Silicon Improves the Production of High Antioxidant or Structural Phenolic Compounds in Barley Cultivars under Aluminum Stress

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Abstract: Aluminum (Al) toxicity is one of the main growth and yield limiting factors for barley grown on acid soils. Silicon (Si) ameliorates Al toxicity as well as it promotes the phenolic compounds production that have antioxidant or structural role. We evaluated the time-dependent kinetics of Al and Si uptake and the impact of Si on the production of antioxidant- or structural- phenols in barley cultivars at the short-term. Two barley cultivars with contrasting Al tolerance (*Hordeum vulgare* 'Sebastian', Al tolerant; and *H. vulgare* 'Scarlett', Al sensitive), exposed to either −Al (0 mM) or +Al (0.2 mM) nutrient solutions without Si (−Si) or with 2 mM (+Si) were cultured for 48 h. Aluminum and Si concentration decreased in plants at all harvest times when Al and Si were simultaneously supplied; this effect was more noticeable in 'Scarlett'. Nevertheless, Si influenced the antioxidant system of barley irrespective of the Al tolerance of the cultivar, decreasing oxidative damage and enhancing radical scavenging activity, the production of phenolic compounds, and lignin accumulation in barley with short-term exposure to Al.

Keywords: aluminum toxicity; barley; phenolic compounds; lignin

1. Introduction

Barley (*Hordeum vulgare* L.) is used as a feedstock due to its elevated nutritional value, particularly carbohydrates, proteins, vitamins, and phenolic compounds [1]. Barley also contains a large amount of fiber, a well-recognized source of β-glucans that reduces cholesterol and the glycemic index [2]. One of the main limitations for the production of barley is its sensitivity to soil acidity, which decreases the yield of grains [3]. In acid soils, aluminum (Al) is solubilized as phytotoxic Al^{3+} ions [4]. This inhibits root growth and nutrient uptake by altering the structure and function of the cell wall and plasma membrane, and therefore limits crop production [4,5]. Furthermore, it has been shown that Al toxicity increases the formation of reactive oxygen species (ROS) triggering oxidative damage in plant cells [5,6]. However, the degree of toxicity differs with the plant species, growth conditions, concentration, and time of exposure to Al stress [4,7]. Barley exhibits considerably higher sensitivity to Al toxicity than rice, rye, oats, and wheat [8,9].

Despite Si is not recognized to be an essential nutrient for vascular plants, it has widely been demonstrated that Si supply increases crop production, root volume, and leaf density, and also decreases diseases and pest attack in several plant species exposed to biotic and abiotic stresses [10–15]. Over recent years, it has been shown that Si enhances the primary metabolism by improving photosynthesis [11,16,17] and nutrient uptake [18], as well as the secondary metabolism through stimulation of the production of phenolic compounds with either antioxidant (e.g., flavonoids) or structural (e.g., lignin) functions [19–21]. Therefore, it has been reported that Si causes improvement in either antioxidant or structural phenols metabolism of plants under stressful environmental conditions [19,20,22,23]. This occurs via the regulation of the transcript level or activity of enzymes involved to phenylpropanoid pathway (e.g., phenylalanine ammonia lyase, peroxidase) [23–25], formation of lignin-carbohydrate complexes [26,27], and direct complexation of Si with polyphenolic compounds [22].

Several reports have suggested that the positive effects of Si on plants are closely related to the high accumulation of this element in different tissues [28,29]. Nevertheless, a differential capacity to take up Si has been reported among plant species and genotypes [28–30]. In general, poaceae species such as barley have been classified as "accumulators", since they can contain up to 1% of Si in dry matter basis [31]. Recent molecular advances associated with the identification and characterization of Si transporters in various plant species have been useful to improve the understanding of the benefits that plants can derive from Si uptake [12,28,29]. Accordingly, influx transporters (Lsi1 and Lsi6; belonging to the subgroup of aquaporins Nodulin 26-like intrinsic proteins III) and efflux transporters (Lsi2 and Lsi3; known as anion transporters) have been reported to be responsible for the uptake and transport of Si in different plant species including barley [29,32–34]. Moreover, the identification of highly conserved features in Si influx transporter determining the functional selectivity for silicic acid provided new insights into the prediction of Si uptake ability of plants [29,35–37].

Silicon is currently viewed as a sustainable alternative to provide tolerance to various metals including zinc (Zn), iron (Fe), copper (Cu), cadmium (Cd), chromium (Cr), manganese (Mn), arsenic (As), and Aluminum (Al) [10,23,38–42]. Accordingly, the amelioration of Al toxicity by Si has so far been demonstrated in numerous crops, including rice, wheat, maize, sorghum, ryegrass, and soybean [12,23,43–45]. In addition, some research has indicated that Si supply could stimulate plant growth processes and decrease the intensity of lipid oxidative damage [23,44,46]. However, the mechanisms induced by Si against Al stress are not yet entirely clear. Nevertheless, it has been demonstrated that Si addition in the presence of Al generates an increase in the culture media pH, and reduces of the availability of phytotoxic Al for plants, due to the formation of hydroxyaluminosilicates in solution [43]. Furthermore, there is evidence showing internal detoxification of Al through the formation of aluminosilicates in plant cell walls [43]. The Si-induced exudation of phenolic compounds with the ability to chelate Al has been also reported [47].

Currently, there is evidence shown that Si could alleviate Al toxicity in barley [46]. Nevertheless, to our knowledge there is a dearth of studies regarding the role of Si in the metabolism of barley, which is one of the most sensitive cereal crops to Al toxicity. Furthermore, the influence of Si on the phenolic metabolism of Al tolerant or sensitive barley genotypes it has not yet been studied. Thus, this study aimed to evaluate the kinetics of Al and Si uptake, as well as the impact of Si on the production of antioxidant or structural phenolic compounds in barley cultivars in the short-term.

