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Ascorbic Acid Mitigates Aluminum Stress Through Improved Antioxidant Mechanism in Highbush Blueberry (*Vaccinium corymbosum* L.)

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Abstract: Ascorbic acid (ASC) is a molecule naturally synthesized in plant cells, protecting against abiotic stresses by reducing reactive oxygen species (ROS), which cause oxidative damage. Aluminum (Al) toxicity is the major limiting factor on crop productivity in acidic soils, increasing ROS within cells and impairing the growth and development of plants. Exogenous antioxidant applications are an effective strategy to promote tolerance to abiotic stress. The objective was to evaluate the effect of foliar ASC applications (0, 50, 100, 200, and 400 mg L^{-1} ASC) and their interaction with Al toxicity (0, 400 µM Al) in Star, an Al-sensitive cultivar of highbush blueberry. Significant increases of 1.6-fold in growth were observed in roots and leaves under treatment with 200 mg L^{-1} ASC. In the same treatment, increased pigments and antioxidant activity (~1.2- to 2.3-fold) were observed concomitant with reduced lipid peroxidation. Positive correlations between organic acid exudation, the ASC/DHA ratio, and calcium levels were observed, whereas a negative correlation between lipid peroxidation and dehydroascorbate (DHA) was observed. Foliar ASC application also increased the ASC/DHA ratio in leaves and enhanced 2.2-fold organic acid exudation in the 200 mg L^{-1} ASC treatment. The results suggest that foliar ASC applications improved redox balance and underscore the potential of ASC as a practical solution to enhance resilience in Al-sensitive plants.

Keywords: ascorbic acid; aluminum toxicity; *Vaccinium corymbosum*; woody plants

1. Introduction

L-ascorbic acid (ascorbate [ASC]), also known as vitamin C, is an important antioxidant that regulates the levels of reactive oxygen species (ROS), as well as a cofactor for several enzymes in animals and plants [1,2]. Four ASC biosynthesis pathways have been proposed in plants, including the L-galactose or Smirnoff–Wheeler (SW), L-glucose, D-galacturonic acid, and myo-inositol biosynthetic pathways [3]. However, the SW is considered the main ASC biosynthesis in plants [4]

In plants, ASC plays important roles in photosynthesis, division, and cellular differentiation, being a crucial molecule in growth, development, and adaptation to stresses [5,6]. Also, it is involved in downstream stress signaling [7]. ASC is mostly found in the cell cytoplasm, and a small part is transported to the apoplast in plants (≈10%) [8]. Akram et al. [9] indicate that the apoplastic fraction of ASC is decisive in the signaling of oxidative stress, and its redox buffering capacity is attributed to the ASC pool. In addition, ASC content in the apoplast influences hormonal balance, growth, *MAPK* (*mitogen-activated protein kinase*) signaling cascades, and antioxidant enzyme activities. The ASC also plays an important role in the perception, physiological and biochemical responses of the plant under normal and abiotic stress conditions [2,9].

Trivalent Al (Al^{3+}) is an important factor that limits plant growth in acid soils (pH < 5.0) [10]. Al can interact with elements of the cell membrane, producing an increase in lipid peroxidation. Also, Al can bond to DNA and multiple enzymes, altering the normal functioning of diverse metabolic and physiological processes [11,12]. Various studies mentioned that Al induces an instantaneous and sustained production of ROS due to the binding of Al to the plasma membrane and the Ca increase in the cytoplasm [13,14]. Plants have developed two main strategies for detoxifying Al. These mechanisms are known as Al-tolerance (translocation of Al^{3+} ions towards the vacuole through the formation of non-phytotoxic complexes) and Al-exclusion or avoidance (plants exudate organic molecules from the roots which chelate the Al^{3+} in the rhizosphere) [15,16]. The main organic acids (OAs) exuded due to the effect of Al-toxicity in plants in order of ionic force OA:Al are citrate, oxalate, and malate [17]. The oxalate is produced for ASC degradation by the oxidation of DHA and 4-O-oxalyl-L-threonic acid [18]. In this sense, the cleavage of ASC between carbon atoms 2 and 3 results in the formation of oxalic acid and L-threonic acid, being proposed as the major pathway of oxalate production in plants [19–21]. Only a few studies are available regarding the dual effect of the ASC and Al-toxicity. For example, in *Oryza sativa* L. plants, the ASC levels were higher in leaves under Al treatment (1 mM $AlCl_3$). Also, it is mentioned that ASC, DHA, and ASC/DHA ratios are key in maintaining the ASC regeneration and in the homeostasis of cellular metabolites involved in ROS removal [22]. Similar results were found in Al-tolerant plants of *Oryza sativa* by Ribeiro et al. [23], where the increase in the ASC content in the cell in the presence of Al^{3+} prevented lipid peroxidation and diminished the H_2O_2 concentration. In *Secale cereale* L., ASC levels in the leaves of the Al-tolerant genotypes were reported to increase by 23% after 24 h of Al exposure (5 mg L^{-1} $AlCl_3$); in contrast, in the Al-sensitive genotypes, the ASC levels remained unaltered [24]. Meanwhile, in *Vaccinium corymbosum*, it is suggested that ASC is a key molecule in generating and synthesizing oxalate under Al-treatment in Al-resistant cultivars (200 mM $AlCl_3$) [25]. Whereas, in *Nicotiana tabacum* plants, it was observed that the overexpression of the *DHAR* gene increased root growth, ASC level, and APX activity under Al exposure compared with wild-type plants [26].

The ASC levels in plants can be affected by abiotic factors such as light or temperature and other aspects such as species, cultivars, and between plant tissues [6,26]. Also, its content can vary among fruits of different species, between varieties or cultivars from the same species, and these differences can be explained by environmental factors, including

cultivation requirements, harvest time, and post-harvest conditions, among others [27,28]. Exogenous ASC application is suggested as an effective approach for alleviating the adverse effects induced by abiotic stress, such as salinity stress, water deficit, and drought, among others [29,30]. It is reported that exogenous application of 100 ppm of ASC enhances photosynthetic pigments, osmoprotective contents, and antioxidant enzymes in *Zea mays* plants under drought conditions [31]. Likewise, in *Linum usitatissimum*, an increase in proline accumulation, water, and photosynthetic pigments was observed with applications of 400 mg L^{-1} ASC and under salinity stress [32], while in *Prunus americana* plants, applying 200 mg L^{-1} of ASC alleviated the negative effect produced by cold stress [33].

Vaccinium corymbosum is a commercially important berry crop because of its high antioxidant capacity, nutritional properties, and health benefits [34,35]. In Chile, this crop is cultivated in volcanic ash-derived soils in southern regions [36]. This soil has strong acidity and high Al availability [37,38]. These conditions promote the increase in toxic Al isoforms in the soil, which alter the normal functioning of the metabolic and physiological processes of plants.

