p-valor (p≤0.05)
Ascorbate (mol g^{-1} FW)	278,3849237 ± 2,156596044 Cb	554,4345888 ± 164,6930575 Aa	367,0916555 ± 25,12102174 Bab	551,9971187 ± 1,04016423 Bb	626,0164677 ± 50,27572219 Ab	1189,419622 ± 141,0047299 Aa	1115,781737 ± 8,980236848 Aa	647,5124279 ± 20,28995251 Ab	1233,124879 ± 69,7985419 Aa	<0.001
ASC/DHA rate	0,579636932 ± 0,002152332 Cb	1,392592738 ± 0,514878078 Aa	0,958340707 ± 0,061077201 Bab	1,110844824 ± 0,012120433 Bb	1,169839904 ± 0,12334091 Ab	4,34728892 ± 0,96418347 Aa	2,275368311 ± 0,041808514 Aab	1,535427498 ± 0,124833479 Ab	3,257088223 ± 0,333515174 Aa	<0.001
Oxalix acid exudate (µmol g^{-1} h^{-1})	1,254660307 ± 0,032434849 Aa	0,44474102 ± 0,04131009 Cb	0,305073386 ± 0,01558323 Cc	0,282846774 ± 0,008277639 Bb	0,690549089 ± 0,036569768 Ba	0,784716958 ± 0,023936145 Ba	1,320170004 ± 0,097093855 Aa	0,855468545 ± 0,030365599 Ab	1,085375126 ± 0,055782332 Aa	<0.001
NAD-MDH (µmol g^{-1} FW min^{-1})	5,885147001 ± 0,797112034 Bb	4,074517915 ± 1,084829179 Bc	17,28654801 ± 0,232748481 Aa	10,43849631 ± 0,453160886 Ab	7,664408207 ± 0,237608141 Ab	16,7431795 ± 1,457131518 Aa	10,75731569 ± 0,270063324 Aa	7,246789963 ± 0,747898601 Aab	5,364090317 ± 0,413412128 Bb	<0.001
NADP-MDH total activity (µmol g^{-1} FW min^{-1})	500,3055195 ± 57,08277007 Ab	1895,139301 ± 67,67888518 Aa	1523,835171 ± 4,675685677 Aa	453,5331291 ± 88,95826518 Aa	417,0947074 ± 63,62844551 Ca	272,4769888 ± 24,22268829 Ca	472,5335456 ± 43,02377496 Ab	897,6905183 ± 95,50952957 Ba	498,5555802 ± 15,94590979 Bb	0,003
NADP-MDH inicial activity (µmol g^{-1} FW min^{-1})	132,2586462 ± 0,704903372 Ab	221,6368333 ± 69,01105978 Aab	449,2655888 ± 53,67302037 Aa	152,877378 ± 0,202369411 Ab	47,30653911 ± 18,71122893 Bc	546,0467005 ± 86,24542425 Aa	120,4207097 ± 50,79650614 Ab	254,8532428 ± 108,5024975 Aa	261,1141303 ± 5,645631386 Aa	0,006
CS (µmol g^{-1} FW min^{-1})	39319,01895 ± 912,5558196 Aa	36462,08483 ± 2837,368145 Aa	3989,415577 ± 1348,329846 Cb	17357,34702 ± 3108,765718 Ba	13237,72088 ± 1442,69159 Bab	10481,72281 ± 1231,708574 Bb	17932,80276 ± 1509,935873 Bb	9889,581688 ± 329,2894647 Bc	39070,17778 ± 968,2717494 Aa	<0.001
NAD-IDH (µmol g^{-1} FW min^{-1})	6,905367882 ± 0,256482139 Aa	1,06185382 ± 0,284809759 Bc	2,376077314 ± 0,122732245 Ab	3,89414516 ± 0,794743776 Aa	4,029361267 ± 0,458772467 Aa	0,730254753 ± 0,054837675 Bb	7,275849507 ± 0,575765821 Aa	2,853882118 ± 0,023334459 Ab	3,792372502 ± 0,261544789 Ab	<0.001
VcNADP-MDH (relative expression)	1,026049605 ± 0,019896591 Ab	0,762809293 ± 0,046004439 Ab	2,104080537 ± 0,557650261 Aa	1,020738447 ± 0,014691131 Ab	0,348010253 ± 0,006663695 Bc	1,900092251 ± 0,489467186 Aa	1,022453029 ± 0,110897499 Aa	0,5423224 ± 0,113937567 ABb	0,815776976 ± 0,152280987 Bab	0,007
VcCS (relative expression)	1,023645176 ± 0,074133921 Aa	0,59552547 ± 0,067008295 Aa	1,888150821 ± 0,86436 Aa	1,017832652 ± 0,026758385 Aa	0,056846577 ± 0,002092473 Ab	0,202005465 ± 0,088940906 Bb	1,025755208 ± 0,046200869 Aa	1,024891234 ± 0,257596383 Aa	0,976941143 ± 0,036027255 Aa	0,006
Glycolic acid [DW (g)]	1,772539025 ± 0,107313436 Aa	2,151040571 ± 0,093936098 Aa	1,293914204 ± 0,025798303 Ab	0,656208724 ± 0,050783929 Ba	0,466483624 ± 0,035191101 Cb	0,635748986 ± 0,058403259 Bab	0,85086519 ± 0,103183529 Ba	0,613573021 ± 0,092176731 Bb	0,663693906 ± 0,00685475 Bab	<0.001

Supplementary Table 2: Data of the determinations evaluated in leaves that show interaction between cultivars and length of Al exposure for each cultivar. The values are the averages of three independent biological replicates (± standard error). Uppercase letters denote significant differences (P<0.05) among the cultivars at a given Al-exposure duration, and lowercase letters show significant differences (P<0.05) among the Al-exposure durations for a given cultivar according to the Tukey test.

