Cultivar Differences in the Biochemical and Physiological Responses of Common Beans to Aluminum Stress

Brigitta Tóth 1,*1, Makoena Joyce Moloi 2, Lóránt Szőke 1, Mátyás Danter 3 and Michael A. Grusak 4

1 Institute of Food Science, University of Debrecen, 138 Bősztúrméni St., 4032 Debrecen, Hungary; szoke.lorant@agr.unideb.hu
2 Department of Plant Sciences, University of the Free State-Main Campus, P.O. Box 339, Bloemfontein 9300, South Africa; MoloiMJ@ufs.ac.za
3 Modern Application Platform Business Unit, VMware, Inc., 3401 Hillview Ave, Palo Alto, CA 94304, USA; mdanter@gmail.com
4 USDA-ARS Edward T. Schafer Agricultural Research Center, 1616 Albrecht Boulevard N., Fargo, ND 58102-2765, USA; mike.grusak@usda.gov
* Correspondence: btoth@agr.unideb.hu; Tel.: +1-720-666-3552

Abstract: Soil conditions leading to high levels of available aluminum are detrimental to plant growth, but data are limited on genotypic differences in tolerance to aluminum stress in some crops. The aim of this study was to examine the morphological, biochemical, and physiological changes in roots and shoots of 25 common bean (Phaseolus vulgaris L.) cultivars (Pinto market class) under aluminum (Al) treatment. Additionally, this study aimed to assess the range of responses amongst the common bean cultivars relative to their Al toxicity tolerance and sensitivity. Plants were grown hydroponically using a simplified nutrient solution with or without 20 µM AlCl₃. Reactive oxygen species (ROS), activities of the antioxidant enzymes superoxide dismutase (SOD) and guaiacol peroxidase (POD), and malondialdehyde (MDA) concentration, an indicator of lipid peroxidation, were measured to establish the effects of Al treatment on the plants. In addition, growth parameters such as shoot and root dry weight, root-to-shoot ratio, root elongation, and root volume changes were also investigated. The cultivar effect was significant for all the measured parameters, except for shoot dry weight. Inhibition of the root and shoot dry weight for selected common bean cultivars shows that the response of common bean to Al stress is genotype-specific. Additionally, Al-induced root elongation inhibition and root volume changes varied among the cultivars. Most cultivars had significantly higher SOD activity (20 of 25 cultivars) and POD activity (12 cultivars) under AlCl₃ treatment compared to the controls. A positive significant correlation was observed between MDA and ROS, showing that Al stress induced the accumulation of ROS along with an increase in lipid peroxidation. According to the results of this study, Arapaho and AC Island cultivars could potentially be used in the future production of common beans under Al stress. Therefore, these two cultivars could also be included in Al tolerance breeding programs.

Keywords: aluminum toxicity; antioxidant enzyme activity; bean; lipid peroxidation; low pH; reactive oxygen species

1. Introduction

Climate change is an important topic that dates back to the early 1950s. Although there were arguments regarding its existence, a consensus was reached about its impact on nature, environment, plants, animals, and humans. Climate change has direct and indirect impacts on crop production and food security. Water imbalance (drought and flooding), elevated CO₂ concentration in the atmosphere, increasing average annual global temperature, extreme weather conditions, and decline in soil properties are the main features of climate change related to agriculture. Agricultural lands are among the first affected by climate change [1]. Climate change affects soil pH and the availability of different macro- and
micro-elements [2]. Anthropogenic features such as increasing greenhouse gas emissions, elevated atmospheric carbon dioxide concentration, nitrogen overfertilization of soils, and acid rain contribute to soil acidity [3] and, consequently, promote aluminum (Al) toxicity. Climate change is also linked to extreme weather conditions such as acidic rainfall. The pH of rain has been changing in many regions in the last decades. The pH of rain is normally slightly acidic and ranges between 5.0 and 6.0 [4]. If the rainfall contains sulfur dioxide or nitrogen oxides—a consequence of anthropogenic environmental pollution—the pH drops to 4.0 or lower in extreme cases [5]. The effects of acid rain on soil acidification depend on soil properties, as well as soil buffer capacity. This soil characteristic is related to the composition of the soil and the soil coverage of the surface. Especially in the northeast part of the United States of America, where the soil is thin and buffer capacity is low, acid rain generates soil acidification and accelerates the dissolution of aluminum salts, which induces aluminum stress [6]. Aluminum mineral salts, i.e., aluminum oxides and aluminosilicates, in soils are not toxic to the plants on their own. Under acidic conditions, Al minerals form toxic Al-hydroxide. The most toxic form of Al is Al$^{3+}$ which has the greatest impact on plant growth [7]. In addition, tropical and subtropical soils—a significant proportion of global soils—are highly sensitive to soil acidification because of their low buffer capacity [8]. The average Al concentration in soil varies between 0.01 and 0.3 ppm [9]. Aluminum becomes toxic when the soil pH drops below 5.5 and Al concentration increases in the soil solution. Soil solution at neutral pH contains 400 µg·L$^{-1}$ Al, while this value can be 5700 µg·L$^{-1}$ in 4.4 pH soil [10]. If the Al concentration in soil solution is higher than 1 mg·L$^{-1}$, aluminum toxicity and reduced yield can occur. A soil aluminum concentration of 2–5 ppm is toxic to the roots of sensitive plant species, and a concentration above 5 ppm is toxic to tolerant species [11]. The Al concentrations measured in plant tissues are different from the soil Al concentrations. The average Al concentration in plants is in the 10 s and 100 s of mg·kg$^{-1}$, while this value is in the 1000 s of mg·kg$^{-1}$ in Al accumulator species. Plants can accumulate or exclude Al from their metabolism. Accumulator species mainly occur in the tropical and subtropical regions, accumulating a minimum of 1 g Al·kg$^{-1}$ in the dry leaf tissue [12]. For instance, buckwheat can accumulate more than 15 g Al·kg$^{-1}$ in leaves growing on acidic soil [13]. The plants which exclude Al from their metabolisms secrete metabolites, i.e., organic acids, which form nontoxic chelates with Al [13]. To protect our crops from the toxic effects of Al, liming is the most widely used agricultural practice [14]. Numerous studies indicate that the toxicity of Al is one of the principal abiotic stressors, especially under acidic growing conditions, which impacts crop production and yield at physiological and morphological levels [15–19]. The most sensitive plant organ is the root, and the first symptom of Al toxicity is reduced root growth [20]. The degree of the effects of Al on plant growth and development depends on the concentration of Al, species, genotype, cultivar, and duration of Al exposure [21,22]. Furthermore, Al can bind with the cell wall, whereby the cell wall becomes rigid [23]. Aluminum can change the lipid peroxidation in the plasma membrane [24] and the homeostasis of calcium ions [25], inhibit the uptake of nutrients and water [7], and decrease the chlorophyll content [26], and photosynthetic rate [27].