Although research conducted in recent years has demonstrated the beneficial effect of ASC in plants, it is still unknown how the ASC molecule interacts with phytotoxic Al and its relationship with Al-resistance mechanisms. Understanding how sensitive species improve their responses to Al stress with ASC application will be essential for designing genetic improvement strategies that increase aluminum tolerance in economically important crops such as highbush blueberry. Therefore, the purpose of this study was to evaluate ASC application on an Al-sensitive cultivar of highbush blueberry under Al-toxicity.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

This study was performed in the South of Chile, in the La Araucanía region, using Star (USOOPP10675P) (*Vaccinium corymbosum* L.) as an Al-sensitive cultivar [25]. One-year-old plants (around 40 cm tall, from in vitro cultures from Global Seedlings Nursery, Maule region, Chile; Supplementary Materials: Figures S1 and S2) were conditioned in plastic pots containing 18 L of Hoagland solution [39] with low ionic strength (50%) and with the initial pH of 5.6 that was lowered daily for two weeks until achieving pH 4.5, and adjusted daily over the 28 days of evaluation with a pH meter (Multiparametric Probe, HI1288, Hanna Instruments, Shanghai, China), and the solution was renewed every three days. 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 $(NH_4)_6Mo_7O_{24}$. The growth greenhouse 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}. In the experiments, we tested six treatments: T1: ontrol (0 mg L^{-1} of ASC + 0 µM Al), T2: Al (0 mg L^{-1} of ASC + 400 µM Al), T3: 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al), T4: 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al), T5: 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al), and T6: 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al). The ASC was foliar sprayed, and their doses were chosen according to preliminary studies [30–32], and Al^{3+} (as $AlCl_3$) doses also were used according to preliminary experiments performed by our group (unpublished data). The measurements were performed on three plants at 0, 7, 14, 21, and 28 days, according to [25]. The harvest was performed in the middle of the light period; samples from fully expanded leaves (from the second or third node of the branch) and root tips (approximately 2 cm from the tips) were snap-frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2. Plant Growth Analysis

Relative growth rate (RGR) was determined according to Hoffmann and Poorter [40]. After conditioning and before the Al treatment started, one plant per replicate was separated for the dry weight (DW) determination (DW_1), and the other plants were subjected to the Al treatment, as mentioned previously. At each time (7, 14, 21, and 28 days), control and Al-exposed plants were harvested for root and shoot DW determination (DW_2). The RGR was calculated as RGR = $(\ln DW_2) - (\ln DW_1)/t2 - t1)$, where t1 correspond to 0 and t2 correspond to 7, 14, 21 and 28 days, respectively.

2.3. Determination of Aluminum and Calcium Concentrations

Aluminum and calcium (Ca) concentrations were analyzed as described by Sadzawka et al. [41], whereby shoot and root samples were dried at 70 °C in a forced-air oven for 72 h. Then, 1.0 g of dried tissue was ground and dry ashed in a muffle furnace at 500 °C for 8 h; ash was dissolved in 2 M HCl. The concentration of Al and Ca was determined using a multi-element atomic absorption spectrophotometer (EAA, Model 969, Unicam, Cambridge, UK).

2.4. Chlorophyll Fluorescence and Gas-Exchange Measurements

Chlorophyll fluorescence and photosynthesis-related parameters were determined in fully expanded leaves from the second or third node of the branch, as described by Reyes-Diaz et al. [42]. In brief, the measurements were performed in the morning between 9 and 11 h using a portable infrared gas analyzer (Licor LI6400xt, Lincoln, NE, USA), equipped with a fluorescence chamber and light source (300 µmol photons m^{-2} s^{-1}), a temperature of 20 °C, and a CO_2 concentration of 400 ppm. The following parameters were assessed: maximum efficiency of PSII in the light (Fv'/Fm'), net photosynthesis (*Pn*) and stomatal conductance (*gs*).

2.5. Determination of Photosynthetic Pigments

Samples of 15 mg of ground leaf tissue were subjected to methanol extraction according to Medeiros et al. [43]. The photosynthetic pigments were determined as described by Porra et al. [44], using a microplate spectrophotometer (Thermo Scientific Varioskan Flash, Wilmington, NC, USA), with absorbances of 653 (chlorophyll *a*), 666 (chlorophyll *b*), and 470 nm (carotenoids).

2.6. Lipid Peroxidation Assay

Lipid peroxidation in leaves and roots of highbush blueberry was determined according to Du and Bramlage [45] modified, using thiobarbituric acid reactive substances (TBARS). Approximately 15 mg of fresh ground material was used for analysis. Absorbance was measured at 440, 530, and 660 nm by a spectrophotometer (UNICOR 2800 UV/VIS, Fairfield, NJ, USA). The lipid peroxidation was expressed in nanomoles of malondialdehyde per gram of fresh weight (nmol MDA g^{-1} FW).

2.7. Antioxidant Determination

The antioxidant activity (AA) in roots and leaves was determined using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, according to the method of Chinnici et al. [46]. The samples were ground and soaked in 1 mL of 80:20 (*v*/*v*) methanol/water. The absorbance was measured at 515 nm in a spectrophotometer (UNICOR 2800 UV/VIS, Spain) using Trolox as standard. The values are expressed in µg Trolox equivalents g^{-1} FW.

2.8. Ascorbate and Dehydroascorbate Determination

Ascorbate (ASC) concentration was quantified in roots and leaves by the protocol described by Kampfenkel et al. [47] with minor modifications [48]. Absorbance was measured at 520 nm in a microplate spectrophotometer (Thermo Scientific Varioskan

Flash, Wilmington, NC, USA). The levels of ASC (ASC-reduced form) were determined from a standard curve using sodium ascorbate as standard. The dehydroascorbate (DHA; ASC-oxidized form) content was determined by subtracting the measurements without N-ethylmaleimide (NEM) and expressed in nmol mg^{-1} FW.

2.9. Superoxide Dismutase (SOD) Activity

The SOD activity was determined through the photo-chemical reduction in nitroblue tetrazolium (NBT). The reaction mixture contained 640 µL of potassium phosphate buffer 0.1 M pH 7.0, 10 µL of ethylenediaminetetraacetic acid (EDTA) 10 mM, 50 µL of methionine 260 mM, 80 µL of NBT 4.2 mM, 170 µL of riboflavine 130 µM and 50 µL of supernatant. The reaction tubes were illuminated for 15 min, and the absorbance of the samples was measured at 560 nm by a spectrophotometer (Thermo Scientific Varioskan Flash, Wilmington, NC, USA). Non-illuminated and illuminated reactions without the supernatant were used as controls. One SOD unit was defined as the amount of enzyme corresponding to 50% inhibition of the NBT reduction. The SOD activity was calculated on a protein basis according to the Bradford [49] method.

2.10. Analysis of Root Exudates

Root exudates were determined according to a modified procedure established by Rosas et al. [50]. The plants were immersed in deionized water with aeration for 1 h, and a 45 mL aliquot of the resulting solution containing the exudates was stored at −80 °C for analysis. In order to quantify the concentration of OAs (oxalate, malate, citrate, and succinate), the aliquot of the resulting solution was lyophilized. The residue was re-suspended in 0.5 mL of deionized water to be analyzed by high-performance liquid chromatography (HPLC, JASCO, LCNet II/ADC, Tokyo, Japan) according to Millaleo et al. [36].

2.11. Experimental Design and Statistical Analyses

The experiment used a completely randomized block design with three replicates and five measurement times for the physiological and biochemical analyses. Due to the lack of difference in the parameters measured at the four time points (7, 14, 21, and 28 days) in the control, 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 a two-way repeated measures analysis of variance (where factors were treatments and sampling times) and Tukey's test for mean comparisons. If data did not pass the Kolmogorov–Smirnov test, we used ANOVA on ranks repeated measures analysis.

To identify the variables explaining the differences between the treatments, we performed principal components analysis (PCA). For PCA, the data of all the variables were normalized [log(2)] to minimize the effect of different units of measurement on the variance of each component. All analyses were performed by XLSTAT-LifeScience v.2024.