2. Materials and Methods

2.1. Hydroponic Experiment

Two barley (*Hordeum vulgare* L.) cultivars with differing Al tolerances (*H. vulgare* 'Scarlett', Al sensitive, and *H. vulgare* 'Sebastian', Al tolerant) were selected from a preliminary hydroponic assay based on the growth parameters and oxidative damage in roots of 11 cultivars subjected Al stress (Supplementary Figure S1). Seeds of 'Scarlett' and 'Sebastian' cultivars were surface sterilized by

immersion in 2% (v/v) sodium hypochlorite for 15 min, and then washed five times with deionized water. The sterilized seeds were germinated on moistened filter paper for 10 days. Once germinated, seedlings were transferred to aerated hydroponic culture pots (3L pots; 48 plants per pot) and cultivated in a basal nutrient solution proposed by Taylor and Foy [48]. During the course of the experiment, dilute HCl or NaOH was used to adjust daily the pH of the solution to 4.5. All plants were precultured for 14 days in a basal nutrient solution and the nutrient solution was changed every 5 days. The plants were then grown in nutrient solutions containing either 0 mM Al (−Al) or 0.2 mM Al (+Al; applied as $AlCl_3$, Merck KGaA, Darmstadt, Germany) in combination with 0 mM Si (−Si) or 2 mM Si (+Si; supplied as Na_2SiO_3, Merck KGaA, Darmstadt, Germany). The free Al^{3+} activity in the nutrient solution was calculated using Geochem-EZ [49] and corresponded to 85 μM. A factorial experimental design completely randomized, considering three replicates per treatment, was used. At harvest, plant tissues (roots and shoots) were sampled at 2, 4, 8, 12, 24, and 48 h after Al/Si treatments. Subsamples of shoots and roots were harvested and stored at either −20 °C or −70 °C for biochemical analyses, measurement of dry weight, and determination of the concentrations of Si and Al.

2.2. Plant Chemical Analyses

Mineral concentration of Al and Si was determined after fresh plant material was dried at 65 °C for 48 h. For Al analysis, root and shoots samples were ashed at 500 °C during 8 h and 2 M hydrochloric acid was added. Flame atomic absorption spectrophotometry (FAAS) was used to quantify Al at 324.7 nm according to Sadzawka et al. [50]. Silicon concentration was determined by the modified method described by Pavlovic et al. [51]. An acid digestion was carried out by using 0.1 g of dried plants samples with 5 mL of HNO_3 on a hot plate at 70 °C for 5 h. Afterward, 1 mL of HF (40%) and 10 mL of distilled water were applied, and the samples were left overnight. Then, 5 mL 2% (w/v) H_3BO_3 was added, the flask volume was adjusted to 25 mL with distilled water, and Si was determined by FAAS at 251.6 nm.

2.3. Plant Biochemical Analyses

Plant samples previously stored at −70 °C were used to analyze the total soluble phenols and radical scavenging activity. Total soluble phenols were assayed according to the method of Slinkard and Singleton [52] using Folin–Ciocalteu reagent. Chlorogenic acid was used as standard, and the absorbance was determined in spectrophotometer at 765 nm. Additionally, radical scavenging activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to Chinnici et al. [53]. The absorbance was spectrophotometrically tested at 515 nm, using Trolox as the standard. The radical scavenging activity was calculated as Trolox equivalents.

Plant lipid peroxidation was monitored by assaying the thiobarbituric acid reactive substances (TBARS) by the method of Du and Bramlage [54]. In this method, the absorbance of the sample is determined at 532, 600, and 440 nm to minimize the interference generated by TBARS-sugar complexes.

2.4. Lignin Visualization Assay

To visualize the lignin distribution in plant tissues, fresh roots, and leaf sections were stained with 0.1% Safranine O and analyzed using Laser Scanning Confocal Microscopy (CLSM; Olympus FV1000, Arquimed, Japan) at λ emission/excitation of 543/590 nm, following the methods of Sant' Anna et al. [55]. The images were analyzed with the Image Processing Software (FV10-ASW v0.200c; Olympus, Tokyo, Japan). In addition, the detection of safranine fluorescence was expressed as Relative Fluorescence Unit (RFU). Thus, different regions of interest in each image were selected and the RFU averages calculated.

2.5. Data Analysis

The data were subjected to analysis of variance (ANOVA), and significantly different means between treatments were tested using the least-significant difference (LSD) at a 0.05 significance level of probability. In addition, Pearson correlation was used to evaluate the relationship between two response variables.