Similar to many abiotic stressors, Al toxicity stimulates the generation of reactive oxygen species (ROS) in plant cells and, consequently, oxidative stress [28–31]. The ROS are highly reactive, and their overproduction is toxic to biomolecules. To protect the biomolecules under Al toxicity, a highly effective antioxidant enzyme system is required [32,33]. One of the key constituents of this defense system is represented by metalloenzymes; superoxide dismutase (SOD) enzymes belong to this group. Elevated activities of SOD were measured in Al-tolerant rice [32], Barbados nut [34], wheat [35], tomato [36], and soybeans [37]. Another antioxidant enzyme, ascorbate peroxidase (APX), is one of the most effective controllers of ROS because it plays a significant role in hydrogen peroxide detoxification [38]. Rajput et al. [39] communicated that the activity of APX increases with activities of other enzymatic antioxidants such as SOD or glutathione reductase (GR), indicating their interdependence. Although reports have indicated increased
APX activity in crops under different abiotic stress conditions such as drought stress in maize [40], wheat [41], and peas [42], there is conflicting evidence under Al stress. For example, Al stress induced an insignificant increase in the APX activity of tolerant rice with higher activity in sensitive plants [32]. Du et al. [37] reported a higher APX activity in ZmAT6 transgenic maize, while no change was observed in the OE-ZmAT6 line after aluminum treatment. On the contrary, Al stress induced a significant increase in the APX activity of sensitive compared to tolerant wheat in their root tips [43]. Noteworthy, however, is that the activity of APX also depends on the duration and intensity of stress [44].

Guaiacol peroxidase (POD) is another member of plants’ detoxifying systems, as it plays a key role in the removal of hydrogen peroxide generated during stress conditions in plants. Peroxidase is not always a biochemical marker of Al tolerance because its activity was found to be notably higher in Al-sensitive than in Al-resistant maize [45]. Additionally, increased POD activity has been documented in several research studies under different environmental conditions [32,46–50].

Common beans (Phaseolus vulgaris L.) are important protein sources in the vegetarian diet and in developing countries. Beans have a high carbohydrate and low fat content, are rich in fiber, and have a low glycemic index [51]. Approximately 8.96 million ha of land is used for growing beans in Latin America, and over 4 million ha is used on the African continent [52,53]. Aluminum toxicity is one of the principal restricting components of common bean production in tropical regions [34], leading to a significant yield reduction in these territories [55]. In the context of bean production, Al soil toxicity mostly affects small-scale farms [55,56]. Since the common bean is sensitive to Al stress [57], several solutions can be implemented to avoid yield loss under Al toxicity. These solutions include cultivation in acidic soil and the use of aluminum-resistant or tolerant lines and genotypes [58,59].

It is difficult to categorize plant species according to their Al tolerance. Pineapple and tea are known as Al-tolerant plants, while most plant species are Al-sensitive, but many wild plant species have adapted to acidic soil and high Al concentration. The degree of sensitivity depends on the origin of plants, the species, and the soil properties [37]. Two geographical origins of the gene pools of common bean are Mesoamerica and the Andes. Accordingly, the common bean cultivars are separated into two groups called Mesoamerican and Andean races beans [60]. Common beans were cultivated on acidic soils in Spain from the 17th century. According to research data, a connection was found between these Spanish beans and the Andean race bean cultivars [61]. On the one hand, these data suggest that common beans adapted to acidic soil conditions, which exist in Central and South America [62]. On the other hand, Lunze et al. [63] stated that the common bean is sensitive to strongly acidic soil conditions; its extremes are soil with pH under 5.0 and above 8.0. In addition, the common bean is an Al-sensitive plant according to Horneck et al.’s [64] data.

The responses of common beans to Al toxicity have been researched widely. The first visible Al toxicity deformation involves root growth retardation [65]. Llugany et al. [66] observed significant root growth inhibition in maize 30–90 min after Al treatment. Similarly, short-term exposure to Al caused root elongation inhibition in common beans [67]. Massot et al. [68] stated that callose synthesis negatively correlated with root elongation rate after 24 h of Al exposure. Additionally, the root elongation rate is the most noticeable parameter used to observe the effect of Al on bean cultivars. The extent of root damage caused by Al is based on the stage of plant development and growth, the concentration of Al, and the degree of Al tolerance of plants [69] at the time of its exposure to Al.

This experiment used 25 common bean genotypes of the Pinto market class. The goal of this research was to examine the physiological, biochemical, and morphological changes in roots, the concentration of reactive oxygen species (ROS), the activity of antioxidant enzymes such as superoxide dismutase (SOD) and guaiacol peroxidase (POD), and the rate of lipid peroxidation in seedlings growing under AlCl₃ toxicity. The additional aim was
to assess the range of responses amongst the common bean cultivars relative to their Al toxicity tolerance and sensitivity.

2. Results

The root dry weight was significantly lower in all Al-treated cultivars, except AC Island and Arapaho. The root dry weight varied between 57 and 104 mg·plant\(^{-1}\) in nontreated plants, while this range was 25–78 mg·plant\(^{-1}\) in plants treated with AlCl\(_3\). More than a 50% decline in root dry weight was measured in Aztec (56%), Burke (51%), Croissantant (56%), Kimberly (57%), Quincy (61%), and TARS-09 (55%). Ouray had the lowest reduction (23%). The effect of Al treatment was more noticeable on the root than the shoot dry weight. The shoot dry weight of Al-treated Aztec was 15% lower than that of the control. In contrast, Al treatment significantly increased shoot dry weight by 11% in Montrose. The root-to-shoot ratio was higher for the controls (ranged between 0.19 and 0.43) than that of the Al-treated cultivars (0.09–0.31). Although the root-to-shoot ratio was significantly reduced for most cultivars under Al treatment, AC Island and Arapaho were not affected (Table 1).