	Star			Camellia			Cargo			Cultivar:Time interacction
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	ANOVA p-valor (p≤0.05)
Dehydroascorbate (mol g^{-1} FW)	3324,7285 ± 223,769815 Ba	2142,97345 ± 251,494544 Bb	2036,69811 ± 34,6337815 Bb	4575,05275 ± 17,1015663 Aa	3940,77396 ± 166,858781 Ab	4567,3957 ± 89,3382564 Aa	2187,83059 ± 7,76181775 Ca	1961,24157 ± 84,9122365 Ba	1932,08929 ± 56,9454572 Ba	0,009
ASC/DHA rate	3,02992318 ± 0,23135893 Bb	4,94012509 ± 0,30109901 Aa	4,697224 ± 0,51138911 Aa	0,71122151 ± 0,02591653 Ca	0,81086115 ± 0,03328426 Ca	0,71229043 ± 0,01289227 Ba	4,19330612 ± 0,00922755 Aa	4,06170757 ± 0,11761845 Aa	4,68054349 ± 0,23806556 Aa	0,014
NAD-MDH (µmol g^{-1} FW min^{-1})	20,5182548 ± 0,61158541 Ca	24,4970229 ± 0,19240519 Ba	22,1531008 ± 0,95950997 Ba	115,88953 ± 0,81609382 Aa	90,0783836 ± 3,61452523 Ab	102,299645 ± 1,83381563 Aab	51,2849468 ± 1,309914 Bb	33,1064158 ± 8,95707684 Bc	67,4569296 ± 9,83517223 Aa	0,013
NADP-MDH total activity (µmol g^{-1} FW min^{-1})	591,490968 ± 2,41890951 Aa	121,58792 ± 3,24744669 Cb	814,695989 ± 99,9058502 Aa	196,253171 ± 8,23120396 Bb	1765,24864 ± 89,4953081 Aa	289,823875 ± 4,63831296 Bb	438,195386 ± 8,97030179 Aa	587,521831 ± 69,5515699 Ba	248,0083 ± 57,5034813 Bb	<0.001
NADP-MDH activation state (%)	56,2395664 ± 0,95926975 Aa	39,0322073 ± 13,1341061 Ba	32,912168 ± 9,01350098 Aa	56,063837 ± 0,85549756 Aa	30,7250232 ± 5,86218057 Ba	65,8866753 ± 18,4842575 Aa	13,9529243 ± 1,33565794 Bb	95,6112617 ± 1,41390425 Aa	58,0233421 ± 23,1075081 Aa	0,002
CS (µmol g^{-1} FW min^{-1})	43377,0748 ± 537,992636 Ca	25131,8617 ± 4875,39084 Bb	12635,3875 ± 1154,97008 Bb	87716,2839 ± 704,034257 Aa	67531,4466 ± 4338,5049 Ab	70856,5873 ± 4308,45696 Ab	64863,8146 ± 1904,42125 Ba	66994,0774 ± 2238,26775 Aa	64782,6851 ± 1660,26552 Aa	<0.001
NAD-IDH (µmol g^{-1} FW min^{-1})	21,0552512 ± 1,53667257 Ba	37,9146667 ± 4,58344456 Aa	54,2980312 ± 9,76666536 Ba	14,146512 ± 3,15845595 Ba	43,153251 ± 16,1962961 Aa	15,2537234 ± 7,69077994 Ca	122,81715 ± 2,66516215 Aab	52,084416 ± 12,0916717 Ab	209,541926 ± 25,0040289 Aa	0,005
VcCS (relative expression)	1,02194472 ± 0,09303102 Aab	1,28117138 ± 0,25001488 Ba	0,66499185 ± 0,02736828 Bb	1,19769664 ± 0,02944411 Aa	1,21943299 ± 0 Ba	0,83875554 ± 0,1047147 Ba	0,99785587 ± 0,06017794 Ab	2,74357784 ± 0,92915684 Aa	1,91863395 ± 0,17010355 Aa	0,009

Supplementary Figure 1: Pearson correlations matrix of root parameters of *V. corymbosum* genotypes exposed to the Al treatment (200 µM Al) for 0, 24 and 48 h; a) genotype Star (Al-sensitive) and b) genotypes Camellia and Cargo (Al-resistant). Values in bold are significant at α=0.05. Positive and negative correlations are distinguished by green and red, respectively. Abbreviations: NAD-dependent malate dehydrogenase activity (NAD-MDH activity), NADP-dependent malate dehydrogenase activation state (NADP-MDH activation state), NADP-dependent malate dehydrogenase total activity (NADP-MDH total), NADP-dependent malate dehydrogenase initial activity (NADP-MDH initial), NAD-dependent isocitrate dehydrogenase activity (NAD-IDH activity), citrate synthase activity (CS activity), NAD-dependent malate dehydrogenase expression (*VcNAD-MDH*), NADP-dependent malate dehydrogenase expression (*VcNADP-MDH*), citrate synthase expression (*VcCS*) and γ-aminobutyric acid (GABA).

A)