3. Results

3.1. Concentration of Si and Al

Aluminum concentration gradually increased in the roots and shoots of barley cultivars after the Al application to the hydroponic solution (Figure 1). In general, at 48 h the Al sensitive cultivar 'Scarlett' showed a higher tissue Al concentration than was observed for the Al tolerant cultivar 'Sebastian'. After 48 h, most of the Al had been accumulated in the shoots of both cultivars, and Al translocation was greater in 'Scarlett' (72%) than it was in 'Sebastian' (58%).
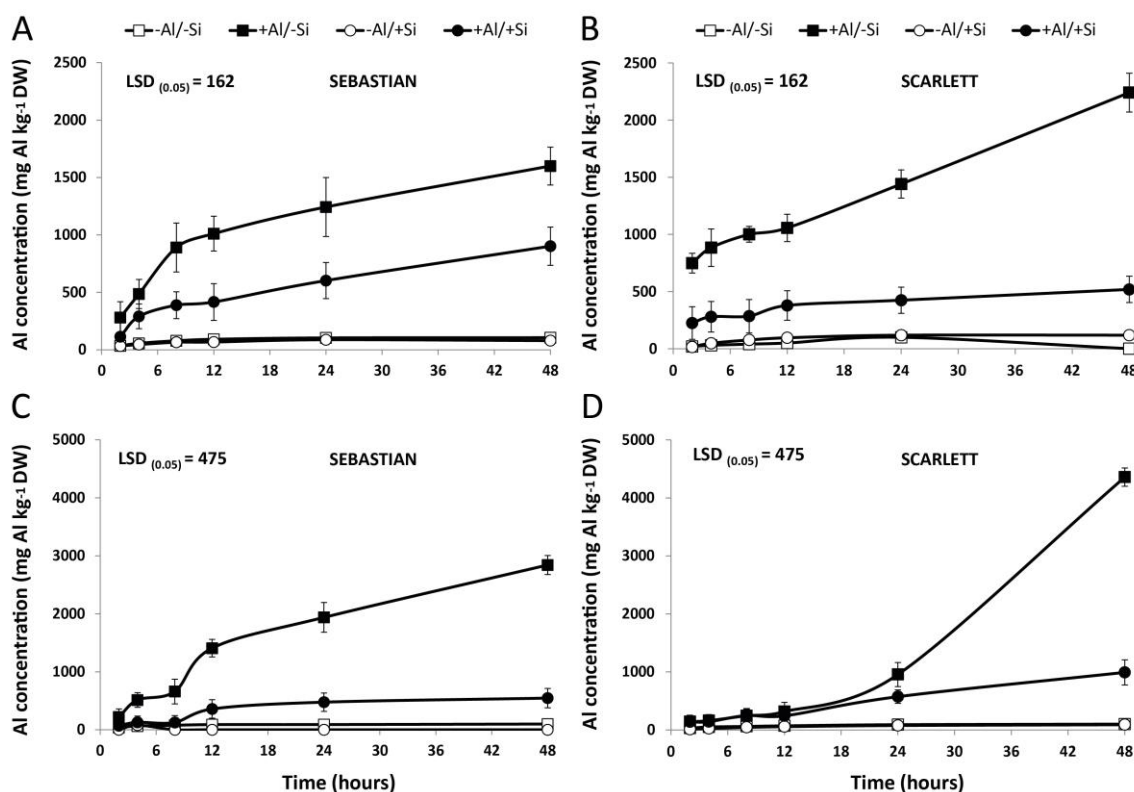

Figure 1. Aluminum concentration of shoots (**A,B**) and roots (**C,D**) in barley cv. 'Sebastian' (Al-tolerant) and 'Scarlett' (Al-sensitive) harvested at 2, 4, 8, 12, 24, and 48 h. The plants were subjected to −Al and +Al (0.2 mM) nutrient solution without (−Si) or with (+Si) supply of 2 mM Si. Values correspond to the average of three replicates ± standard deviation. Differences between treatments were examined using the LSD test ($p \leq 0.05$).

In plants supplied with Al, a rapid and significant reduction in the Al concentration in the roots and shoots was induced by Si, respect to plants treated with Al alone. Thus, the Al sensitive cultivar 'Scarlett' showed further reductions in Al concentration of about 63% (shoots), and 76% (roots) due to the addition of Si after 48 h. Likewise, in the Al tolerant cultivar 'Sebastian', Si addition reduced Al uptake by about 59% and 43% in shoots and roots, respectively. Silicon concentration in the shoots and roots (Figure 2) increased progressively over time by the addition of Si, but when both Al and Si were applied, this increase was lower in both cultivars. The highest Si concentration was recorded after 48 h

in plants treated solely with Si (−Al/+Si). This increased the Si concentration 9.2-fold (shoots) and 8.8-fold (roots) for the Al tolerant cultivar 'Sebastian' and 12.5-fold (shoots) and 15.2-fold (roots) for the Al sensitive cultivar 'Scarlett', compared with the control (−Al/−Si).
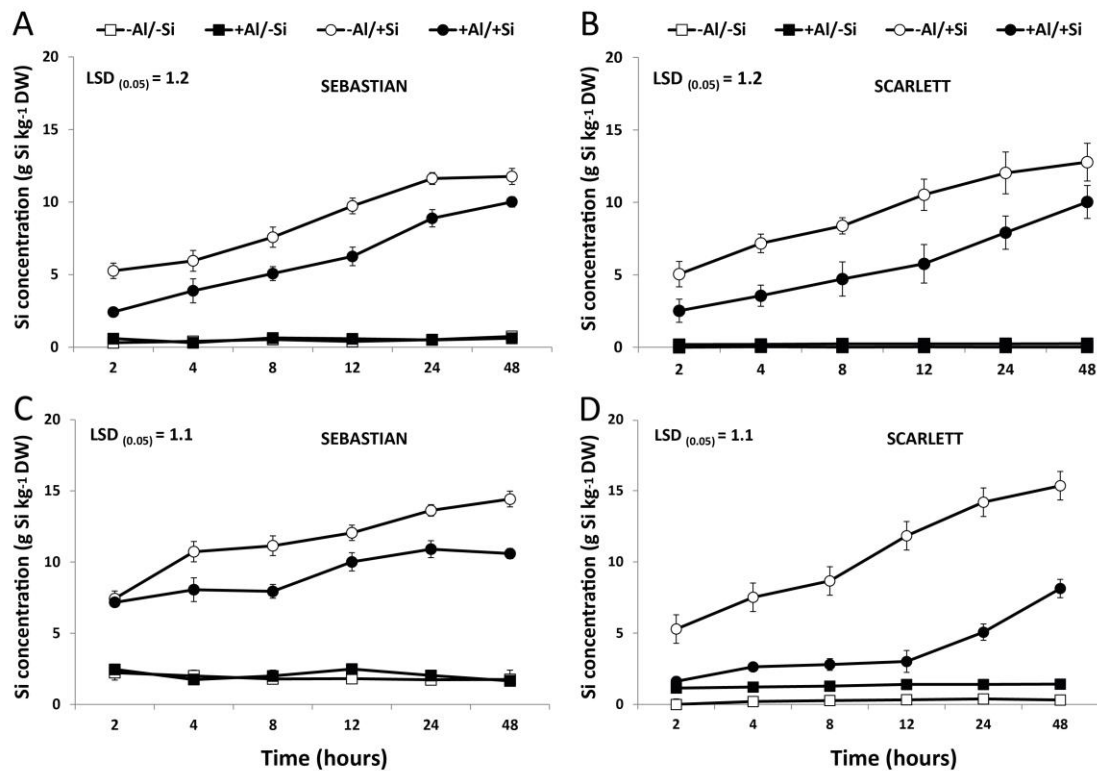