### Table 1. The effect of AlCl\(_3\) on the dry weight of roots and shoots (DW) (mg·plant\(^{-1}\)), as well as the root-to-shoot ratio, of 25 common bean cultivars grown for 3 days in hydroponics.

| Cultivars   | Root DW (mg plant\(^{-1}\)) | Shoot DW (mg plant\(^{-1}\)) | Root/Shoot Ratio |
|-------------|-----------------------------|-------------------------------|------------------|
|             | 0 \(\mu\)M Al | 20 \(\mu\)M Al | 0 \(\mu\)M Al | 20 \(\mu\)M Al |                          |
| AC Island   | 76 ± 18          | 69 ± 11             | 273 ± 52    | 282 ± 28    | 0.28 ± 0.24              |
| Apache      | 77 ± 2           | 41 ± 4*             | 269 ± 33    | 267 ± 52    | 0.28 ± 0.15*              |
| Arapaho     | 71 ± 27          | 52 ± 11*            | 227 ± 44    | 238 ± 48    | 0.26 ± 0.22*              |
| Aztec       | 71 ± 7           | 31 ± 1*             | 261 ± 20    | 227 ± 38*   | 0.27 ± 0.14*              |
| Bill Z      | 85 ± 22          | 53 ± 5*             | 224 ± 46    | 240 ± 40    | 0.38 ± 0.22*              |
| Buckskin    | 80 ± 24          | 45 ± 6*             | 271 ± 70    | 254 ± 18    | 0.29 ± 0.18*              |
| Burke       | 64 ± 4           | 31 ± 5*             | 263 ± 21    | 272 ± 37    | 0.24 ± 0.11*              |
| Croissant   | 104 ± 15         | 46 ± 1*             | 268 ± 36    | 250 ± 24    | 0.39 ± 0.18*              |
| Flint       | 58 ± 13          | 33 ± 6*             | 208 ± 47    | 237 ± 44    | 0.28 ± 0.14*              |
| Fargo       | 90 ± 15          | 57 ± 7*             | 271 ± 59    | 303 ± 26    | 0.33 ± 0.19*              |
| Grand Mesa  | 77 ± 11          | 49 ± 3*             | 241 ± 24    | 246 ± 8     | 0.32 ± 0.09*              |
| Kimberly    | 60 ± 15          | 26 ± 6*             | 307 ± 45    | 288 ± 19    | 0.19 ± 0.09*              |
| Kodiak      | 93 ± 14          | 53 ± 14*            | 305 ± 34    | 264 ± 40    | 0.30 ± 0.20*              |
| Max         | 103 ± 12         | 59 ± 12*            | 271 ± 28    | 270 ± 5     | 0.34 ± 0.22*              |
| Montrose    | 59 ± 10          | 32 ± 6*             | 250 ± 19    | 28 ± 6*     | 0.24 ± 0.11*              |
| La Paz      | 75 ± 11          | 45 ± 6*             | 206 ± 19    | 229 ± 37    | 0.36 ± 0.20*              |
| Ouray       | 103 ± 19         | 78 ± 15*            | 237 ± 45    | 251 ± 25    | 0.43 ± 0.31*              |
| Poncho      | 78 ± 20          | 48 ± 7*             | 304 ± 48    | 326 ± 52    | 0.26 ± 0.15*              |
| Pinto       | 65 ± 10          | 35 ± 2*             | 247 ± 30    | 268 ± 26    | 0.26 ± 0.13*              |
| Quincy      | 67 ± 5           | 26 ± 4*             | 276 ± 25    | 271 ± 37    | 0.24 ± 0.10*              |
| Santa Fe    | 93 ± 11          | 53 ± 1*             | 308 ± 36    | 279 ± 17    | 0.30 ± 0.19*              |
| Sierra      | 89 ± 13          | 50 ± 6*             | 240 ± 21    | 236 ± 19    | 0.37 ± 0.21*              |
| TARS-09     | 68 ± 7           | 30 ± 8*             | 189 ± 34    | 221 ± 38    | 0.36 ± 0.14*              |
| Topaz       | 78 ± 4           | 46 ± 1*             | 234 ± 26    | 247 ± 35    | 0.33 ± 0.19*              |
| Windbreaker | 72 ± 7           | 46 ± 1              | 258 ± 29    | 264 ± 2     | 0.28 ± 0.18*              |

Values in columns are means ± standard deviation (\(n = 7\); DW: dry weight. *Significant differences compared to control based on Shapiro–Wilk test (\(p \leq 0.05\)).

Aluminum treatment had less of an effect on the percentage change in the root volume of Arapaho for the entire experimental period. The changes ranged between +10% (Arapaho) and −81% (Burke) 24 h after Al treatment. Forty-eight hours after Al treatment, Arapaho exhibited the lowest change in root percentage (−39%) and Kodiak exhibited the highest (−90%). Similarly, after 72 h of Al treatment, Arapaho had the lowest percentage change (−52%) while Kodiak had the highest (−92%) (Table 2).
Table 2. The change in root volume percentages of 25 Pinto bean cultivars 24, 48, and 72 h after AlCl\textsubscript{3} treatment, relative to non-stressed controls.

| Cultivars    | % ∆ of Root Volume | 24 h after Al Treatment | 48 h after Al Treatment | 72 h after Al Treatment |
|--------------|--------------------|-------------------------|-------------------------|-------------------------|
| AC Island    | −41                | −53                     | −65                     |
| Apache       | −68                | −72                     | −83                     |
| Arapaho      | +11                | −39                     | −53                     |
| Aztec        | −49                | −62                     | −77                     |
| Bill Z       | −47                | −53                     | −66                     |
| Buckskin     | −70                | −58                     | −69                     |
| Burke        | −82                | −84                     | −89                     |
| Croissant    | −81                | −80                     | −83                     |
| Fargo        | −54                | −67                     | −69                     |
| Flint        | −55                | −70                     | −69                     |
| Grand Mesa   | −35                | −53                     | −59                     |
| Kimberly     | −73                | −90                     | −92                     |
| Kodiak       | −46                | −60                     | −71                     |
| La Paz       | −61                | −72                     | −77                     |
| Max          | −69                | −74                     | −76                     |
| Montrose     | −56                | −73                     | −80                     |
| Ouray        | −46                | −59                     | −63                     |
| Poncho       | −63                | −73                     | −81                     |
| Pinto        | −65                | −82                     | −87                     |
| Quincy       | −26                | −79                     | −86                     |
| Santa Fe     | −31                | −60                     | −76                     |
| Sierra       | −36                | −63                     | −68                     |
| TARS-09      | −69                | −85                     | −85                     |
| Topaz        | −37                | −69                     | −66                     |
| Windbreaker  | −55                | −68                     | −73                     |

The Al-induced primary root inhibition varied between 15.25% (Burke) and 72.39% (Buckskin) 4 h after Al treatment. Root growth inhibition was higher 8 h after Al treatment, increasing from 36.67% to 86.00%, compared to the values 4 h after Al treatment. The lowest inhibition was measured in Arapaho 24 h after the Al treatment, while the highest was recorded in Poncho (91.35%). The root length inhibition was between 7.88% and 95.00% 48 h, and between 35.43% and 96.15% 72 h after Al treatment (Table 3).