	Oxalic acid	Dehydroascorbate	Ascorbate	Total ascorbate	ASC/DHA ratio	Glycolic acid	Alanine	Valine	Isoleucine	Glyceric acid	Serine	Succinic acid	Threonine	Malic acid	GABA	Threonic acid	Ornithine	Asparagine	Citric acid	*VcNAD-MDH*	*VcNADP-MDH*	*VcCS*	NAD-MDH activity	NADP-MDH activation state	NADP-MDH total	NADP-MDH inicial	NAD-IDH activity
Al concentration roots	-0,98																										
Oxalic acid	-0,81	0,86																									
Dehydroascorbate	0,55	-0,52	-0,61																								
Ascorbate	-0,03	0,08	0,04	0,74																							
Total ascorbate	0,65	-0,64	-0,77	0,97	0,59																						
ASC/DHA ratio	-0,24	0,37	0,28	0,38	0,66	0,24																					
Glycolic acid	-0,13	0,21	0,03	0,44	0,63	0,35	0,27																				
Alanine	-0,50	0,49	0,28	0,13	0,53	0,04	0,57	0,29																			
Valine	-0,51	0,50	0,32	-0,05	0,39	-0,12	0,35	0,35	0,92																		
Isoleucine	-0,42	0,43	0,30	0,27	0,54	0,16	0,78	0,08	0,68	0,36																	
Glyceric acid	-0,26	0,24	0,06	0,14	0,36	0,12	0,50	0,07	0,92	0,82	0,64																
Serine	-0,41	0,47	0,43	0,03	0,44	-0,07	0,70	0,25	0,80	0,69	0,73	0,81															
Succinic acid	-0,38	0,38	0,26	0,16	0,48	0,07	0,71	0,00	0,91	0,71	0,85	0,92	0,84														
Threonine	-0,72	0,69	0,54	-0,25	0,16	-0,32	0,49	-0,09	0,77	0,60	0,82	0,74	0,77	0,84													
Malic acid	-0,15	0,17	0,07	0,45	0,62	0,36	0,75	0,14	0,73	0,44	0,93	0,77	0,79	0,89	0,70												
GABA	-0,57	0,55	0,32	-0,05	0,27	-0,11	0,58	0,04	0,90	0,74	0,81	0,90	0,83	0,93	0,93	0,79											
Threonic acid	0,17	-0,19	-0,21	0,16	-0,11	0,20	0,20	-0,41	0,06	-0,26	0,57	0,29	0,27	0,37	0,42	0,60	0,38										
Ornithine	-0,25	0,33	0,20	-0,18	0,03	-0,21	-0,01	0,63	0,36	0,58	-0,15	0,28	0,40	0,06	0,08	-0,04	0,23	-0,38									
Asparagine	-0,65	0,63	0,52	-0,35	0,02	-0,37	0,32	0,04	0,60	0,49	0,66	0,62	0,79	0,64	0,90	0,58	0,81	0,44	0,31								
Citric acid	0,22	-0,31	-0,20	0,21	0,30	0,23	-0,35	0,21	0,22	0,43	-0,31	0,21	-0,03	0,02	-0,21	-0,10	-0,11	-0,46	0,19	-0,22							
VcNAD-MDH	0,28	-0,43	-0,38	-0,08	-0,26	0,05	-0,84	-0,05	-0,23	0,00	-0,63	-0,17	-0,46	-0,43	-0,40	-0,50	-0,40	-0,31	0,07	-0,27	0,77						
VcNADP-MDH	-0,03	-0,11	-0,06	-0,07	0,08	-0,03	-0,54	0,11	0,14	0,37	-0,39	0,07	-0,19	-0,12	-0,14	-0,30	-0,14	-0,53	0,14	-0,14	0,92	0,86					
VcCS	0,36	-0,52	-0,54	0,00	-0,40	0,16	-0,87	-0,13	-0,45	-0,37	-0,49	-0,35	-0,61	-0,55	-0,40	-0,43	-0,44	0,11	-0,13	-0,23	0,35	0,82	0,49				
NAD-MDH activity	0,24	-0,21	-0,02	0,04	0,18	0,03	-0,22	0,37	0,15	0,42	-0,39	0,19	0,22	-0,05	-0,25	-0,13	-0,13	-0,47	0,60	-0,06	0,77	0,51	0,60	0,09			
NADP-MDH activation state	0,95	-0,89	-0,77	0,66	0,14	0,71	0,01	-0,08	-0,38	-0,43	-0,29	-0,18	-0,33	-0,24	-0,68	-0,04	-0,48	0,11	-0,29	-0,71	0,13	0,05	-0,16	0,11	0,14		
NADP-MDH total	0,75	-0,75	-0,54	0,11	-0,28	0,24	-0,59	-0,01	-0,67	-0,46	-0,78	-0,48	-0,49	-0,71	-0,81	-0,58	-0,76	-0,23	0,05	-0,55	0,38	0,61	0,30	0,52	0,53	0,58	
NADP-MDH inicial	-0,79	0,67	0,50	-0,52	-0,16	-0,53	-0,30	0,11	0,22	0,28	0,11	0,00	0,01	0,01	0,45	-0,13	0,26	-0,15	0,17	0,48	0,04	0,25	0,38	0,27	-0,17	-0,90	-0,43
NAD-IDH activity	-0,62	0,71	0,59	0,03	0,57	-0,13	0,85	0,30	0,75	0,66	0,65	0,56	0,71	0,73	0,63	0,56	0,68	-0,16	0,22	0,41	-0,15	-0,66	-0,22	-0,89	-0,07	-0,38	-0,74	0,08
CS Activity																											

B)