Figure 2. Silicon concentration of shoots (**A,B**) and roots (**C,D**) in barley cv. 'Sebastian' (Al-tolerant) and 'Scarlett' (Al-sensitive) harvested at 2, 4, 8, 12, 24, and 48 h. The plants were subjected to −Al and +Al (0.2 mM) nutrient solution without (−Si) or with (+Si) supply of 2 mM Si. Values correspond to the average of three replicates ± standard deviation. Differences between treatments were examined using the LSD test ($p \leq 0.05$).

3.2. Antioxidant and Structural Phenols

The concentration of phenols in plants showed a tendency to increase due to Si addition in both cultivars studied. In the Al tolerant 'Sebastian' cultivar, the highest root phenol concentration was detected in plants supplied with both Al and Si (+Al/+Si) at 48 h, showing a 2-fold increase compared with the control (Figure 3A). In contrast, the lowest concentration of phenols in the roots of the Al tolerant cultivar 'Sebastian' was found in the −Al/+Si treatment at all harvest times. During the course of the experiment, the total phenols of the 'Sebastian' shoots tended to increase with the supply of both Si and Al; the highest shoot phenol concentration was found after 48 h (47% increase, compared with the control) as shown in Figure 3A.
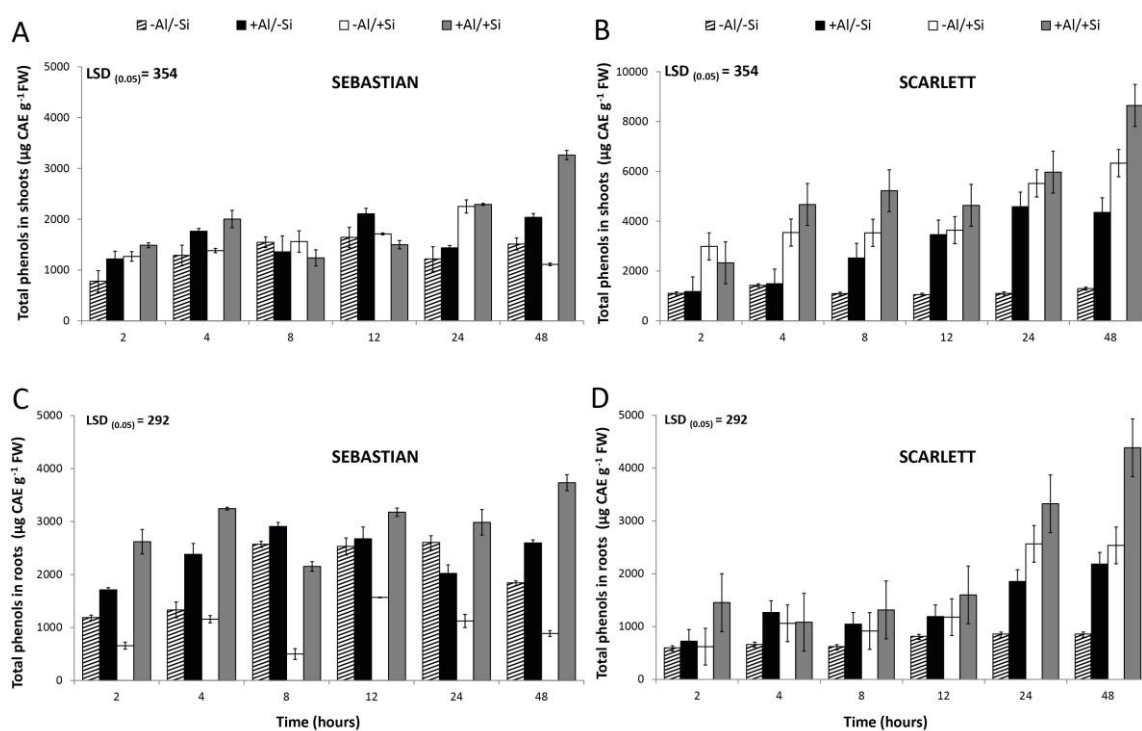
Figure 3. Total phenols of shoots (**A,B**) and roots (**C,D**) in barley cv. 'Sebastian' (Al-tolerant) and 'Scarlett' (Al-sensitive) harvested at 2, 4, 8, 12, 24, and 48 h. The plants were subjected to −Al and +Al (0.2 mM) nutrient solution without (−Si) or with (+Si) supply of 2 mM Si. Values correspond to the average of three replicates ± standard deviation. Differences between treatments were examined using the LSD test ($p \leq 0.05$).