The concentration of reactive oxygen species (ROS) varied widely in the control bean cultivars: 261,711 RFU·g\textsuperscript{−1} FW (Bill Z control) and 1,661,220 RFU·g\textsuperscript{−1} FW (Windbreaker control). The concentration of total ROS was 1.5 times (i.e., significantly) lower in Montrose grown in nutrient solution containing 20 µM AlCl\textsubscript{3}, compared to the control treatment. The effect of Al treatment was by far the highest in TARS09 (72.99% increase) and AC Island (64.49% increase) compared to the controls. In contrast, Flint and Montrose had remarkably lower ROS under Al treatment (41.93% and 34.84%, respectively) (Figure 1).

Higher superoxide dismutase (SOD) activities were measured in the Al-treated roots of common beans compared to the controls. The activity of SOD was significantly higher in Arapaho, Aztec, Bill Z, Burke, Fargo, Flint, Grand Mesa, Kimberly, La Paz, Max, Ouray, Poncho, Pinto, Quincy, Santa Fe, Sierra, TARS09, and Topaz cultivars. Flint had the highest increase in SOD activity (45.5%) under Al treatment (Figure 2).
Table 3. The root length inhibition percentage of 25 common bean cultivars 4, 8, 24, 48, and 72 h after AlCl$_3$ treatment, relative to non-stressed controls.

| Cultivars   | 0–4 h | 4–8 h | 8–24 h | 24–48 h | 48–72 h |
|------------|-------|-------|--------|---------|---------|
| AC Island  | 30    | 58    | 19     | 31      | 56      |
| Apache     | 55    | 74    | 81     | 89      | 91      |
| Arapaho    | 49    | 48    | –6     | 8       | 35      |
| Aztec      | 62    | 46    | 82     | 95      | 97      |
| Bill Z     | 30    | 36    | 7      | 22      | 51      |
| Buckskin   | 72    | 79    | 84     | 85      | 77      |
| Burke      | 15    | 76    | 78     | 87      | 96      |
| Croissant  | 46    | 77    | 68     | 86      | 75      |
| Fargo      | 40    | 80    | 83     | 74      | 81      |
| Flint      | 65    | 69    | 86     | 81      | 82      |
| Grand Mesa | 54    | 46    | 26     | 31      | 39      |
| Kimberly   | 67    | 85    | 90     | 93      | 96      |
| Kodiak     | 39    | 78    | 68     | 78      | 71      |
| La Paz     | 37    | 71    | 73     | 75      | 79      |
| Max        | 57    | 68    | 77     | 84      | 82      |
| Montrose   | 53    | 77    | 80     | 92      | 88      |
| Ouray      | 24    | 55    | 21     | 19      | 48      |
| Poncho     | 39    | 79    | 9      | 94      | 92      |
| Pinto      | 42    | 57    | 85     | 85      | 76      |
| Quincy     | 51    | 7     | 76     | 75      | 78      |
| Santa Fe   | 56    | 83    | 76     | 79      | 74      |
| Sierra     | 62    | 84    | 80     | 90      | 87      |
| TARS-09    | 56    | 77    | 80     | 42      | 89      |
| Topaz      | 35    | 86    | 77     | 94      | 90      |
| Windbreaker| 49    | 85    | 82     | 78      | 86      |

Figure 1. The total reactive oxygen species (ROS) of 25 common bean cultivars treated for 72 h with AlCl$_3$. Values are the means of five biological and technical repetitions ± S.D. * Significant difference between treatments based on Shapiro–Wilk test ($p \leq 0.05$). Lowercase letters denote a significant difference among the AlCl$_3$-treated cultivars. FW: fresh weight, RFU: relative fluorescence unit.
Plants Flint had the highest increase in SOD activity (45.5%) under Al treatment (Figure 2).

Figure 2. The superoxide (SOD) activity of 25 common bean cultivars 72 h after AlCl₃ treatment. Values are the averages of five biological and technical repetitions ± SD. * Significant difference between treatments based on Shapiro–Wilk test (p ≤ 0.05). Lowercase letters denote a significant difference among the AlCl₃-treated cultivars. FW: fresh weight.

Peroxidase (POD) activity varied among the cultivars under Al treatment. The lowest activity was measured in Grand Mesa (0.878 g⁻¹ FW·min⁻¹), while the highest was measured in Windbreaker (10.848 g⁻¹ FW·min⁻¹). Kodiak had the greatest reduction in POD activity (52.76%) under Al treatment, followed by Kimberly (37.33%) and Arapaho (29.62%). In contrast, POD activity was higher by more than 30% in Al-treated cultivars of Apache, Aztec, Burke, Fargo, Flint, Quincy, and TARS-09 compared to the control plants (Figure 3).

To evaluate the rate of lipid peroxidation, the concentration of malondialdehyde (MDA) was measured in the roots of common beans. Max had the lowest (4.197 nmol·g⁻¹ FW) and Topaz had the highest (39.02 nmol·g⁻¹ FW) MDA content under Al treatment. Compared to the control, the highest increases were measured in the Ouray (76.05%), Grand Mesa (51.54%), and Topaz (50.42%) cultivars. In contrast, Al treatment led to significant reductions in the MDA contents of Arapaho (39.78%), Aztec (52.65%), and TARS-09 (44.66%) (Figure 4).
To evaluate the rate of lipid peroxidation, the concentration of malondialdehyde (MDA) was measured in the roots of common beans. Max had the lowest (4.197 nmol·g⁻¹ FW) and Topaz had the highest (39.02 nmol·g⁻¹ FW) MDA content under Al treatment. Compared to the control, the highest increases were measured in the Ouray (76.05%), Grand Mesa (51.54%), and Topaz (50.42%) cultivars. In contrast, Al treatment led to significant reductions in the MDA contents of Arapaho (39.78%), Aztec (52.65%), and TARS-09 (44.66%) (Figure 4).

Table 4 contains the mean values of the measured parameters of 25 common bean cultivars. The average root dry weight was significantly lower (37.5%), while the average shoot dry weight was not affected by the Al treatment. Furthermore, the average value of root-to-shoot ratio also was significantly lower (by 42%) when Al treatment was examined. The average root volume and average root length significantly declined, with this rate of reduction decreasing as time went on. The changes in root volume were significant 24 (51.70%), 48 (72.50%), and 72 h (82.36%) after the Al treatment was applied. Compared to the control, the length of the primary root was significantly shorter 4, 8, 24, 48, and 72 h after Al treatment (49.81%, 66.46%, 66.51%, 73.00%, and 76.80%, respectively).