	Oxalic acid	Dehydroascorbate	Ascorbate	Total ascorbate	ASC/DHA ratio	Glycolic acid	Alanine	Valine	Isoleucine	Glyceric acid	Serine	Succinic acid	Threonine	Malic acid	GABA	Threonic acid	Ornithine	Asparagine	Citric acid	*VcNAD-MDH*	*VcNADP-MDH*	*VcCS*	NAD-MDH activity	NADP-MDH activation state	NADP-MDH total	NADP-MDH inicial	NAD-IDH activity
Al concentration roots	0,86																										
Oxalic acid	-0,59	-0,41																									
Dehydroascorbate	0,82	0,67	-0,78																								
Ascorbate	0,16	0,50	0,32	0,01																							
Total ascorbate	0,82	0,60	-0,91	0,94	-0,20																						
ASC/DHA ratio	-0,22	-0,55	-0,19	0,11	-0,56	0,18																					
Glycolic acid	0,32	0,51	0,12	0,23	0,30	0,03	-0,72																				
Alanine	-0,30	0,16	0,55	-0,38	0,58	-0,57	-0,70	0,60																			
Valine	-0,41	0,01	0,58	-0,54	0,38	-0,66	-0,65	0,50	0,95																		
Isoleucine	-0,45	-0,69	0,05	-0,33	-0,38	-0,18	0,52	-0,56	-0,53	-0,48																	
Glyceric acid	-0,10	0,26	0,43	-0,34	0,38	-0,44	-0,73	0,55	0,87	0,93	-0,72																
Serine	-0,65	-0,65	0,66	-0,73	0,09	-0,73	-0,01	-0,11	0,18	0,22	0,66	-0,06															
Succinic acid	-0,19	0,22	0,53	-0,39	0,52	-0,53	-0,71	0,53	0,94	0,96	-0,68	0,98	0,04														
Threonine	-0,55	-0,56	0,59	-0,32	0,07	-0,51	0,25	0,13	0,19	0,11	0,45	-0,19	0,70	-0,05													
Malic acid	-0,20	-0,18	0,64	-0,12	0,20	-0,40	-0,01	0,50	0,38	0,28	-0,08	0,14	0,35	0,25	0,81												
GABA	-0,56	-0,51	0,87	-0,61	0,18	-0,76	0,01	0,14	0,32	0,29	0,37	0,05	0,79	0,17	0,87	0,79											
Threonic acid	0,37	0,33	-0,05	0,52	0,13	0,31	0,01	0,48	-0,07	-0,28	-0,16	-0,26	-0,28	-0,21	0,33	0,58	0,20										
Ornithine	-0,04	0,07	0,52	-0,09	0,59	-0,30	-0,16	0,28	0,24	0,03	0,19	-0,13	0,45	0,03	0,66	0,66	0,73	0,57									
Asparagine	-0,35	-0,46	-0,28	0,15	-0,45	0,12	0,41	-0,02	-0,24	-0,28	0,53	-0,52	0,19	-0,48	0,50	0,16	0,10	0,28	0,01								
Citric acid	-0,18	0,03	-0,07	-0,35	0,23	-0,19	-0,46	-0,10	0,26	0,32	0,17	0,25	0,34	0,25	-0,29	-0,58	-0,20	-0,73	-0,21	-0,14							
VcNAD-MDH	0,34	-0,05	-0,46	0,56	-0,55	0,59	0,58	-0,02	-0,58	-0,57	0,20	-0,54	-0,17	-0,59	0,16	0,14	-0,15	0,23	-0,17	0,41	-0,42						
VcNADP-MDH	-0,56	-0,84	-0,11	-0,29	-0,79	-0,11	0,75	-0,67	-0,58	-0,41	0,74	-0,57	0,28	-0,60	0,22	-0,20	0,06	-0,29	-0,37	0,59	-0,06	0,32					
VcCS	0,61	0,22	-0,62	0,73	-0,49	0,79	0,47	0,00	-0,68	-0,70	0,09	-0,57	-0,39	-0,66	-0,10	-0,03	-0,34	0,33	-0,17	0,25	-0,43	0,93	0,16				
NAD-MDH activity	0,26	-0,20	-0,58	0,50	-0,72	0,62	0,76	-0,35	-0,86	-0,83	0,43	-0,79	-0,21	-0,86	0,02	-0,12	-0,26	0,19	-0,27	0,49	-0,42	0,88	0,60	0,88			
NADP-MDH activation state	-0,35	-0,16	0,27	-0,36	0,41	-0,34	0,24	-0,65	0,03	-0,01	0,17	-0,10	0,13	0,02	-0,01	-0,22	0,12	-0,26	0,22	-0,31	0,16	-0,53	0,06	-0,50	-0,32		
NADP-MDH total	0,47	0,08	-0,64	0,71	-0,52	0,76	0,61	-0,13	-0,78	-0,84	0,28	-0,78	-0,36	-0,84	0,02	-0,02	-0,28	0,49	-0,05	0,46	-0,52	0,85	0,33	0,92	0,93	-0,34	
NADP-MDH inicial	-0,82	-0,54	0,80	-0,95	0,25	-0,96	-0,26	-0,13	0,57	0,65	0,20	0,45	0,68	0,53	0,34	0,16	0,60	-0,45	0,20	-0,16	0,40	-0,73	0,10	-0,90	-0,70	0,45	-0,85
NAD-IDH activity	-0,86	-0,72	0,65	-0,62	-0,11	-0,75	0,24	-0,06	0,43	0,50	0,11	0,26	0,42	0,35	0,64	0,54	0,61	-0,07	0,10	0,30	-0,23	-0,19	0,36	-0,48	-0,24	0,19	-0,39	0,64
CS Activity																											

C)