3. Results

3.1. Relative Growth Rate

RGR in roots showed a significant interaction ($p < 0.001$) between the treatment and the time of the evaluation (Table 1). In contrast, the differences were observed individually in shoots in each factor (treatment and time) (Table 1). In leaves, the T3 (50ASC+Al) treatment promoted significant differences, showing a 1.6-fold increase at 14 days compared with the T5 (200ASC+Al) treatment. However, the tendency to final evaluation showed higher growth in T1 (control) and T4 (100ASC+Al) treatments. Furthermore, in roots, the main

significant differences were observed in T5 (400ASC+Al) at 21 days, contrary to that observed in T3 (50ASC+Al), which showed a low RGR at 14 days.

Table 1. Relative growth rate (RGR; g day^{-1}) in shoots and roots of Star cultivar exposed to different doses of ASC and Al toxicity: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al) for 7, 14, 21 and 28 days.

Shoots															
Treatment	**7 d**				**14 d**				**21 d**				**28 d**			
	Average		**SE**		**Average**		**SE**		**Average**		**SE**		**Average**		**SE**	
T1	1.42	±	0.06	Aa	1.44	±	0.15	Aab	1.77	±	0.17	Aa	1.95	±	0.14	Aa
T2	1.54	±	0.13	Aa	1.42	±	0.07	Aab	1.73	±	0.29	Aa	1.72	±	0.13	Aa
T3	1.26	±	0.16	Aa	1.62	±	0.12	Aa	1.78	±	0.08	Aa	1.56	±	0.30	Aa
T4	1.32	±	0.22	Aa	1.41	±	0.21	Aab	1.41	±	0.07	Aa	2.04	±	0.15	Aa
T5	1.41	±	0.10	Aa	1.00	±	0.24	Ab	1.69	±	0.14	Aa	1.80	±	0.27	Aa
T6	1.22	±	0.13	Aa	1.47	±	0.24	Aab	1.54	±	0.15	Aa	1.60	±	0.12	Aa
Roots																
Treatment	7 d				14 d				21 d				28 d			
	Average		SE		Average		SE		Average		SE		Average		SE	
T1	0.84	±	0.04	ABCDE	0.83	±	0.14	ABCDE	0.84	±	0.07	ABCDE	1.11	±	0.16	ABCD
T2	0.63	±	0.03	DE	0.75	±	0.08	BCDE	1.05	±	0.12	ABCD	0.99	±	0.07	ABCD
T3	0.83	±	0.06	ABCDE	0.30	±	0.02	F	1.29	±	0.14	AB	1.02	±	0.07	ABCD
T4	1.05	±	0.13	ABCD	1.2	±	0.15	ABC	0.71	±	0.14	CDE	1.26	±	0.11	ABC
T5	0.87	±	0.06	ABCDE	1.1	±	0.09	ABCD	1.35	±	0.12	A	1.35	±	0.07	AB
T6	0.49	±	0.03	EF	0.76	±	0.11	BCDE	1.02	±	0.13	ABCD	1.05	±	0.11	ABCD

The values are the average of three independent biological replicates (±standard error). Statistical analysis without interactions: uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment compared to different times of evaluation, and lowercase letters indicate significant differences ($p \leq 0.05$) between treatments in the same time evaluation (ANOVA two-way). Statistical analysis with interactions: uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment × time evaluation (ANOVA one-way). Both analyses are according to the Tukey test.

3.2. Determination of Al and Ca Content

The Al and Ca content in shoot and root tissues was highest, influenced by the interaction between treatment and duration of the evaluation ($p < 0.01$). In the leaves, high Al content was observed in the T2 (Al) treatment at 21 d, increased significantly 1.9-fold compared with T1 (control) treatment. Moreover, at the same time, the T3 (50ASC+Al), T4 (100ASC+Al), T5 (200ASC+Al), and T6 (400ASC+Al) treatments showed significantly low levels of 24, 39, 41 and 46%, respectively, compared with the T2 (Al) treatment (Figure 1A). In the roots (Figure 1B), significant differences were observed in the T5 (200ASC+Al) treatment with a 1.6-fold increase at 28 days compared with T1 (control) treatment; whereas the T5 (200ASC+Al) treatment significantly diminished the Al content by 5% at 14 d compared to the T2 (Al) treatment. Furthermore, all treatments showed significant differences compared to T1 (control), with an increase between 1.5- and 1.6-fold under Al conditions.

On the other hand, in the leaves of the Star cultivar, the main significant differences in Ca content were observed in T6 (400ASC+Al) at 7 and 14 days, diminishing by 33 and 31%, respectively, compared with T1 (control). While the T4 (100ASC+Al) treatment showed a high level of Ca (5136 mg Ca kg^{-1} DW) compared with T1 (control, 4408 mg Ca kg^{-1} DW) and T6 (400ASC+Al, 2968 mg Ca kg^{-1} DW) (Figure 1C). Unlike the leaves, the Ca content in the roots showed a significantly lesser reduction of ~13.5% in all treatments (T2 to T6) during the time evaluation, compared with T1 (control, 8.4 mg Ca kg^{-1} DW) (Figure 1D).

3.3. Gas Exchange Parameters

For net photosynthesis (*Pn*) and stomatal conductance (*gs*), significant interaction was observed between the treatment × the times of evaluation ($p \leq 0.05$) (Table 2). The main differences were observed in T6 (400ASC+Al) at 7 days, with a 1.5-fold increase with respect to T2 (Al) and 1.8-fold to T4 (100ASC+Al). However, T6 (400ASC+Al) showed a significant increase of 1.6- and 1.5-fold at 21 days compared to T1 (control) and T3 (50ASC+Al),

respectively, unlike those observed at 28 days, where all treatments exhibited a significantly low level of *Pn* compared with other treatments and times (Table 2). Regarding *gs*, T4 (100ASC+Al) showed an increase of 7.6-fold compared with T1 (control) and 3.6-fold with respect to T2 (Al) at 28 days, while that low level is observed at 14 days, showing the T3 (50ASC+Al) treatment value of 0.07 mol H_2O m^{-2} s^{-1} (Table 2).

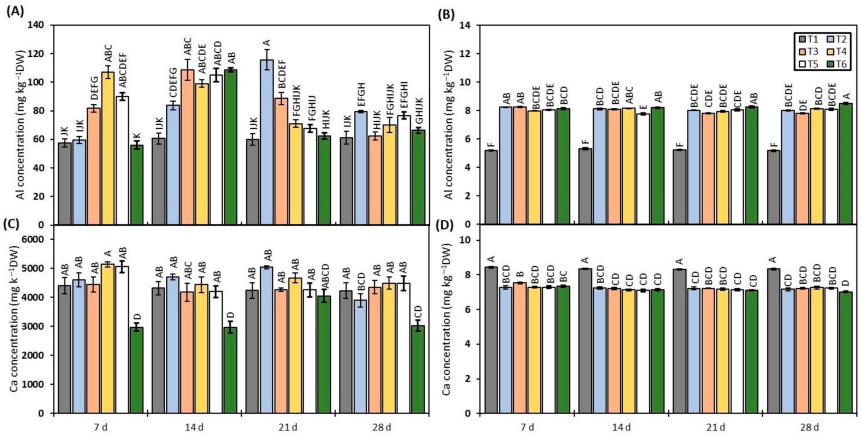

Figure 1. Aluminum and calcium concentration (mg kg^{-1}) in leaves (**A**,**C**) and roots (**B**,**D**) of Star cultivar of *V. corymbosum* exposure to different doses of ASC and Al toxicity. Treatments: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al); for 7, 14, 21 and 28 days. The values are the averages of three independent biological replicates (±standard error). Statistical analysis with interactions: uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment × time evaluation interaction (ANOVA one-way), according to the Tukey test.