On the other hand, in the Al sensitive cultivar 'Scarlett', the lowest concentration of phenols in the roots was observed in the control treatment (−Al/−Si) at all harvest times (Figure 3D). The total phenols of the roots steadily increased over time with the addition of Al; when both Al and Si were added (+Al/+Si), the total root phenols doubled after 48 h, compared to the Al-treated plants that did not have Si added (+Al/−Si).

Similarly, the total phenols of the Al sensitive cultivar 'Scarlett' augmented in the shoots after 2 h as a consequence of the addition of Si, irrespective of the presence or absence of Al (Figure 3B). The highest shoot phenol concentration was found after 48 h in the +Al/+Si treatment (6.7-fold increase, compared with the control), whereas the lowest concentration was recorded in +Al/−Si and in the control treatments at 2 and 4 h after treatment.

During the time-course of the experiment, confocal microscopy analysis showed a higher lignin accumulation in the roots of Al sensitive cultivar 'Scarlett' than the Al tolerant cultivar 'Sebastian', irrespective of Al/Si added (Figure 4). Compared with non-treated plants, an increment of lignin content was detected in both cultivars as a consequence of 0.2 mM Al supply, with a further increase being observed in plants simultaneously supplied with Al and Si.
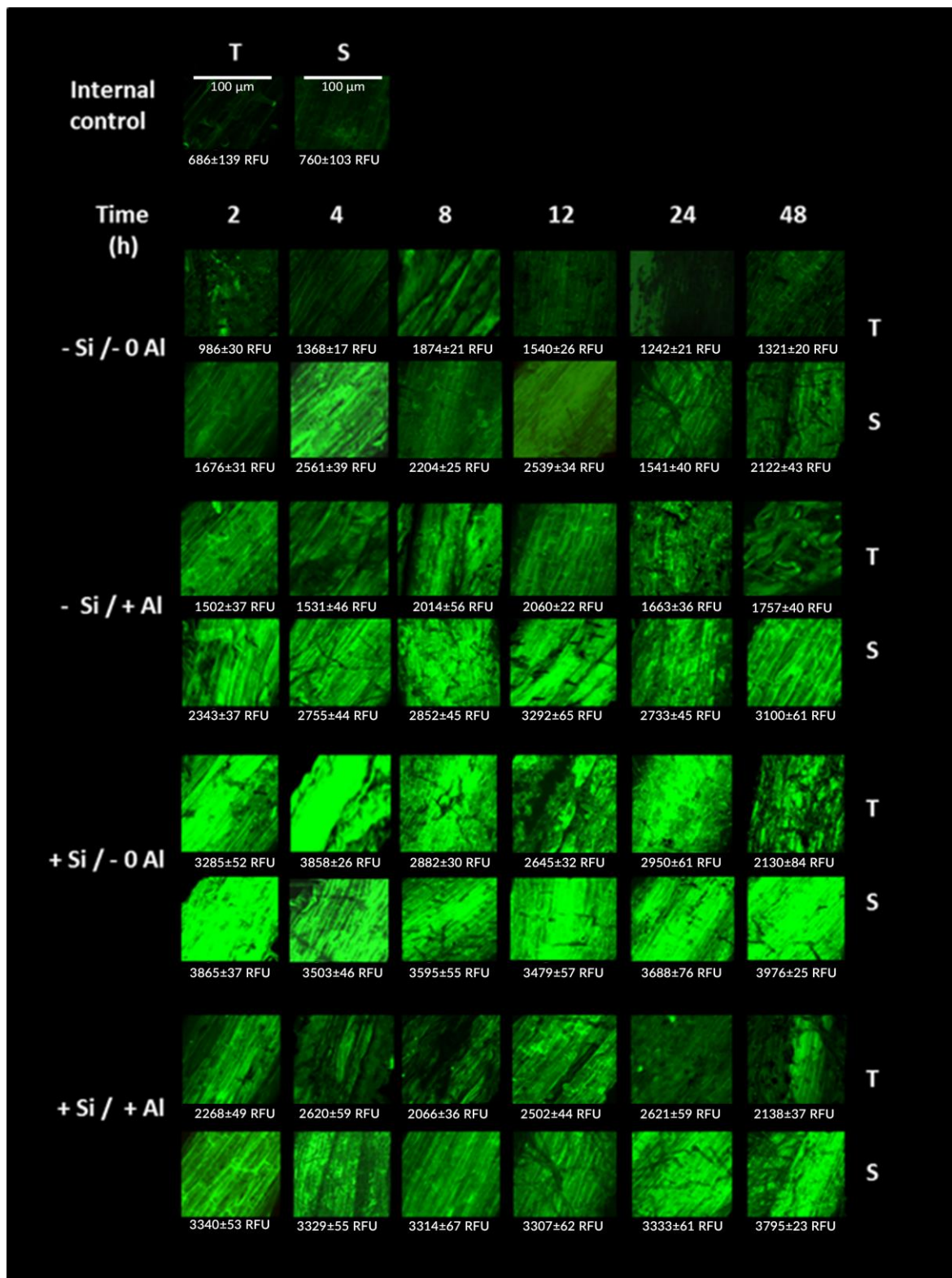
Figure 4. Visualization of lignin contents in barley roots of 'Sebastian', Al-tolerant (T); and 'Scarlett', Al-sensitive (S) harvested at 2, 4, 8, 12, 24, and 48 h. The plants were subjected to −Al and +Al (0.2 mM) nutrient solution without (−Si) or with 2 mM Si (+Si). The detection of safranine fluorescence was expressed as relative fluorescence unit (RFU).