	Oxalic acid	Dehydroascorbate	Ascorbate	Total ascorbate	ASC/DHA ratio	Glycolic acid	Alanine	Valine	Isoleucine	Glyceric acid	Serine	Succinic acid	Threonine	Malic acid	GABA	Threonic acid	Ornithine	Asparagine	Citric acid	*VcNAD-MDH*	*VcNADP-MDH*	*VcCS*	NAD-MDH activity	NADP-MDH activation state	NADP-MDH total	NADP-MDH inicial	NAD-IDH activity
Al concentration roots	0,93																										
Oxalic acid	-0,81	-0,89																									
Dehydroascorbate	-0,03	0,13	-0,06																								
Ascorbate	-0,25	-0,11	0,14	0,74																							
Total ascorbate	0,29	0,46	-0,45	0,91	0,63																						
ASC/DHA ratio	-0,62	-0,48	0,23	0,40	0,37	0,29																					
Glycolic acid	-0,02	-0,03	0,25	0,47	0,64	0,34	-0,19																				
Alanine	0,23	0,13	0,04	0,40	0,67	0,38	-0,24	0,83																			
Valine	0,41	0,27	-0,09	0,31	0,48	0,34	-0,39	0,65	0,94																		
Isoleucine	-0,75	-0,66	0,49	0,18	-0,03	-0,05	0,79	-0,33	-0,51	-0,56																	
Glyceric acid	-0,23	-0,26	0,36	0,42	0,73	0,26	0,04	0,92	0,85	0,66	-0,15																
Serine	-0,85	-0,85	0,63	0,00	0,06	-0,25	0,57	-0,19	-0,28	-0,33	0,82	0,08															
Succinic acid	-0,08	-0,12	0,06	0,37	0,73	0,36	0,16	0,70	0,84	0,70	-0,17	0,89	0,08														
Threonine	-0,93	-0,78	0,70	0,26	0,45	-0,04	0,60	0,29	-0,05	-0,30	0,65	0,45	0,73	0,28													
Malic acid	-0,16	-0,12	0,23	0,60	0,88	0,47	0,05	0,87	0,87	0,71	-0,19	0,93	0,04	0,86	0,40												
GABA	-0,87	-0,71	0,68	0,41	0,59	0,10	0,58	0,42	0,12	-0,13	0,59	0,56	0,65	0,38	0,97	0,55											
Threonic acid	0,11	0,14	0,12	0,71	0,62	0,59	-0,18	0,82	0,76	0,72	-0,19	0,71	-0,12	0,54	0,14	0,81	0,32										
Ornithine	-0,29	-0,33	0,59	0,34	0,65	0,09	-0,13	0,88	0,76	0,60	-0,19	0,87	0,03	0,60	0,42	0,83	0,55	0,72									
Asparagine	-0,90	-0,78	0,76	0,32	0,35	-0,02	0,61	0,27	-0,11	-0,32	0,75	0,37	0,72	0,14	0,96	0,31	0,95	0,20	0,42								
Citric acid	0,15	0,21	-0,08	0,77	0,25	0,70	0,18	0,15	0,17	0,28	0,28	0,07	0,05	0,02	-0,05	0,23	0,09	0,64	0,07	0,14							
VcNAD-MDH	-0,41	-0,37	0,45	0,52	0,15	0,27	0,44	-0,04	-0,09	-0,03	0,71	0,01	0,54	-0,11	0,35	0,09	0,43	0,37	0,13	0,55	0,79						
VcNADP-MDH	-0,06	-0,04	-0,14	0,11	0,17	0,17	0,36	-0,08	-0,16	-0,33	-0,04	-0,08	-0,15	-0,03	0,07	-0,12	-0,02	-0,39	-0,15	-0,02	-0,32	-0,37					
VcCS	-0,92	-0,77	0,64	-0,06	0,07	-0,32	0,61	-0,12	-0,42	-0,58	0,80	0,05	0,78	-0,06	0,87	-0,01	0,80	-0,22	0,07	0,86	-0,16	0,35	-0,08				
NAD-MDH activity	0,86	0,77	-0,48	0,16	0,06	0,34	-0,70	0,36	0,57	0,72	-0,81	0,13	-0,83	0,12	-0,75	0,22	-0,61	0,49	0,21	-0,70	0,28	-0,22	-0,23	-0,89			
NADP-MDH activation state	0,28	0,18	-0,05	-0,84	-0,56	-0,73	-0,66	-0,22	-0,17	-0,09	-0,52	-0,31	-0,41	-0,36	-0,41	-0,41	-0,47	-0,44	-0,11	-0,46	-0,69	-0,61	-0,20	-0,16	0,23		
NADP-MDH total	0,74	0,69	-0,35	-0,12	-0,13	0,02	-0,83	0,23	0,34	0,47	-0,83	-0,05	-0,83	-0,16	-0,69	0,03	-0,58	0,29	0,18	-0,65	0,02	-0,34	-0,28	-0,71	0,89	0,57	
NADP-MDH inicial	-0,33	-0,30	0,29	0,41	0,77	0,31	0,56	0,44	0,57	0,40	0,14	0,65	0,12	0,72	0,40	0,63	0,51	0,28	0,53	0,34	0,04	0,13	0,17	0,20	-0,09	-0,36	-0,27
NAD-IDH activity	0,42	0,55	-0,42	0,87	0,48	0,94	0,14	0,31	0,37	0,40	-0,10	0,16	-0,38	0,20	-0,22	0,36	-0,06	0,63	0,09	-0,12	0,82	0,38	0,02	-0,44	0,52	-0,62	0,22	0,22
CS Activity																											

Negative		Positive	
-0,75	-1,00	0,75	1,00
-0,50	-0,74	0,50	0,74
-0,25	-0,49	0,25	0,49
0,00	-0,24	0,00	0,24

Supplementary Figure 2: Pearson correlations matrix of leaves parameters of *V. corymbosum* genotypes exposed to the Al treatment (200 µM Al) for 0, 24 and 48 h; a) genotype Star (Al-sensitive) and b) genotypes Camellia and Cargo (Al-resistant). Values in bold are significant at α=0.05. Positive and negative correlations are distinguished by green and red, respectively. Abbreviations: NAD-dependent malate dehydrogenase activity (NAD-MDH activity), NADP-dependent malate dehydrogenase activation state (NADP-MDH activation state), NADP-dependent malate dehydrogenase total activity (NADP-MDH total), NADP-dependent malate dehydrogenase initial activity (NADP-MDH initial), NAD-dependent isocitrate dehydrogenase activity (NAD-IDH activity), citrate synthase activity (CS activity), NAD-dependent malate dehydrogenase expression (*VcNAD-MDH*), NADP-dependent malate dehydrogenase expression (*VcNADP-MDH*), citrate synthase expression (*VcCS*) and γ-aminobutyric acid (GABA).