Table 2. Net photosynthetic (*Pn*; µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1}) in fully expanded leaves of plants from Star cultivar exposed to different doses of ASC and Al toxicity. Treatments: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al) for 7, 14, 21 and 28 days.

***Pn* (µmol CO_2 m^{-2} s^{-1})**															
Treatment	**7 d**				**14 d**				**21 d**				**28 d**			
	Average		**SE**		**Average**		**SE**		**Average**		**SE**		**Average**		**SE**	
T1	2.60	±	0.19	ABC	1.82	±	0.08	DEF	1.82	±	0.08	DEF	1.18	±	0.09	G
T2	2.30	±	0.07	BCD	2.20	±	0.12	BCD	2.50	±	0.07	ABCD	1.30	±	0.08	G
T3	2.53	±	0.06	ABC	2.02	±	0.03	CDE	1.94	±	0.12	CDEF	1.31	±	0.10	G
T4	1.95	±	0.06	CDEF	2.61	±	0.09	BCD	2.19	±	0.14	BCD	1.55	±	0.04	EFG
T5	2.41	±	0.18	BCD	2.13	±	0.07	BCDE	2.66	±	0.25	BCD	1.31	±	0.13	G
T6	3.40	±	0.20	A	2.16	±	0.19	BCD	2.93	±	0.12	AB	1.46	±	0.01	FG
***gs* (mol H_2O m^{-2} s^{-1})**																
Treatment	7 d				14 d				21 d				28 d			
	Average		SE		Average		SE		Average		SE		Average		SE	
T1	0.20	±	0.06	ABCDE	0.11	±	0.01	BCDE	0.20	±	0.09	ABCDE	0.09	±	0.04	CDE
T2	0.15	±	0.04	ABCDE	0.10	±	0.01	BCDE	0.20	±	0.08	ABCDE	0.19	±	0.05	ABCDE
T3	0.30	±	0.10	ABCDE	0.06	±	0.02	E	0.14	±	0.04	ABCDE	0.09	±	0.03	BCDE
T4	0.43	±	0.08	ABC	0.10	±	0.03	BCDE	0.22	±	0.05	ABCDE	0.68	±	0.04	A
T5	0.36	±	0.14	ABCD	0.11	±	0.04	BCDE	0.27	±	0.03	ABCDE	0.40	±	0.14	ABCD
T6	0.49	±	0.08	AB	0.06	±	0.01	DE	0.41	±	0.10	ABC	0.26	±	0.03	ABCDE

The values are the averages of three independent biological replicates (±standard error). Statistical analysis with interactions: uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment × time evaluation (ANOVA one-way), according to the Tukey test.

3.4. Photosynthetic Pigments

The chlorophyll *a* (*Chla*), *b* (*Chlb*), and ratio showed a significant interaction between the treatments × times of evaluation ($p \leq 0.01$) (Supplementary Table S1). The chlorophyll concentrations increased in *Chla* 1.6-fold at 7 days and *Chlb* 1.2-fold at 14 days in the T5 (200ASC+Al) treatment, compared with the T4 (100ASC+Al) treatment at 7 days. Whereas the chlorophyll *a*/*b* ratio was found to have a significant interaction in the T4 (100ASC+Al) treatment at 28 days (1.7 ratio), compared with the low value observed in T3 (50ASC+Al) treatment at 21 d (1.3 ratio) (at 24% in relation to T4) (Figure 2). About carotenoids, a high interaction between the treatment × time evaluation was observed in T4 (100ASC+Al) and T6 (400ASC+Al), with an increase ~1.4-fold at 28 days compared with T3 (0.93 μg mg^{-1} FW) treatment at 7 days (Figure 2).

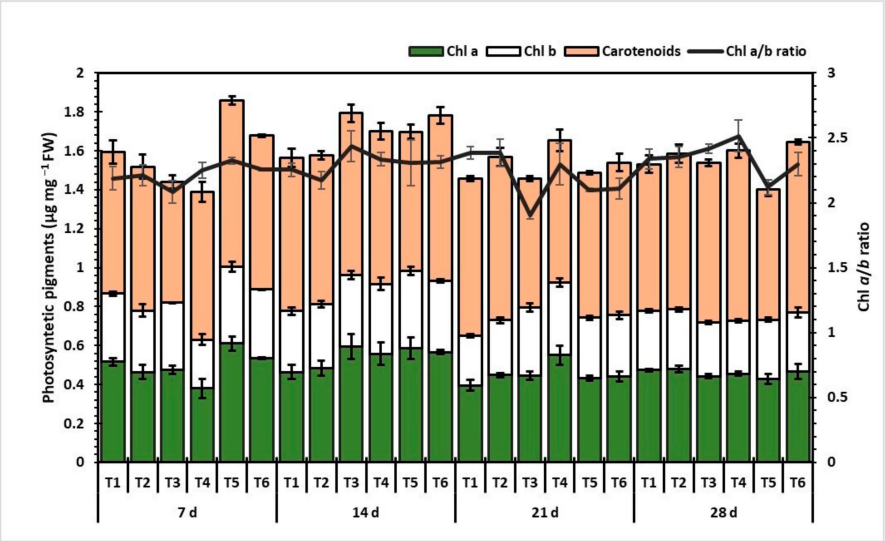

Figure 2. Photosynthetic pigments levels in fully expanded leaves of plants from Star cultivar of *V. corymbosum* exposed to different doses of ASC and Al toxicity. Applied treatments: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 μM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 μM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 μM Al; T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 μM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 μM Al) for 7, 14, 21 and 28 days. The values are the averages of three independent biological replicates (±standard error). For complete information from statistical analysis, please see Supplementary Table S1.

3.5. Antioxidants and Lipid Peroxidation Assay

We performed antioxidant activity and lipid peroxidation determinations on the roots and leaves to determine the plant stress responses and/or their mechanisms to environmental conditions. Regarding the total antioxidant activity in leaves, a significant interaction between ASC doses and the evaluation times ($p < 0.001$) was observed (Figure 3A). In treatment 5 (200ASC+Al), the main differences were observed at 21 and 28 days with increases of 1.9- and 2.3-fold, respectively, compared with T1 (control). Whereas, when compared with T2 (Al), the increase in T5 (200ASC+Al) was 1.3-fold higher at 28 days. On the other hand, in the roots, the antioxidant activity showed significant differences between the times of evaluation ($p < 0.001$) (Figure 3B). T1 (control) showed a reduction of 29% at 28 d; while T4 (100ASC+Al) exhibited reductions of 27, 29 and 35% at 14, 21 and 28 days, respectively. The T5 (200ASC+Al) treatment showed reduced antioxidant activity by 33 and 42% at 21 and 28 days, all compared with 7 days. In T3 (50ASC+Al), significant decreases were observed at 21 and 28 d, of 32% and 28%, respectively, compared with 14 days. Even if no significant differences had been observed in T2 (Al), the trend showed a reduction with the time in the roots (Figure 3B).