3.3. Plant Antioxidant Capacity and Oxidative Damage

Radical scavenging activity increased with the Al treatment at all harvest times in both Al sensitive and Al tolerant cultivars. Moreover, DPPH and Al concentration were positively correlated in roots ('Sebastian', $r = 0.436$; $p \leq 0.01$) and shoots ('Sebastian', $r = 0.684$; $p \leq 0.01$; 'Scarlett', $r = 0.401$; $p \leq 0.01$). A simultaneous application of Al and Si further increased DPPH compared with the control (Figure 5). For the Al tolerant cultivar 'Sebastian', the highest antioxidant capacity (7-fold higher in shoots, and 5-fold higher in roots, compared with the control) was observed after 48 h of exposure to both Al and Si (Figure 5A,C). For the Al-sensitive cultivar 'Scarlett', the highest increase in the antioxidant capacity was also observed after 48 h of exposure, as a consequence of the simultaneous addition of Si and Al (9-fold higher in shoots and 10-fold higher in roots as compared to the control; Figure 5B,D).
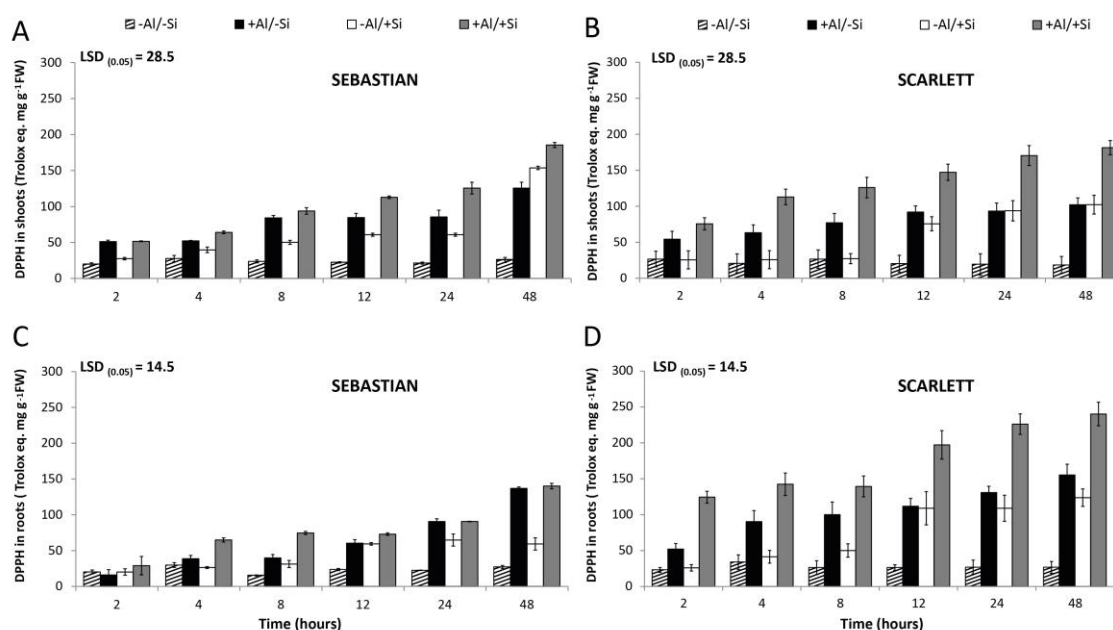

Figure 5. Free radical scavenging of shoots (**A**,**B**) and roots (**C**,**D**) in barley cv. 'Sebastian' (Al-tolerant) and Scarlett (Al-sensitive) harvested at 2, 4, 8, 12, 24, and 48 h. The plants were subjected to −Al and +Al (0.2 mM) nutrient solution without (−Si) or with (+Si) supply of 2 mM Si. Values correspond to the average of three replicates ± standard deviation. Differences between treatments were examined using the LSD test ($p \leq 0.05$).