A)

	Dehydroascorbate	Ascorbate	Total ascorbate	ASC/DHA ratio	Glycolic acid	Alanine	Valine	Proline	Glyceric acid	Serine	Succinic acid	Malonic acid	Malic acid	GABA	Threonic acid	Glutamic acid	Quinic acid	Glycine	*VcNAD-MDH*	*VcNADP-MDH*	*VcCS*	NAD-MDH activity	NADP-MDH activation state	NADP-MDH total	NADP-MDH initial	NAD-IDH activity
Dehydroascorbate	**-0,88**																									
Ascorbate	0,26	-0,30																								
Total ascorbate	-0,18	0,32	**0,69**																							
ASC/DHA ratio	**0,80**	**-0,87**	**0,70**	0,15																						
Glycolic acid	0,17	-0,06	0,01	0,33	0,16																					
Alanine	0,37	-0,34	0,08	-0,14	0,32	-0,33																				
Valine	0,28	-0,40	0,15	-0,30	0,33	**-0,72**	**0,83**																			
Proline	0,38	**-0,69**	0,21	-0,29	0,58	-0,30	0,60	**0,76**																		
Glyceric acid	0,15	-0,27	-0,08	-0,32	0,18	-0,42	**0,88**	**0,86**	**0,72**																	
Serine	0,20	-0,39	0,09	-0,26	0,34	-0,40	**0,83**	**0,87**	**0,84**	**0,97**																
Succinic acid	0,29	-0,35	-0,13	-0,41	0,21	-0,42	**0,87**	**0,85**	**0,71**	**0,95**	**0,93**															
Malonic acid	0,04	-0,28	0,01	-0,25	0,24	-0,21	0,60	0,63	**0,75**	**0,85**	**0,91**	**0,84**														
Malic acid	0,54	-0,49	0,27	-0,26	0,45	**-0,69**	0,58	**0,82**	0,50	0,52	0,53	0,58	0,26													
GABA	0,24	-0,48	0,27	-0,12	0,43	-0,46	**0,72**	**0,81**	**0,86**	**0,67**	**0,76**	**0,67**	0,57	0,48												
Threonic acid	0,41	-0,43	-0,10	-0,47	0,23	-0,54	**0,85**	**0,82**	0,58	**0,72**	**0,69**	**0,83**	0,48	0,66	**0,73**											
Glutamic acid	0,13	-0,46	-0,09	-0,39	0,29	-0,19	0,40	0,58	**0,90**	**0,69**	**0,77**	0,66	**0,81**	0,24	0,65	0,36										
Quinic acid	0,40	-0,48	0,26	-0,01	0,52	-0,21	**0,89**	**0,81**	**0,82**	**0,87**	**0,91**	**0,85**	**0,75**	0,53	**0,80**	**0,66**	**0,67**									
Glycine	0,13	0,05	0,38	0,27	0,15	-0,43	**0,71**	0,57	0,07	0,46	0,42	0,48	0,22	0,53	0,37	0,60	-0,22	0,48								
VcNAD-MDH	-0,06	0,12	0,23	0,37	0,08	0,36	0,13	-0,20	-0,26	-0,06	-0,12	-0,28	-0,26	-0,29	-0,11	-0,21	-0,36	-0,01	0,18							
VcNADP-MDH	-0,17	0,03	0,58	0,50	0,22	0,00	-0,03	-0,05	-0,02	-0,22	-0,16	-0,41	-0,32	-0,15	0,24	-0,18	-0,24	-0,04	0,16	**0,70**						
VcCS	-0,31	0,14	0,47	0,32	0,00	-0,45	-0,15	0,08	-0,02	-0,30	-0,21	-0,35	-0,34	0,07	0,32	-0,01	-0,23	-0,19	0,19	0,12	**0,76**					
NAD-MDH activity	0,48	-0,61	**0,78**	0,19	**0,82**	-0,14	0,47	0,49	0,51	0,33	0,47	0,28	0,33	0,48	0,52	0,35	0,17	0,51	0,49	0,32	0,48	0,27				
NADP-MDH activation state	-0,43	0,41	-0,48	-0,42	-0,66	-0,56	-0,41	-0,08	-0,34	-0,33	-0,41	-0,28	-0,44	0,03	-0,19	-0,01	-0,25	-0,59	-0,21	-0,37	-0,09	0,40	-0,55			
NADP-MDH total	-0,11	0,26	-0,46	-0,11	-0,35	0,21	-0,28	-0,26	-0,17	-0,05	-0,10	0,08	0,14	-0,17	-0,44	-0,26	0,14	-0,14	-0,35	-0,63	**-0,86**	-0,66	**-0,67**	0,06		
NADP-MDH initial	**-0,75**	**0,90**	-0,50	0,05	**-0,94**	-0,17	-0,49	-0,43	**-0,73**	-0,42	-0,56	-0,43	-0,48	-0,39	-0,55	-0,38	-0,50	-0,67	-0,13	-0,12	-0,14	0,15	**-0,79**	**0,72**	0,35	
NAD-IDH activity	**0,90**	**-0,90**	-0,06	-0,57	0,63	0,07	0,28	0,30	0,47	0,21	0,25	0,34	0,14	0,49	0,24	0,45	0,33	0,31	-0,14	-0,22	-0,32	-0,38	0,30	-0,20	-0,03	**-0,68**
CS activity	**-0,86**	0,66	0,05	0,34	-0,54	-0,28	-0,39	-0,19	-0,21	-0,28	-0,25	-0,41	-0,20	-0,39	0,02	-0,38	-0,09	-0,35	-0,13	0,05	0,49	**0,70**	-0,27	0,48	-0,17	0,59
	AI concentration leaves	**Dehydroascorbate**	**Ascorbate**	**Total ascorbate**	**ASC/DHA ratio**	**Glycolic acid**	**Alanine**	**Valine**	**Proline**	**Glyceric acid**	**Serine**	**Succinic acid**	**Malonic acid**	**Malic acid**	**GABA**	**Threonic acid**	**Glutamic acid**	**Quinic acid**	**Glycine**	***VcNAD-MDH***	***VcNADP-MDH***	***VcCS***	**NAD-MDH activity**	**NADP-MDH activation state**	**NADP-MDH total**	**NADP-MDH initial**	**NAD-IDH activity**

B)