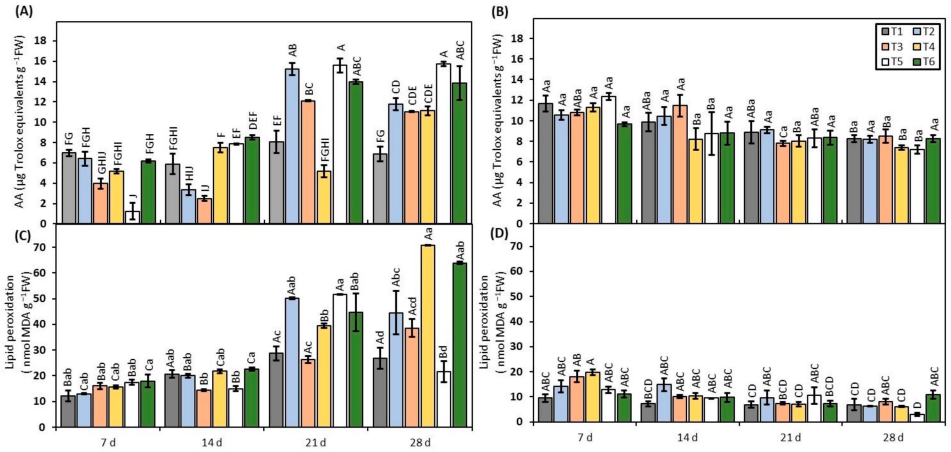

Figure 3. Antioxidant activity (AA: µg Trolox equivalents g^{-1} FW) (**A**,**B**) and lipid peroxidation (nmol MDA g^{-1} FW) (**C**,**D**) in leaves (**A**,**C**) and roots (**B**,**D**) of plants from Star cultivar of *V. corymbosum* exposed to different ASC doses and Al toxicity. Applied treatments: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al) for 7, 14, 21 and 28 days. The values are the averages of three independent biological replicates (±standard error). The significant differences ($p \leq 0.05$) in the time and treatment of factors are shown as: uppercase letters, differences between the evaluation times in the same treatment and lowercase letters, differences between treatments in the same evaluation (statistical analysis without interactions, ANOVA two-way), whereas, uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment × time evaluation interaction, both according to the Tukey test (statistical analysis with interactions, ANOVA one-way).

Lipid peroxidation in the leaves showed significant differences in ASC doses and evaluation times ($p \leq 0.01$). In T5 (200ASC+Al), it was observed that even though an increase of 1.8-fold occurred at 21 d, it diminished by 19% at 28 d, compared with T1 (control) (Figure 3C). In the roots, significant interaction between ASC doses × evaluation times was found ($p < 0.001$) diminishing significantly by 64% in the treatment with 200ASC+Al at 28 d (Figure 3D). The highest level of lipid peroxidation was observed at 7 d in all treatments, showing reductions at the end of the experiment, T5 (200ASC+Al) being the treatment with a significant reduction of 56%, compared with 7 days.

3.6. Ascorbate Determination

To explore the changes experienced by the non-enzymatic antioxidant mechanism, we analyzed the reduced and oxidized form of ASC. Regarding both ASC forms, the levels in leaves and roots showed a significant interaction between doses of ASC × times of evaluation ($p < 0.05$) (Figure 4). In the leaves, ASC content was increased significantly by 1.6-fold in T6 (400ASC+Al) at 28 days compared with 7d. Also, T5 (200ASC+Al) showed similar significant changes, increasing 1.4-fold with respect to 7 days (Figure 4A). In relation to oxidate form, in T6 (400ASC+Al), the DHA content increased by 2.4-fold compared with the same treatment at 7 days (Figure 4C). Unlike the leaves, in the roots, both forms of ASC diminished until the end of the experiment, although in T2 (Al), the tendency was first to increase 1.2-fold at 14 days and later decrease significantly by 53% at 28 days. Whereas in T5 (200ASC+Al), a significant decrease was observed of about 49% compared with itself but at 7 d (Figure 4B). Furthermore, similar results were observed in DHA content; T3 (50ASC+Al) and T4 (100ASC+Al) showed an increased level at 14 days, but as the experiment advanced, both decayed significantly by ~76% (Figure 4D).

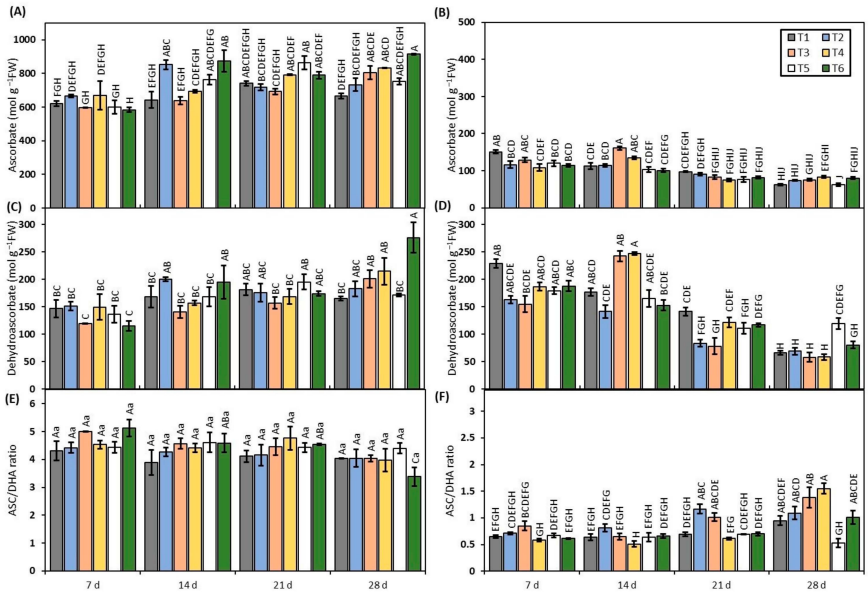

Figure 4. Ascorbate (ASC) (**A**,**B**), dehydroascorbate (DHA) (**C**,**D**) (mol g^{-1} FW) and ASC/DHA ratio (**E**,**F**) in leaves (**A**,**C**,**E**) and roots (**B**,**D**,**F**) from plants of the Star cultivar of *V. corymbosum* exposed to different doses of ASC and Al toxicity. Applied treatment: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al) for 7, 14, 21 and 28 days. The values are the averages of three independent biological replicates (±standard error). The significant differences ($p \leq 0.05$) in the time and treatment of factors are shown as: uppercase letters, differences between the evaluation times in the same treatment, and lowercase letters, differences between treatments in the same evaluation (statistical analysis without interactions, ANOVA two-way). Whereas, only uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment × time evaluation interaction (statistical analysis with interactions, ANOVA one-way), both according to the Tukey test.

In addition, we determined the ASC/DHA ratio, an important indicator of the redox state of the ASC pool and indicative of the antioxidant capacity of the plant. The results observed in the leaves indicate significant differences in treatments with different doses of ASC and evaluation times ($p < 0.001$). In the roots, we observed a strong interaction between doses of ASC × times of evaluation ($p < 0.01$) (Figure 4E,F). In the leaves, the T6 (400ASC+Al) leads to differences at 28 days, decreasing by 34% at 7 days. Unlike the results observed in roots, T4 (100ASC+Al) increased the ASC/DHA ratio at 28 days by ~2.7-fold compared with the T5 (200ASC+Al) treatment.