The activities of both antioxidant enzymes and the concentrations of ROS and MDA were higher under Al treatment. Al stress also significantly induced the activities of SOD and POD (26.32% and 10.00%, respectively). In addition, the amount of ROS also was significantly elevated by 16.10%, while the amount of MDA did not change significantly (8%) when plants were grown in a simplified nutrient solution containing 20 µM AlCl₃ (Table 4).

The correlations between the measured parameters under Al treatment for 25 bean cultivars showed that ROS positively correlated with SOD (p ≤ 0.01), POD (p ≤ 0.05), and MDA (p ≤ 0.01). Additionally, positive correlations were observed between ROS and root length 4, 8, and 24 h after the Al treatment. A highly significant correlation (p ≤ 0.001) was observed between SOD and POD (positive). MDA positively correlated with root DW and root volume change 72 h after Al treatment (Table 5).

**Figure 3.** The peroxidase (POD) activity of 25 common bean cultivars 72 h after AlCl₃ treatment. Values are the averages of five biological and technical repetitions ± SD. * Significant difference between treatments based on Shapiro–Wilk test (p ≤ 0.05). Lowercase letters denote a significant difference among the AlCl₃-treated cultivars. FW: fresh weight.

**Figure 4.** The malondialdehyde (MDA) concentrations of 25 common bean cultivars 72 h after AlCl₃ treatment. Values are the averages of five biological and technical repetitions ± SD. * Significant difference between treatments based on Shapiro–Wilk test (p ≤ 0.05). Lowercase letters denote a significant difference among the AlCl₃-treated cultivars. FW: fresh weight. MDA: malondialdehyde.
To demonstrate the impact of Al on the measured parameters more noticeably, Table 4 contains the mean values of the measured parameters of 25 common bean cultivars. The average root dry weight was significantly lower (37.5%), while the average shoot dry weight was not affected by the Al treatment. Furthermore, the average value of root-to-shoot ratio also was significantly lower (by 42%) when Al treatment was examined. The average root volume and average root length significantly declined, with this rate of reduction decreasing as time went on. The changes in root volume were significant 24 (51.70%), 48 (72.50%), and 72 h (82.36%) after the Al treatment was applied. Compared to the control, the length of the primary root was significantly shorter 4, 8, 24, 48, and 72 h after Al treatment (49.81%, 66.46%, 66.51%, 73.00%, and 76.80%, respectively).

Table 4. Average values of measured parameters of 25 Pinto cultivars based on the applied treatments (0 µM AlCl$_3$ and 20 µM AlCl$_3$): root and shoot dry weight $n = 175 \pm SD$; root volume and root length $n = 300 \pm SD$; SOD, POD, ROS, and MDA, $n = 125 \pm SD$.

| Treatment          | 0 µM AlCl$_3$  | 20 µM AlCl$_3$ |
|--------------------|----------------|----------------|
| Root DW            | 0.08 ± 0.02    | 0.05 ± 0.02 *  |
| Shoot DW           | 0.26 ± 0.05    | 0.26 ± 0.04 ns |
| Root:shoot         | 0.31 ± 0.07    | 0.18 ± 0.06 *  |
| $\Delta$ root volume cm$^3$/24 h | 0.29 ± 0.15    | 0.14 ± 0.11 *  |
| $\Delta$ root volume cm$^3$/48 h | 0.40 ± 0.16    | 0.11 ± 0.09 *  |
| $\Delta$ root volume cm$^3$/72 h | 0.34 ± 0.17    | 0.06 ± 0.05 *  |
| PRG 4 h after Al (mm/h) | 5.14 ± 2.14    | 2.58 ± 1.44 *  |
| PRG 8 h after Al (mm/h) | 7.99 ± 3.25    | 2.68 ± 1.51 *  |
| PRG 24 h after Al (mm/h) | 27.68 ± 3.96   | 9.27 ± 8.99 *  |
| PRG 48 h after Al (mm/h) | 42.64 ± 6.97   | 11.53 ± 11.61 |
| PRG 72 h after Al (mm/h) | 41.20 ± 6.78   | 9.56 ± 8.66 *  |
| SOD                | 0.14 ± 0.03    | 0.19 ± 0.04 *  |
| POD                | 4.41 ± 2.89    | 4.88 ± 2.61 *  |
| ROS                | 712,864.92 ± 407,069.50 | 849,014.63 ± 352,566.80 * |
| MDA                | 13.97 ± 9.61   | 15.12 ± 10.05 ns |

DW: dry weight, PRG: primary root growth, SOD: superoxide dismutase, POD: peroxidase, ROS: reactive oxygen species, MDA: malondialdehyde. * Significant differences compared to control based on Shapiro–Wilk test ($p \leq 0.05$); ns: not significant.

The activities of both antioxidant enzymes and the concentrations of ROS and MDA were higher under Al treatment. Al stress also significantly induced the activities of SOD and POD (26.32% and 10.00%, respectively). In addition, the amount of ROS also was significantly elevated by 16.10%, while the amount of MDA did not change significantly (8%) when plants were grown in a simplified nutrient solution containing 20 µM AlCl$_3$ (Table 4).

The correlations between the measured parameters under Al treatment for 25 bean cultivars showed that ROS positively correlated with SOD ($p \leq 0.01$), POD ($p \leq 0.05$), and MDA ($p \leq 0.01$). Additionally, positive correlations were observed between ROS and root length 4, 8, and 24 h after the Al treatment. A highly significant correlation ($p \leq 0.001$) was observed between SOD and POD (positive). MDA positively correlated with root DW and root volume change 72 h after Al treatment (Table 5).
Table 5. Correlations based on the average values for all the cultivars combined under AlCl₃ treatment.

| Char I | Characteristics II |
|--------|--------------------|
|        | Root DW | Root Shoot | Root Volume 24 h | Root Volume 48 h | Root Volume 72 h | Root Length 4 h | Root Length 24 h | Root Length 48 h | Root Length 72 h | ROS | SOD | POD | MDA |
| Root DW | 1       | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | +0.151 * |
| Root:Shoot | 1     | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root volume 24 h | 1    | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root volume 48 h | 1    | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | +0.169 * |
| Root volume 72 h | 1    | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root length 4 h | 1    | +0.231 *** | +0.206 **         | ns               | −0.345 ***       | +0.165 *         | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root length 8 h | 1    | +0.173 *   | +0.135 *         | ns               | +0.121 *         | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root length 24 h | 1    | ns         | ns               | +0.179 *         | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root length 48 h | 1    | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| Root length 72 h | 1    | ns         | ns               | +0.126 *         | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| ROS | 1    | +0.195 ** | +0.179 *         | +0.202 **        | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| SOD | 1    | +0.256 *** | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| POD | 1    | −0.430 ** | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |
| MDA | 1    | ns         | ns               | ns               | ns               | ns               | ns               | ns               | ns               | ns   | ns   | ns   | ns   |