	Dehydroascorbate	Ascorbate	Total ascorbate	ASC/DHA ratio	Glycolic acid	Alanine	Valine	Proline	Glyceric acid	Serine	Succinic acid	Malonic acid	Malic acid	GABA	Threonic acid	Glutamic acid	Quinic acid	Glycine	*VcNAD-MDH*	*VcNADP-MDH*	*VcCS*	NAD-MDH activity	NADP-MDH activation state	NADP-MDH total	NADP-MDH initial	NAD-IDH activity
Dehydroascorbate	-0,28																									
Ascorbate	0,00	0,57																								
Total ascorbate	-0,11	**0,88**	**0,73**																							
ASC/DHA ratio	0,29	-0,52	0,41	-0,22																						
Glycolic acid	0,23	-0,01	**0,70**	0,14	**0,74**																					
Alanine	0,06	**0,72**	0,37	**0,70**	-0,42	0,20																				
Valine	**-0,77**	-0,15	-0,12	-0,25	0,07	-0,10	-0,33																			
Proline	**-0,85**	0,00	0,03	-0,03	0,05	0,04	-0,07	**0,91**																		
Glyceric acid	0,39	0,12	0,14	0,32	0,00	-0,02	0,30	-0,17	-0,18																	
Serine	-0,32	-0,43	-0,25	-0,35	0,23	-0,19	-0,46	**0,79**	0,59	0,31																
Succinic acid	0,23	0,59	0,14	0,64	-0,52	-0,10	**0,81**	-0,63	-0,35	0,35	-0,62															
Malonic acid	0,02	0,37	0,34	0,24	-0,06	0,38	0,53	0,15	0,12	0,45	0,16	0,07														
Malic acid	0,39	-0,22	0,05	-0,12	0,28	0,11	-0,28	-0,38	-0,37	0,40	-0,08	0,07	-0,12													
GABA	0,04	-0,42	-0,51	-0,41	-0,06	-0,27	-0,34	-0,27	-0,20	-0,65	-0,38	0,00	**-0,82**	-0,08												
Threonic acid	-0,23	0,06	-0,09	-0,05	-0,14	-0,46	-0,45	0,32	0,04	-0,32	0,24	-0,46	-0,30	-0,40	0,04											
Glutamic acid	0,13	0,40	0,55	0,49	0,13	0,65	**0,80**	-0,10	0,14	0,35	-0,18	0,47	0,64	-0,16	-0,51	-0,58										
Quinic acid	0,22	0,40	0,16	0,50	-0,28	0,08	**0,74**	-0,44	-0,16	0,16	-0,53	**0,84**	-0,05	-0,13	0,08	-0,37	0,61									
Glycine	0,37	0,12	-0,05	0,14	-0,19	0,09	0,39	**-0,79**	-0,57	-0,25	**-0,85**	0,62	-0,28	0,06	0,62	-0,45	0,10	0,49								
VcNAD-MDH	-0,35	**0,68**	**0,83**	**0,77**	0,12	0,49	0,53	0,12	0,39	0,11	-0,18	0,33	0,29	-0,04	-0,48	-0,20	0,65	0,42	-0,11							
VcNADP-MDH	0,43	-0,42	0,21	-0,04	**0,68**	0,21	-0,47	-0,27	-0,28	0,11	0,02	-0,15	-0,60	0,48	0,24	0,08	-0,22	0,01	0,01	0,00						
VcCS	-0,53	-0,34	0,11	-0,15	0,50	0,08	-0,61	0,58	0,62	-0,27	0,47	-0,54	-0,50	0,10	0,18	0,19	-0,32	-0,35	-0,41	0,21	0,49					
NAD-MDH activity	**-0,70**	**0,83**	0,31	0,60	-0,59	-0,09	0,59	0,26	0,43	-0,16	-0,21	0,36	0,36	-0,42	-0,27	0,00	0,31	0,21	0,02	0,60	**-0,70**	-0,11				
NADP-MDH activation state	-0,16	**0,69**	0,22	0,47	-0,54	-0,09	0,64	0,11	0,13	0,44	0,01	0,41	**0,71**	-0,12	**-0,75**	-0,05	0,51	0,30	-0,30	0,43	-0,61	-0,51	0,60			
NADP-MDH total	0,47	**-0,84**	-0,05	-0,56	**0,87**	0,46	-0,55	-0,01	-0,10	0,00	0,29	-0,52	-0,24	0,26	0,20	-0,13	-0,07	-0,25	-0,10	-0,32	**0,68**	0,36	**-0,87**	**-0,68**		
NADP-MDH initial	0,53	-0,47	0,37	-0,13	**0,90**	**0,73**	-0,16	-0,12	-0,10	0,13	0,10	-0,25	-0,02	0,17	-0,07	-0,21	0,36	0,09	-0,06	0,12	**0,67**	0,24	-0,65	-0,40	**0,86**	
NAD-IDH activity	0,07	-0,57	-0,15	-0,29	0,48	-0,07	**-0,69**	-0,06	-0,03	-0,09	0,14	-0,26	**-0,77**	0,53	0,51	-0,01	-0,54	-0,23	0,05	-0,20	**0,80**	**0,71**	-0,57	**-0,78**	0,55	0,31
CS activity	**-0,79**	0,42	-0,06	0,26	-0,52	-0,58	-0,14	0,40	0,41	-0,24	0,12	-0,03	-0,25	-0,13	0,07	0,42	-0,48	-0,25	-0,20	0,18	-0,26	0,38	0,62	0,13	**-0,66**	**-0,77**
	AI concentration leaves	**Dehydroascorbate**	**Ascorbate**	**Total ascorbate**	**ASC/DHA ratio**	**Glycolic acid**	**Alanine**	**Valine**	**Proline**	**Glyceric acid**	**Serine**	**Succinic acid**	**Malonic acid**	**Malic acid**	**GABA**	**Threonic acid**	**Glutamic acid**	**Quinic acid**	**Glycine**	***VcNAD-MDH***	***VcNADP-MDH***	***VcCS***	**NAD-MDH activity**	**NADP-MDH activation state**	**NADP-MDH total**	**NADP-MDH initial**	**NAD-IDH activity**

C)