3.7. Superoxide Dismutase Activity

In evaluating the SOD enzyme by exploring the changes in the enzymatic antioxidant mechanism, the protein content was also exposed. Superoxide dismutase in leaves and roots showed a significant interaction between doses of ASC × times of evaluation ($p < 0.001$) (Figure 5A,B). In leaves, all treatments were diminished in the later experiments; T1 (control), T2 (Al), T3 (50ASC+Al), and T6 (400ASC+Al) showed significant decreases of 35, 25, 26 and 33%, respectively, at 28 days compared with 7 days (Figure 5A). In contrast, in roots, a decrease of 36% in SOD activity in the T2 (Al) treatment was observed between 7 and 28 days (Figure 5B). Regarding protein content, a significant interaction was found between doses of ASC × times of evaluation ($p < 0.001$) in the leaves of the Star cultivar (Figure 5C). In T5 (200ASC+Al), a high protein content (105.72 mg g^{-1} FW) was observed at 21 days, compared with all doses × times evaluations (Figure 5C). Unlike in the roots, which showed significant differences between the doses of ASC and the times of evaluation ($p < 0.001$) (Figure 5D). Protein levels diminished significantly throughout the evaluation. Furthermore, T1 (control) showed a high

level of proteins over the 7 to 21 day period, with an increase of ~1.2-fold, compared with other treatments in the same evaluation period.

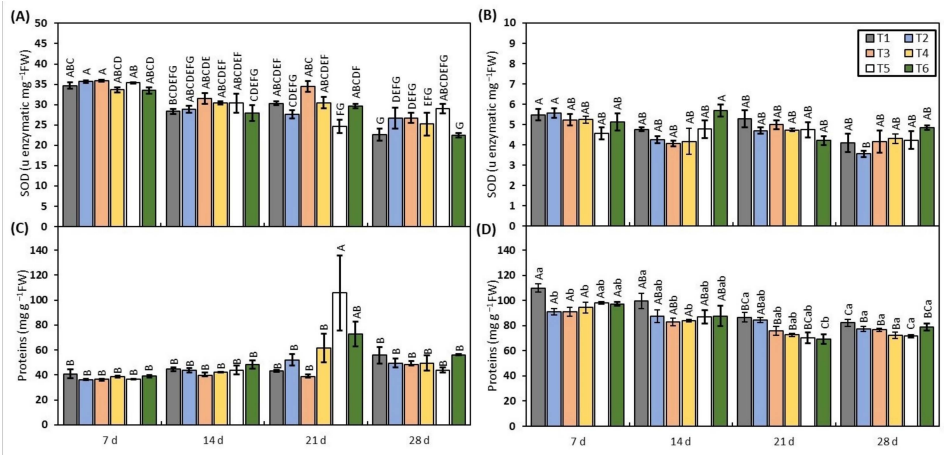

Figure 5. Superoxide dismutase (SOD) (**A**,**B**) (U enzymatic mg^{-1} FW) and proteins (**C**,**D**) (mg g^{-1} FW) in leaves (**A**,**C**) and roots (**B**,**C**) of the Star cultivar of *V. corymbosum* exposure to different doses of ASC and Al toxicity. Applied treatment: T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al) for 7, 14, 21 and 28 days. The values are the averages of three independent biological replicates (±standard error). The significant differences ($p \leq 0.05$) in the time and treatment of factors are shown as: uppercase letters, differences between the evaluation times in the same treatment, and lowercase letters, differences between treatments in the same evaluation (statistical analysis without interactions, ANOVA two-way). Whereas, only uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment × time evaluation interaction (statistical analysis with interactions, ANOVA one-way), both according to the Tukey test.

3.8. Organic Acid Exudates

Root organic acid exudation is one of the main strategies of Al-resistance in plants. In Star cultivar plants, the prevalent exudated organic acid was oxalate, which exhibited significant differences at different times of evaluation ($p < 0.001$) (Table 3). In T5 (200ASC+Al), the oxalate exudation increased 2.2-fold at 28 days, with respect to 14 days.

Table 3. Oxalate concentration (µmol g^{-1} h^{-1}) in root exudate of Star cultivar of *V. corymbosum* exposure to different doses of ASC and Al toxicity. Applied treatments; T1, control; T2, Al (0 mg L^{-1} of ASC + 400 µM Al); T3, 50ASC+Al (50 mg L^{-1} of ASC + 400 µM Al); T4, 100ASC+Al (100 mg L^{-1} of ASC + 400 µM Al); T5, 200ASC+Al (200 mg L^{-1} of ASC + 400 µM Al); and T6, 400ASC+Al (400 mg L^{-1} of ASC + 400 µM Al) for 7, 14, 21 and 28 days.

Treatment	Oxalate (µmol g^{-1} h^{-1})															
	7 d				14 d				21 d				28 d			
	Average		SE		Average		SE		Average		SE		Average		SE	
T1	54.2	±	7.7	Aa	41.6	±	4.9	Aa	51.5	±	6.4	Aa	52.0	±	4.4	Aa
T2	53.2	±	5.0	Aa	52.4	±	7.6	Aa	76.0	±	24.8	Aa	88.0	±	12.8	Aa
T3	75.1	±	10.1	Aa	66.0	±	6.9	Aa	61.9	±	27.0	Aa	87.4	±	17.5	Aa
T4	70.1	±	5.2	Aa	41.2	±	20.4	Aa	57.9	±	9.8	Aa	65.8	±	3.2	Aa
T5	69.6	±	12.0	Aab	51.0	±	4.7	Ab	61.2	±	4.6	Aab	112.5	±	24.5	Aa
T6	66.1	±	13.8	Aa	56.2	±	18.5	Aa	66.7	±	18.5	Aa	93.0	±	10.4	Aa

The values are the averages of three independent biological replicates (±standard error). The significant differences ($p \leq 0.01$) between treatment factors: uppercase letters indicate significant differences ($p \leq 0.05$) in the treatment compared to different times of evaluation, lowercase letters indicate significant differences ($p \leq 0.05$) between treatments in the same time evaluation (ANOVA two-way), according to the Tukey test.

3.9. Multivariate Analysis

To identify potential associations between the variables evaluated, we performed Pearson correlation analysis and calculated the coefficients for all determinations at 7, 14, 21, and 28 days, with the different treatments of ASC and Al toxicity in leaves and roots (Figure 6). In the leaves of Star, 23 positive and 21 negative significant correlations ($p < 0.05$) were obtained. The significant negative correlations were observed mainly between the RGR with Chl*a*, Chl*b*, total chlorophylls, SOD, and *Pn*, and AA with *Chla*, *Chlb*, total chlorophylls and SOD (Figure 6A). In contrast, significant positive correlations were observed between LP with ASC, DHA, AA, RGR, total chlorophylls and proteins, and DHA with AA, RGR, carotenoids, proteins and LP (Figure 6A). On the other hand, in the roots 14 positive and 10 negative significant correlations were registered, showing a significant positive correlation between proteins with Ca content, ASC, DHA, AA, LP and SOD (Figure 6B) and significantly negative correlations between Ca content with Al content and OA exudate, and OA exudate with ASC and DHA (Figure 6B).