A steady increase in the lipid peroxidation of both cultivars was found across all harvest times as a result of the application of Al, and this increase was more apparent in the Al sensitive cultivar 'Scarlett' than in the Al tolerant cultivar 'Sebastian' (Figure 6). A positive correlation was found for both cultivars between Al uptake and lipid peroxidation in the roots ('Sebastian', $r = 0.828$; $p \leq 0.01$; 'Scarlett', $r = 0.546$; $p \leq 0.01$) and shoots ('Sebastian', $r = 0.741$; $p \leq 0.01$; 'Scarlett', $r = 0.713$; $p \leq 0.01$). For 'Sebastian', the highest increase of TBARS content relative to the control was observed after 12 h, showing an increase of 54% (shoots) and 46% (roots) due to Al addition (Figure 6A,C). In contrast, the lowest TBARS concentration occurred at 48 h in plants treated simultaneously with Si and Al. For 'Scarlett', the highest augment of TBARS relative to the control was observed at 48 h in the Al treatment, with an increase of 52% (shoots) and 45% (roots) relative to the control (Figure 6B,D). Silicon treatment significantly diminished lipid peroxidation in plants supplied with Al, and the lowest TBARS concentration was registered at 48 h after treatment in both cultivars. Furthermore, a negative correlation was observed between Si uptake and lipid peroxidation in either roots ('Sebastian', $r = -0.701$; $p \leq 0.01$; 'Scarlett', $r = -0.299$; $p \leq 0.05$) or shoots ('Sebastian', $r = -0.655$; $p \leq 0.01$).
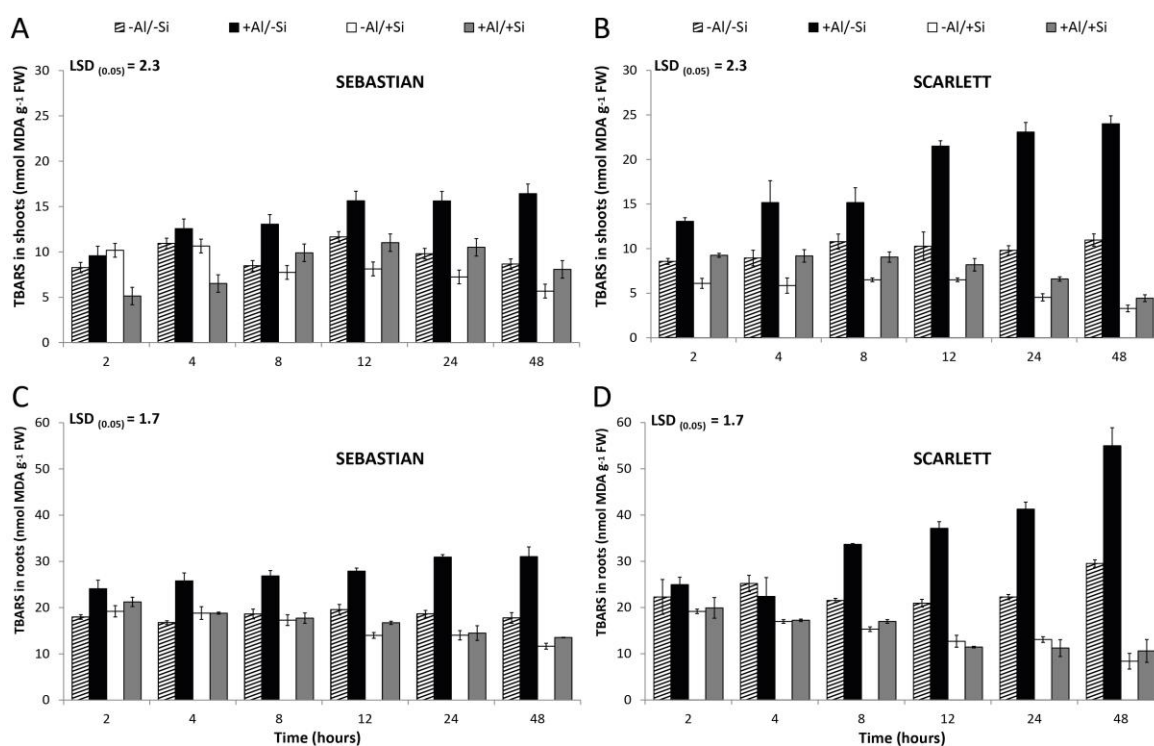
Figure 6. Lipid peroxidation of shoots (**A**,**B**) and roots (**C**,**D**) in barley cv. 'Sebastian' (Al-tolerant) and 'Scarlett' (Al-sensitive) harvested at 2, 4, 8, 12, 24, and 48 h. The plants were subjected to −Al and +Al (0.2 mM) nutrient solution without (−Si) or with (+Si) supply of 2 mM Si. Values correspond to the average of three replicates ± standard deviation. Differences between treatments were examined using the LSD test ($p \leq 0.05$).

4. Discussion

Although numerous researches have shown that Si stimulates the growth and development of plants under Al stress [12,43,45], only very few studies have been undertaken using barley. So far, there have not been any studies conducted on the secondary metabolism response of barley to Si/Al interactions. This is the first report about the impact of Si on the production of phenolic compounds with antioxidant or structural functions in barley cultivated under Al stress conditions.

Previous studies have shown the differential accumulation of Al between different plant species exposed to Al stress [4,56]. In our study, barley cultivars showed rapid incorporation of Al into their tissues (Figure 1). However, 'Scarlett' exhibited the highest Al accumulation and translocation from roots to shoots (about 72%). These findings denote the greater sensitivity of 'Scarlett' to Al toxicity compared with 'Sebastian', which is in agreement with previous reports on the differences in Al tolerance among genotypes [6,57].

We conducted our kinetic study over a period of only 48 h, since metabolic disruptions at the physiological and biochemical level occur as early responses triggered by Al toxicity [4,56]. The metabolic responses induced by Al at the short-term could result in decreased yields and quality of crops, due to long-term alterations of plant homeostasis [4,56,58]. An increment in both the damage to lipid membranes (Figure 6) and the production of phenols (Figure 3) as a consequence of the addition of Al were found after the second hour of treatment. In addition, the Al concentration in the roots and shoots were found to be positively correlated with either lipid peroxidation or antioxidant activity in both cultivars, which is consistent with the informed by Xu et al. [59]. Thus, as Al triggered oxidative damage to biological membranes, the plants increased the production of phenolic compounds to counteract the stress induced by Al, as had already been observed by Valentinuzzi et al. [60].

In the same way, an increase in the lignin staining intensity was observed in the roots at all harvest times in Al treated plants, compared with the control (Figure 4). In the Al sensitive cultivar 'Scarlett', the intensity of staining was stronger than in the Al tolerant cultivar 'Sebastian'. Ma et al. [61] also found higher lignin accumulation in an Al sensitive rice cultivar, compared to an Al tolerant rice cultivar; this effect was related with increased hydrogen peroxide production and peroxidase activity. Furthermore, You et al. [62] showed that the expression of lignin genes in the cell walls of soybean was induced by Al supply; this was associated with root growth inhibition by Al stress. Nevertheless, the mechanism involved in the accumulation of lignin in the roots induced by Al toxicity still needs to be clarified.