DW: dry weight, SOD: superoxide dismutase, POD: peroxidase, ROS: reactive oxygen species, MDA: malondialdehyde, Char: characteristics. * p ≤ 0.001, ** p ≤ 0.01, *** p ≤ 0.05; ns: not significant.
3. Discussion

Anthropogenic pollution is becoming more relevant than ever in the 21st century because of the constantly growing industrial production to satisfy the demand for food, energy, and living conditions of the continuously growing human population [70,71]. Aluminum toxicity is one of the side effects of soil acidification, where the soil pH is less than or reaches 5.5, which can cause growth inhibition, i.e., reduced growth in the roots, stems, and shoots, decreased biomass production, and serious yield loss [72]. This phenomenon occurs particularly in the subtropical and tropical regions, but soil acidification is also observed in European soils [72].

According to several research studies, the root is the most sensitive plant organ to Al toxicity [7,20,65,67,69,72] because of the direct contact it has with Al in the soil. Results of this experiment confirm such observations as lower root dry biomass and root-to-shoot ratio were observed in 23 of 25 common bean cultivars (except AC Island and Arapaho) under Al treatment (Table 1). The reduced root biomass could be associated with damage to the root cell wall [73] and plasma membrane [74], a decrease in the amount of hemicellulose and pectin fractions in the roots [75], which makes them rigid [76], calmodulin changes in the symplast [77], or imbalanced nutrient uptake [78]. Such factors have a significant impact on plant growth. Selective inhibition of the root and shoot dry weight of the common bean cultivars further show that the response of common beans to Al stress is genotype-specific. Considering the stability of root-to-shoot dry mass of Arapaho and AC Island under Al treatment, these cultivars could potentially be used in the future production of common beans under Al toxic soils and be included in Al tolerance breeding programs. In addition, the change in root volume percentage was very low for Arapaho at 24, 48, and 72 h after Al treatment in contrast to other cultivars (Table 2), which further suggests this cultivar is a better alternative for cultivation under Al toxicity.

Growth requires two main constituents: cell division and cell elongation. A short-term exposure (30–90 min) to Al treatment inhibited root elongation in common beans [79]. Aluminum-inhibited root elongation is an appropriate parameter for the classification of the investigated genotypes related to Al sensitivity or Al resistance [80]. In a set of 28 Andean and Mesoamerican common bean genotypes, nine genotypes were Al-sensitive (Al-inhibited root elongation >50%), 12 genotypes had intermediate sensitivity (Al-inhibited root elongation 30–50%), and seven genotypes were Al-resistant (Al-inhibited root elongation ≤30%) [80]. In this study, six cultivars were Al-sensitive and indicated ≥90% Al-induced primary root elongation inhibition 72 h after Al treatment (Aztec was among these cultivars). Additionally, Al-induced root elongation inhibition was between 70% and 90% in 14 cultivars, two cultivars had 50–70% inhibition, and three were intermediate Al-sensitive (30–50%). In agreement with the results on root and shoot dry weight, as well as on the percentage change in root volume above, Arapaho had the least root inhibition (8–72 h after Al treatment) compared to the 24 other cultivars (Table 3). This further confirms that this cultivar is less sensitive to Al stress, which could result in better growth.

Zheng and Yang [81] stated that Al toxicity modified cell-wall characteristics and caused oxidative stress. Aluminum toxicity leads to oxidative stress; its degree depends on the concentration of Al and the sensitivity of plants. When antioxidant systems are insufficient, more reactive oxygen species (ROS) are generated, leading to oxidative stress in plants [82]. Nahar et al. [83] studied the effect of Al on ROS production in mung beans. They found that Al toxicity caused higher ROS production, resulting in higher lipid peroxidation in membranes. In addition, elevated ROS quantities were found in pea roots, and the ROS production increased with the duration of exposure. Yamamoto et al. [84] suggested that increased amounts of ROS inhibit the root elongation of peas. In this study, ROS production under Al stress varied with cultivars, whereby nine cultivars had significantly higher accumulation (23.62–72.99% increase). Noticeably lower ROS concentrations were measured in Flint and Montrose (27.81% and 46.55%, respectively) under Al treatment in this study. Interestingly, Arapaho was among the cultivars with low ROS accumulation under Al treatment (Figure 1). This corresponds well with the observed decrease in the
inhibition of the root parameters, implying that this cultivar had a better mechanism of avoiding excessive production of ROS. Although excessive ROS accumulation could be detrimental to plants, when produced in lower quantities, they (especially hydrogen peroxide) could act as signaling molecules to switch on the plant’s defense responses during abiotic stress conditions [85]. This could be the case for Arapaho because of the significant correlation between ROS and root length between 4 and 24 h post Al treatment (Table 5).

Antioxidant enzymes are very important in regulating the ROS levels in plants. Zhang et al. [86] found that the activity of SOD was significantly higher in fava bean roots 6 days after 50 and 100 µM Al treatments, and 9 days after 100 µM Al treatment relative to non-Al-treated control. Additionally, the activity of POD also increased after 100 µM Al treatment compared to the control. An Al-tolerant broad bean cultivar had significantly higher SOD activity 12 and 24 h after 50 µM Al treatment, while the activity decreased in an Al-sensitive cultivar. The activity of POD was higher in both cultivars 2, 4, 8, and 12 h after Al treatment, and it was notably higher in the Al-sensitive cultivar than the Al-tolerant one [87]. However, in maize, the activities of both SOD and POD were noticeably higher in Al-sensitive maize and did not change in the Al-resistant cultivar after Al treatment [45]. This further confirms the importance of a cultivar type and its sensitivity to Al. In the current study, SOD activity was higher in all cultivars under Al stress conditions. Twenty of the 25 cultivars had significantly higher activity, varying between 17.86% and 45.50% increases. Although SOD activity was significantly increased by Al treatment in Arapaho, the level was not high (Figure 2). This suggests that the lower level of ROS observed above for this cultivar could be maintained by other antioxidant systems. However, this needs to be elucidated further. Another explanation could be that the low ROS level was not caused by higher antioxidant activity but by lower Al uptake due to better Al chelation in the rhizosphere and apoplast by organic acid exudations [88]. Although there was a positive correlation ($p \leq 0.01$) between SOD and the ROS, it is unlikely that SOD activity was directly involved in the defense against Al toxicity because it did not correlate well with any of the root and shoot parameters (Table 5).