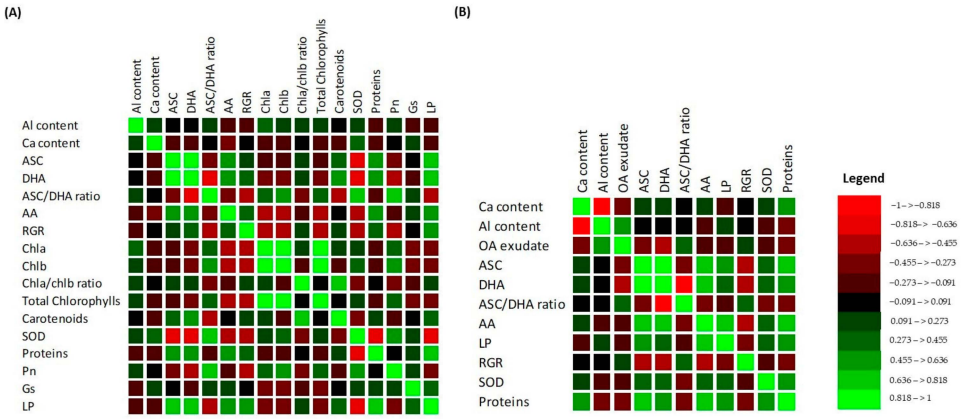

Figure 6. Pearson correlation of leaves (**A**) and roots (**B**), of Star cultivar of *V. corymbosum* exposure to different ASC and Al toxicity doses. Abbreviations: ASC: ascorbic acid, DHA: dehydroascorbate, OA exudate: organic acids exudate, AA: antioxidant activity, RGR: relative growth rate, Chl*a*: chlorophyll *a*, Chl*b*: chlorophyll *b*, SOD: superoxide dismutase, *Pn*: photosynthesis net, *gs*: stomatal conductance, LP: lipid peroxidation.

Furthermore, we performed a hierarchical clustering analysis and present the results as a heat map (Figure 7). This analysis revealed a clear trend in both tissues concerning time, ASC dose, and physiological and biochemical parameters. A positive pattern was observed in the leaves in Chl*a*, Chl*b*, *Pn*, and Fv′/Fm′ (Supplementary Table S2), particularly in treatments with high ASC doses, mainly at 7 and 14 days. In contrast, antioxidant parameters such as DHA, ASC, and AA showed higher levels at 21 and 28 days, along with RGR and proteins. Additionally, the clustering of Al and Ca indicated higher levels (green) at 7 and 14 days, while lower levels (red) were recorded at 21 and 28 days (Figure 7A). In roots, OA, AA, and the ASC/DHA ratio exhibited a strong negative response at 7 and 14 days, the opposite of that observed at 21 and 28 days, where positive responses were recorded (Figure 7B).

Regarding time-based clustering, which reflects the evolution of physiological and biochemical responses, distinct patterns were identified in both tissues between early (7 days) and late (28 days) time points, suggesting an effect of temporal dynamics on physiological and biochemical responses, with antioxidant and photosynthetic parameters playing a key role in this differentiation.

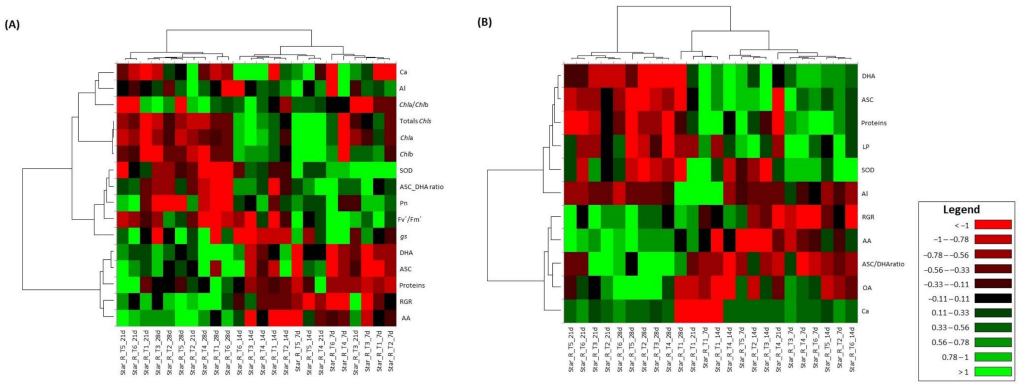

Figure 7. Clustering analyses in leaves (**A**) and roots (**B**) of Star cultivar of *V. corymbosum* exposure to different doses of ASC and Al toxicity. Hierarchical clustering was based on Euclidean distances and average as the metric parameter and linkage method. Abbreviations: ASC: ascorbic acids, DHA: dehydroascorbate, OA: organic acids exudate, AA: antioxidant activity, RGR: relative growth rate, Chl*a*: chlorophyll *a*, Chl*b*: chlorophyll *b*, Total Chls: total chlorophylls, SOD: superoxide dismutase, *Pn*: photosynthesis net, Fv′/Fm′: maximum efficiency of PSII in the light, *gs*: stomatal conductance and, LP: lipid peroxidation.

4. Discussion

Currently, 50% of the world's agricultural lands have a pH value below 5 [15,51]. This condition facilitates the solubility of Al into monomeric and toxic forms (Al $(OH)^{2+}$, Al^{3+}, Al $(OH)_2{}^{+}$, and Al $(OH)_4{}^{-}$) [15]. It has been documented that the trivalent form (Al^{3+}) is the most deleterious to plants, as it disrupts growth and productivity, induces cell wall damage, increases the ROS levels, and reduces plant development, ultimately leading to negative effects on plant growth and production [52,53]. In this study, a positive effect of applying high doses of ASC in highbush blueberry enhanced the plant growth of Star, an Al-sensitive cultivar, with 200 mg L^{-1} of ASC and 400 µM of Al. Similar results were previously observed in a study performed by Ye et al. [54]. In this study, using rice plants, a positive effect of ASC on seed germination was observed in its role in regulating phytohormone synthesis such as ethylene, ABA, and gibberellin (GA). For instance, ASC can influence ABA and GA synthesis, thereby regulating plant growth, development processes, and abiotic stress tolerance [9,55,56]. An effective alleviating effect was observed in foliar-applied ASC (150 mg L^{-1}) to cauliflower plants under water deficit conditions, improving plant growth due to a reduction in H_2O_2 content and membrane permeability [57]. Likewise, Paciolla et al. [58] reported ASC as a positive factor influencing plant cell growth, division, differentiation, and metabolism.

Aluminum is a non-essential metal for plants; it is documented that toxic levels of aluminum in plants reduce root and shoot growth and increase its content in both tissues in Al-sensitive cultivars under acidic conditions [59]. In this study, the treatments with Al always increased Al content at all times. In contrast, foliar ASC applications registered a minor content of Al in roots, as compared with leaves where the observed trend was the opposite (major Al-content, minor Ca-content, or vice versa) (view Figures 1 and 6). These differences could be an effect of the competition between Al and cations (Ca^{2+} and Mg^{2+}) on the root surface in plants. Additionally, altered levels of Ca were observed in our study under Al and ASC treatments, as well as an increase in Ca content in plant roots from the Star cultivar in the first days of treatment. This response agrees with the findings by [60]. This study indicated that ASC application enhanced the level of carbohydrates, protein, and nutrients (N, P, K, and Ca content) in *Pisum sativum* plants. Furthermore, it has been indicated that ASC serves as a substrate for oxalate biosynthesis, which can influence cell wall elongation and pectin crosslinking by binding Ca^{2+} [6]. In this context, DHA is

converted in the cell wall to oxalate, by binding with Ca^{2+} through crystallization, thereby regulating Ca^{2+} levels within the cell [6,61]. On the other hand, under other abiotic stresses, ASC application induces changes in chlorophyll pigments and regenerates the ASC pool by oxidizing carotenoids and tocopherols [62,63].