The shoot Si accumulation has been mainly associated to the root capacity to take up Si [12,28]. We observed that Si taken up by barley roots was translocated rapidly (after 2 h) to the shoots of both cultivars, irrespective of the Al addition (Figure 2) as expected considering that barley is a Si accumulator species [32–34,63]. Accordingly, considering the importance of the plant genetic predisposition to accumulate Si [29,64], molecular aspects related with the uptake and transport of Si in barley plants subjected to Al stress deserves to be investigated.

Silicon benefits have been demonstrated for plants grown under stress conditions, such as pest attack, lodging, nutrient imbalance, salinity, metal, water stress, and low temperatures [12–15]. In our study, the sole application of Si stimulated the antioxidant system of both barley cultivars, compared with the control treatment. In this way, the lipid peroxidation was reduced by Si in roots and shoots from 12 h for 'Sebastian', and at all harvest times for 'Scarlett', as shown in Figure 6. These responses might imply a direct effect of Si in attenuating the deleterious effect of low pH in the culture media (pH 4.5), as barley is very sensitive to acid conditions.

Both the shoots and the roots of the Al sensitive cultivar 'Scarlett' showed an increase in the concentrations of phenols from the Si application after 24 h (Figure 3B,D). However, this effect was not detected in the Al tolerant cultivar 'Sebastian' (Figure 3 A,C). Moreover, structural phenols such as lignin also showed an increase in root staining intensity in both cultivars studied at all harvest times (Figure 4), when Si was applied. Our results suggest that there is a direct relationship between Si and phenolic metabolism, as has previously been proposed by Filha et al. [19], Shetty et al. [20], and Schaller et al. [21], but it seems that this effect is variable between cultivars. Based on this premise, further research is needed to clarify the mechanisms underlying the potential role of Si in the modulation of phenolic metabolism, and whether these mechanisms vary between different species and different cultivars.

There are evidences have shown that Si can counteract the effects of excess of certain metals (e.g., Zn, Fe, Cu, Cd, Cr, Mn, As) [10,38–42] by promoting: (i) cell wall-binding [10], (ii) modification of gene expression of PAL enzyme [38], (iii) phenotypical structural alterations that increase root length [39], (iv) changes to the number of leaves per plant and leaf area [40], and (v) changes to the thickness of epidermal layers of the leaf [41]. With respect to Al toxicity in particular, some studies have proposed that Si may reduce plant stress by promoting: (i) increases in the solution pH [43], (ii) the formation of aluminosilicates in the growth media and cell wall [43,65,66], (iii) the release of phenolic compounds by the root tips [47,60], (iv) increment of carotenoids and chlorophyll in leaves [67], and (v) stimulation of antioxidant enzyme activities and antioxidant compound production [23,44]. Nevertheless, there is still a controversial debate regarding the mechanisms implicated in the Si-mediated the attenuation of Al toxicity. In our study, when Al was applied in combination with Si, both barley cultivars showed a decrease in oxidative damage at all harvest times, compared to those treated with Al alone (Figure 6). This reduction was accompanied by an improvement of the antioxidant capacity (Figure 5) and total phenol concentration (Figure 3). In this context, some studies have found an enhancement in the antioxidant system due to the action of Si under different stresses. Consequently, the increased production of flavonoids [47], activated antioxidant enzymes [17,23,44,46], decreased lipid peroxidation of the membranes [68], regulation of gene expression, and activation of key enzymes of the phenylpropanoid pathway [16,20,44,69] have all been identified.

Our results also showed that Si increased lignin accumulation, mainly in the roots, when treated with both Al and Si compared to control (Figure 4). This is in agreement with previous studies, that have shown a stress mitigation effect from Si due to stimulation of lignin production [19,70], and the alteration of lignin composition [23] under diverse stresses. Additionally, it has been demonstrated that Si increases the activities of lignin metabolic pathway enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase [20,25,69]. This increase in lignin appears to be playing an important role in counteracting Al induced damage, since reactive oxygen species can react with lignin in the apoplast to generate signal molecules under stress conditions [71]. Moreover, Si may exert an important function in plant signaling by inducing changes in both the C/N balance [11] and the lignification-related genes [24].

5. Conclusions

Silicon decreases Al uptake in barley plants by providing reductions in oxidative damage and improvements in antioxidant activity at the short-term. In addition, Si stimulated phenolic metabolism in Al stressed plants, as demonstrated by the increase in both the phenol concentrations and the lignin accumulation at the root level. Further studies are needed to clarify the function of Si in the modulation of phenylpropanoid pathway, as well as the potential role of Si induced lignin production in the generation of signaling molecules in barley cultivars grown under conditions of Al toxicity.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/7/388/s1, Figure S1: Dry weight, length and lipid peroxidation in roots of different barley cultivars hydroponically grown without (−Al) or with (+Al) supply of 0.2 mM Al during 21 days.

Author Contributions: Data curation, I.V.; Formal analysis, I.V. and S.P.; Funding acquisition, P.C.; Investigation, P.C.; Methodology, I.V., S.P., and K.G.; Supervision, P.C.; Visualization, I.V.; Writing—original draft, I.V.; Writing—review & editing, I.V., M.N., S.P., K.G., M.d.L.L.M., and P.C.

Conflicts of Interest: The authors declare no conflict of interest.

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