A differential response for POD activity under Al treatment was observed in common bean cultivars. Some cultivars had significantly high POD activity (12 cultivars) while others had lower activity under Al treatment. The role of POD in improving the tolerance of common bean cultivars to Al stress is uncertain because Arapaho (which was found to be less Al-sensitive) was among the cultivars with reduced POD activity. Moreover, a cultivar with the highest POD activity (cultivar Windbreaker) (Figure 3) had the highest recorded ROS accumulation, showing that POD did not effectively reduce ROS accumulation. Furthermore, a significantly positive correlation between POD activity (Table 5) and ROS shows that its activity increased with an increase in ROS accumulation under Al stress. Therefore, there is no strong evidence that POD has a concrete role in improving the performance of common bean plants during Al stress.

The measurement of lipid peroxidation is one of the indices of stressed conditions. A higher rate of lipid peroxidation was observed in many plants under Al stress, e.g., pea roots [89], soybean root tips [90], and fava bean roots [86,87]. The generated amount of MDA during lipid peroxidation increased with the applied Al concentration [91]. In contrast, Yamamoto et al. [89] observed higher MDA concentration only in the root apex of peas under Al toxicity, and they stated that the elevated rate of lipid peroxidation and the higher MDA levels are not the direct cause of Al toxicity. Zhang et al. [86] investigated the impacts of Al toxicity on fava bean in a 9 day hydroponic experiment. They used 10, 50, and 100 µM Al concentrations and found that MDA content was significantly higher in the leaves and roots after 6 and 9 days of 50 and 100 µM Al treatment. Additionally, the amount of MDA in broad bean roots also increased 2, 4, 8, 12, and 24 h after 50 µM Al application. The increase was higher in the case of the Al-sensitive cultivar compared to the Al-tolerant one [87]. Chen et al. [87] concluded that oxidative stress was generated by Al toxicity, through elevated lipid peroxidation. As with many abiotic stresses, MDA levels increased with Al stress for most cultivars (Figure 4). Arapaho had the highest reduction
in MDA concentration under Al treatment, as well as the highest MDA concentration under nontoxic (control) conditions. This suggests that Arapaho is sensitive to acidic pH growing conditions and that Al alleviated its proton toxicity stress [92]. Furthermore, a positive significant correlation was observed between MDA and ROS, showing that Al stress induced the accumulation of ROS along with an increase in lipid peroxidation. Although POD had no clear role in lessening the sensitivity of common beans to Al toxicity, it somehow contributed to reducing lipid peroxidation (Table 5). Although lipid peroxidation is crucial for the cellular membranes, it does not always have a direct impact on root growth and elongation [91]. A highly significant positive correlation was observed between POD and SOD \((p \leq 0.001)\), and significant positive correlations were observed between ROS and SOD, and between SOD and MDA \((p \leq 0.01)\). In addition, MDA and POD were significantly \((p \leq 0.01)\) negatively correlated in common beans under Al treatment, which is in agreement with such findings.

4. Materials and Methods

4.1. Growth Conditions

The surface of 25 common bean cultivar seeds (from US commercial lines that were developed by US bean breeders) were scratched to abrade the seed coat and were germinated for 5 days between two wet sheets of filter paper oriented vertically. Seedlings were transferred to a simplified nutrient solution with compositions of 0.5 mM \(\text{CaCl}_2\), 0.5 mM \(\text{KCl}\), and 8 \(\mu\text{M}\) \(\text{H}_3\text{BO}_3\) [80] in black plastic pots holding 4.5 L of solution and equipped with an aerator. Seedling roots were placed in the nutrient solution, and shoots were maintained above the solution using one-holed cups placed in the lids on top of the pots. The chemical composition of the hydroponic solution ensured optimal root growth for a minimum of 3 days. The pH of the nutrient solution was lowered with 0.1 M HCl to pH 5.0 after 24 h. The pH was adjusted to 4.5 after another 24 h and monitored daily, while being maintained at 4.5 using 0.1 M HCl or 0.1 M KOH, as needed, throughout the study. There were two treatments (0 and 20 \(\mu\text{M}\) \(\text{AlCl}_3\)), each consisting of three pots that contained four plants. Seven plants were used for morphological and weight measurements, and five were used for enzymes assays. The pots were placed in a grid-like fashion while making sure that there was no set order to them with respect to the cultivar or the treatment in order to create a completely random design for the experiment. Common beans were cultivated in a growth chamber with the following environmental conditions: daytime lighting 16 h, dark period 8 h, day temperature 20 °C, night temperature 15 °C, relative humidity 50% ± 5%, and photon flux density at the top of plants of 574 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\).

4.2. Root Elongation

In order to prevent damage, the primary root of each plant was marked 2 cm from the tip of the root with a permanent marker 2 h before the Al treatment. Subsequently, beans were placed into a nutrient solution that either contained 20 \(\mu\text{M}\) \(\text{AlCl}_3\) or none. Measurements (in mm) of root elongation of the primary root were taken 4, 8, 24, 48, and 72 h after Al treatment and rounded to the nearest mm. Measurements were made from the permanent marker to the root tip.

Change in root volume was estimated using the Archimedes method on a day-to-day basis. This is a quick and precise technique to measure root growth without any root damage [92].

4.3. Dry Weight

The dry weight of each sample was determined using the thermogravimetry method. The fresh roots and shoots of 25 common beans per plant were oven-dried for 72 h at 60 °C. After the samples cooled to room temperature, they were weighed using an analytical scale. The root/shoot ratios were calculated for each cultivar and both treatments. The ratios are based on the dry weight of the roots to the dry weight of the shoots.
4.4. Enzyme Assays

The following procedure was used for the preparation of the enzyme extract: liquid nitrogen was used to grind frozen bean root tissue (400 mg) to a fine powder and homogenized in 50 mM phosphate buffer (pH 7.8, 2 mL) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1% polyvinylpyrrolidone (PVP) (w/v), and 1 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged at 10,000× g for 15 min, and the supernatant was used as the enzyme extract for superoxide dismutase (SOD), guaiacol peroxidase (POD), and reactive oxygen species (ROS).

The activity of SOD was measured according to the methods of Giannopolities and Ries [93] and Beyer and Fridovich [94]. One SOD unit was determined as the amount of enzyme needed to inhibit 50% of nitroblue tetrazolium (NBT) light-induced reduction compared to the test tubes that did not contain the plant extract. After centrifugation, 25 µL of supernatant as plant extract, 25 µL of NBT (9 mM), 25 µL of riboflavin (0.25 mM), 250 µL of methionin (0.16 M), and 2.675 mL of phosphate buffer (pH 7.8, 50 mM) were mixed and kept at room temperature; then, the mixture’s absorbance was measured after 15 min at 560 nm. The blank tubes contained 2.7 mL of phosphate buffer and no plant extract; all other components were the same as described above.