In fact, at the physiological level, it has been suggested that ASC acts as an alternative electron donor for PSII because it is able to donate and accept electrons from the electron transport chain (ETR) [64]. Our results showed a high net photosynthesis (*Pn*) level at 7 d under 200 mg L^{-1} ASC treatment (T5), accompanied by increases in chlorophyll levels. However, both declined by the end of the evaluation period. Regarding carotenoids, in this study, higher levels were observed in 100 mg ASC and 400 mg L^{-1} ASC (T4 and T6) treatments at 7 days, with significant increases, particularly at 28 d (Figure 3). Ascorbate has been described as being associated with the xanthophyll cycle, where it scavenges ROS and facilitates the smooth transport of electrons during photosynthesis, as well as contributing to the dissipation of excess energy under heavy metal and oxidative stress conditions in plants [65,66]. Furthermore, ASC has been described to indirectly promote the conservation and regeneration of oxidized carotenoids and tocopherols in plants [67]. Furthermore, ASC is a potent inhibitor of *2CPA* expression, influencing signaling pathways between the chloroplast and nucleus via redox-sensitive transcription factor *Rap2.4a* [68,69]. For example, under water stress conditions, foliar application of 400 mg L^{-1} of ASC increased carotenoid levels in *Phaseolus vulgaris* plants [70]. Otherwise, the production and activation of antioxidant mechanisms to alleviate Al-induced ROS accumulation are associated with enzymatic and non-enzymatic processes that form part of the defense system, helping to mitigate Al stress in plants [15].

Regarding the enzymatic pathway, SOD is recognized as the first line of the antioxidant defense mechanism. This enzyme, along with catalase (CAT) and glutathione peroxidase (GPX), is one of the molecules that collectively act against free ROS (H_2O_2/alcohol and O_2) induced by abiotic and biotic stress [15]. Meanwhile, the non-enzymatic system includes carotenoids, tocopherols, phenolic compounds, and flavonoids [71]. Indeed, it is documented that ASC is essential for flavonoid synthesis, as it acts as a cofactor in flavonoid biosynthesis pathways. Ascorbate has an indispensable bioactive role in maintaining the active center of the 2 oxoglutarate/Fe (II)-dependent dioxygenase (2-ODD) protein family, which participates in hydroxylation, desaturation, and demethylation during stages of plant growth, development, fruit ripening, and abiotic stress responses [68,72]. Our study showed that 200 mg L^{-1} and 400 mg L^{-1} of ASC increased AA and reduced LP. This was observed concurrently with no significant changes in or low activity of SOD, which might be attributed to the increased ASC levels (both reduced and oxidized forms), suggesting the activation of a non-enzymatic defense response at 28 days. Conversely, under Pb toxicity in wheat plants, exogenous application of ASC in plant exposure to 2 mM of Pb mitigated Pb-induced oxidative damage [73]. This mitigation was mediated by enhanced activities of antioxidant enzymes, such as SOD, CAT, and glutathione reductase (GR), as observed in *Triticum aestivum* plants [73].

Al-stress promotes the exudation of organic acid anions, such as citrate and malate, as a resistance strategy against Al-toxicity. The organic acids have the ability to bind Al and compete with the binding capability of Ca-transporters and pectin to Al, thereby reducing the entry of this element into the plant cell [74]. Our study showed that oxalic acid was exudated under 200 mg L^{-1}ASC foliar treatment in Al toxicity conditions by the end of the experiment (Table 3). This result may be related to variations in the concentration of both forms of ASC (reduced and oxidated) in T5 at 28 d in roots (Figure 4), suggesting activation of the ASC recycled pathway. In this pathway, DHA is degraded to generate oxalate alongside the inhibition of DHA reductase (DHAR) expression [75]. This mechanism appears to be identified as a crucial strategy for Al tolerance in crops [76–78]. In addition,

the ASC/DHA ratio is considered critical for understanding redox balance because ASC-DHA redox signaling transmits the cellular redox state in response to changes in ROS levels under abiotic and biotic conditions in plants [79]. In this study, a strong positive correlation was observed between the ASC/DHA ratio and Chlb, SOD, and *Pn* in the T5 treatment (200 mg ASC+Al) (Figure 6A). This response aligns with the findings of Xiao et al. [2] who reported that a high ASC/DHA ratio can is associated with a strong ability of plants to cope with abiotic stress. Furthermore, the ASC/DHA ratio exhibits a strong negative correlation with DHA in both tissues, leaves, and roots (Figure 6). This may be due to the fact that Al^{3+} induces the generation of ROS in plants, leading to the oxidation of ASC to DHA [2,80]. Consequently, higher levels of DHA accumulate in plant cells as the increased ROS demands greater ASC for naturalization. Another possible reason for elevated DHA levels could be the toxic effect of aluminum on plants, which inhibits the activity of enzymes such as DHAR and GR, which are responsible for recycling DHA back into ASC [81].

Finally, multivariate analyses show a higher relation with key determinations such as aluminum content, photosynthesis, and organic acid, among others, which are identified as part of the Al-resistance mechanism. For example, leaf trends are found as negative metabolic responses dominate among pigments and some antioxidants (e.g., carotenoids). In contrast, positive responses are observed in protein content and certain antioxidants (e.g., ASC, DHA, and AA), while in the roots, the specific trends were found to increase in specific parameters like ASC and OA exudates, but with important reductions in growth-related metrics like RGR. These results suggested a specific response in each organ, highlighting differential metabolic and biochemical adjustments between the leaves and roots, likely tied to their respective roles in stress mitigation and resource acquisition. Mehmood et al. [64] indicate that ASC can stimulate some physiological processes and inhibit others; this response depends on the ASC doses used, the plant species, development stage, and environmental conditions to which different plants are subjected.

5. Conclusions

The results suggest that foliar application of exogenous ASC in doses of 200 mg L^{-1} can mitigate the oxidative damage promoted by Al in toxic levels. The ASC treatment improves growth and regulates the physiological responses in plants of the blueberry cultivar Star, an Al-sensitive cultivar under Al stress conditions. Furthermore, it is important to highlight the interaction between ASC application and organic acid exudation, underscoring its role in Al-resistance mechanisms and as a practical solution to improve plant resilience in stress environments. These findings provide insights to optimize the use of ASC to improve the performance of plants grown under Al-toxicity conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11030330/s1. Table S1: Analysis statistics. Photosynthetic pigments. Table S2. Analysis statistics: Fluorescence parameter. Figure S1: Star cultivar plants in 18 L pots in Hoagland's solution and controlled conditions. Figure S2: Images of the roots before starting the experiment.

Author Contributions: Conceptualization, P.C.-F.; methodology, P.C.-F., J.T.-M. and R.M.-S.; software, P.C.-F.; formal analysis, P.C.-F.; investigation, P.C.-F. and M.R.-D.; resources, P.C.-F., M.R.-D. and C.I.-B.; writing—original draft preparation, P.C.-F.; writing—review and editing, P.C.-F., M.R.-D., C.I.-B., A.N.-N., B.S.-C. and R.T.-N.; supervision, M.R.-D.; visualization, P.C.-F. and M.R.-D.; project administration, P.C.-F., M.R.-D. and C.I.-B.; funding acquisition, P.C.-F., M.R.-D. and C.I.-B. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

ASC	Ascorbic acid/ascorbate
DHA	Dehydroascorbate
OA	Organic acids
LP	Lipid peroxidation
AA	Antioxidant activity
SOD	Superoxide dismutase
CAT	Catalase
GPX	Glutathione peroxidase

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