	Dehydroascorbate	Ascorbate	Total ascorbate	ASC/DHA ratio	Glycolic acid	Alanine	Valine	Proline	Glyceric acid	Serine	Succinic acid	Malonic acid	Malic acid	GABA	Threonic acid	Glutamic acid	Quinic acid	Glycine	*VcNAD-MDH*	*VcNADP-MDH*	*VcCS*	NAD-MDH activity	NADP-MDH activation state	NADP-MDH total	NADP-MDH initial	NAD-IDH activity
Dehydroascorbate	-0,60																									
Ascorbate	0,06	0,32																								
Total ascorbate	-0,35	0,64	0,49																							
ASC/DHA ratio	0,45	-0,46	**0,68**	-0,02																						
Glycolic acid	0,32	-0,12	-0,52	-0,37	-0,46																					
Alanine	0,44	-0,25	-0,18	0,20	0,02	-0,04																				
Valine	0,51	-0,44	-0,36	-0,49	0,00	0,39	0,48																			
Proline	0,57	-0,15	0,10	-0,32	0,18	0,03	0,41	**0,74**																		
Glyceric acid	0,65	-0,40	0,05	-0,28	0,30	0,54	0,01	0,54	0,43																	
Serine	0,32	-0,23	0,06	-0,21	0,17	0,25	0,10	0,34	0,45	0,60																
Succinic acid	0,30	-0,31	**-0,71**	-0,16	-0,48	0,66	0,51	0,30	-0,04	0,19	0,28															
Malonic acid	-0,33	0,08	0,40	0,28	0,34	-0,28	-0,65	**-0,75**	**-0,75**	-0,13	-0,40	-0,48														
Malic acid	0,50	-0,37	-0,60	-0,35	-0,30	**0,77**	0,47	**0,79**	0,33	0,53	0,12	**0,67**	-0,56													
GABA	0,66	-0,43	-0,13	-0,08	0,18	0,18	**0,89**	0,60	0,47	0,18	0,19	0,52	-0,65	0,58												
Threonic acid	0,37	-0,20	-0,64	-0,55	-0,46	0,63	0,27	**0,82**	0,59	0,41	0,14	0,44	**-0,72**	**0,83**	0,34											
Glutamic acid	-0,01	0,14	-0,10	0,17	-0,15	-0,41	0,61	0,28	0,36	-0,50	-0,48	-0,08	-0,47	0,10	0,43	0,25										
Quinic acid	0,48	-0,12	0,18	0,10	0,20	0,55	0,26	0,25	0,15	0,62	0,63	0,44	-0,20	0,41	0,48	0,05	-0,47									
Glycine	0,48	-0,29	0,34	-0,54	0,50	-0,03	-0,29	0,29	0,65	0,54	0,54	-0,37	-0,12	-0,12	-0,09	0,17	-0,28	0,09								
VcNAD-MDH	**0,68**	-0,50	-0,39	-0,46	-0,04	0,36	0,33	0,14	0,16	0,10	0,01	0,58	-0,29	0,37	0,49	0,33	0,07	0,11	0,11							
VcNADP-MDH	0,56	-0,41	-0,17	-0,15	0,11	0,22	0,34	-0,14	-0,16	-0,08	-0,19	0,50	0,01	0,21	0,51	-0,04	0,04	0,20	-0,17	**0,88**						
VcCS	**0,73**	-0,51	-0,14	-0,32	0,21	0,19	0,43	0,09	0,15	0,04	-0,03	0,45	-0,21	0,26	0,63	0,12	0,13	0,21	0,09	**0,93**	**0,94**					
NAD-MDH activity	0,14	0,40	**0,81**	0,28	0,43	-0,38	-0,18	-0,06	0,51	0,25	0,22	**-0,69**	0,06	-0,41	-0,20	-0,18	0,03	0,03	0,61	-0,35	-0,41	-0,25				
NADP-MDH activation state	0,51	-0,37	-0,13	-0,48	0,08	0,56	-0,18	-0,09	-0,07	0,32	0,34	0,44	0,02	0,17	0,16	0,06	-0,58	0,48	0,30	**0,70**	**0,67**	**0,67**	-0,23			
NADP-MDH total	-0,44	0,31	-0,16	0,17	-0,39	0,06	-0,36	**-0,78**	**-0,81**	-0,62	-0,49	0,14	0,49	-0,35	-0,37	-0,42	-0,18	-0,23	-0,54	0,22	0,44	0,18	-0,45	0,33		
NADP-MDH initial	-0,06	0,13	-0,08	-0,52	-0,22	0,33	**-0,74**	-0,29	-0,12	-0,03	-0,02	-0,12	0,21	-0,20	-0,50	0,08	-0,44	-0,15	0,39	0,28	0,14	0,14	0,03	0,61	0,46	
NAD-IDH activity	0,27	-0,03	0,62	0,06	0,63	-0,56	0,26	0,34	0,66	0,06	0,04	-0,61	-0,22	-0,18	0,31	-0,05	0,48	-0,06	0,47	-0,25	-0,26	-0,03	**0,67**	-0,41	**-0,67**	-0,34
CS activity	0,03	0,38	-0,03	-0,04	-0,38	0,64	-0,56	-0,23	-0,17	0,34	0,01	0,08	0,24	0,13	-0,45	0,20	-0,54	0,21	0,13	0,11	0,03	-0,07	0,14	0,45	0,34	**0,67**
	AI concentration leaves	**Dehydroascorbate**	**Ascorbate**	**Total ascorbate**	**ASC/DHA ratio**	**Glycolic acid**	**Alanine**	**Valine**	**Proline**	**Glyceric acid**	**Serine**	**Succinic acid**	**Malonic acid**	**Malic acid**	**GABA**	**Threonic acid**	**Glutamic acid**	**Quinic acid**	**Glycine**	***VcNAD-MDH***	***VcNADP-MDH***	***VcCS***	**NAD-MDH activity**	**NADP-MDH activation state**	**NADP-MDH total**	**NADP-MDH initial**	**NAD-IDH activity**

Negative		Positive	
-0,75	-1,00	0,75	1,00
-0,50	-0,74	0,50	0,74
-0,25	-0,49	0,25	0,49
0,00	-0,24	0,00	0,24