The POD assay as proposed by Zieslin and Ben-Zaken [95] was adopted for evaluating the activity of guaiacol peroxidase. The modified mixture had 50 µL of 0.2 M H2O2, 100 µL of 50 mM guaiacol, 340 µL of distilled H2O, 500 µL of 80 mM phosphate buffer (pH 5.5), and 10 µL of enzyme. The POD activity was determined on the basis of the concentration of generated tetraguaiacol. The absorbance of the reaction mixture was read at 470 nm for 3 min at 30 °C. All the above-mentioned chemicals were used for the blank, but 50 mM phosphate buffer was used instead of the enzyme. The concentration of tetraguaiacol was determined using a 25.5 mM−1·cm−1 extinction coefficient.

The method used for the determination of the total amount of ROS (reactive oxygen species) was adopted from Keller et al. [96]. This method is based on the oxidation of the nonfluorescent 2,7-dichlorodihydrofluorescein to the highly fluorescent 2,7-dichlorofluorescein by ROS. Ten microliters of 2,7-dichlorofluorescein diacetate (10 µM) and 100 µL of plant extract were transferred to a 96-well flat-bottom microplate and incubated for 1 h at room temperature; then, fluorescence intensity was measured using a Synergy HT microplate reader (Biotek Instrument, Inc., Winooski, VT, USA) with 485 nm as the excitation and 530 nm as the emission wavelength.

To determine the amount of malondialdehyde (MDA) in bean roots, the thiobarbituric acid (TBA) test was used according to Heath and Packer [97]. For measurement, 100 mg of frozen bean roots were crushed with liquid nitrogen and homogenized to a fine pulp on ice in 1 mL of 0.25% TBA and 10% trichloroacetic acid (TCA), followed by centrifugation (10,800× g for 25 min at 4 °C). Thereafter, the supernatant (0.2 mL) was transferred to clean Eppendorf tubes. To this, a 0.8 mL mixture of 20% TCA and 0.5% TBA was added to the tubes. The mixtures were vortexed and warmed at 95 °C for 30 min and cooled on ice. The tubes were centrifuged again at 10,800× g for 10 min at 4 °C, and the absorbances were measured at 532 nm and 600 nm. An extinction coefficient of 155 mM−1·cm−1 was used to quantify MDA.

4.5. Data Evaluation

Research data were analyzed for normal distribution using the Kolmogorov–Smirnov and Shapiro–Wilk tests [98]. The Mann–Whitney U nonparametric test (p < 0.05) was used to compare the mean values [99]. Significant differences were denoted by lowercase letters. Relationships between two measured parameters were computed by Pearson correlation [100]. The statistical analysis was performed using IBM SPSS Statistics 25 software (Armonk, NY, USA).
5. Conclusions

To conclude, 20 µM AlCl₃ treatment had significant negative effects on most of the measured characteristics, except shoot dry weight and MDA concentration, as well as biochemical and physiological parameters of the different cultivars’ roots. The average values in Table 4 of Al-treated plants show that the activities of SOD and POD, and the total number of ROS values were significantly higher, while the root dry biomass, root-to-shoot ratio, the growth of primary root length, and root volume were significantly lower. According to the results of this study, Arapaho and AC Island cultivars could potentially be used in the future production of common beans in aluminum-toxic soils. Therefore, these two cultivars could also be included in Al tolerance breeding programs.

Author Contributions: Conceptualization, B.T. and M.A.G.; methodology, M.A.G.; validation, M.J.M.; formal analysis, L.S.; investigation, B.T.; resources, M.A.G.; data curation, M.D.; writing—original draft preparation, B.T. and M.J.M.; writing—review and editing, M.A.G. and M.D.; visualization, B.T.; supervision, M.A.G.; funding acquisition, B.T. and M.A.G. All authors have read and agreed to the published version of the manuscript.

Funding: B.T. is supported by the Hungarian State Post-Doctoral Scholarship by the Hungarian Government. This study was supported through funding to M.A.G. from the US Department of Agriculture, Agricultural Research Service, Project no. 101-3060-526. Project no. TKP2020-1KA-04 was implemented with the support provided from the National Research, Development, and Innovation Fund of Hungary, financed under the 2020-1.1.1-TKP2020 funding scheme.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. All data, tables and figures presented in this manuscript are original.

Acknowledgments: The authors thank Ana-Flor Milan Lopez, Chee-Ming Li, David Dworak, and Ashley Hudson for providing excellent technical assistance.

Conflicts of Interest: M.D. was employed by the company VMware, Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does the mention of trade names, commercial products, or organizations imply endorsement by the US Government.

References

1. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhan, X.; Lv, Y.; Xu, J. Impact of climate change on crops adaptation and strategies on trackle its outcome: A review. Plants 2019, 8, 34. [CrossRef]
2. Rengel, Z. Soil pH, soil health and climate change. In Soil Health and Climate Change; Singh, B., Cowie, A., Chan, K., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; Volume 29, pp. 69–85. [CrossRef]
3. Tóth, B.; Moloi, M. The interdependence between low pH and heavy metal stress and its crucial role in crop production efficiency. In Contemporary Studies in Sciences; Efe, R., Cürebal, I., Eds.; Cambridge Scholars Publishing: Newcastle upon Tyne, UK, 2020; pp. 2–17. ISBN 1527554244.
4. Smith, W.H. Pollution, Overview. Encylo. Biodiver. 2001, 731–743. [CrossRef]
5. Lake Scientist. Available online: https://www.lakescientist.com/acid-rain/ (accessed on 16 August 2021).
6. EPA. United States Environmental Protection Agency: Effects of Acid Rain. Available online: https://www.epa.gov/acidrain/effects-acid-rain (accessed on 16 August 2021).
7. Bojórquez-Quintal, E.; Escalante-Magana, C.; Echevarría-Machado, I.; Martínez-Estévez, M. Aluminum, a friend or foe of higher plants in acid soils. Front. Plant Sci. 2017, 8, 1767. [CrossRef]
8. Nguyen, T.; Tran, T.T.H. The contribution of various components to pH buffering capacity of Acrisols in southeastern Vietnam. Commun. Soil Sci. Plant Anal. 2019, 50, 1170–1177. [CrossRef]
9. Lindsay, W.L. Chemical Equilibria in Soils; John Wiley and Sons: New York, NY, USA, 1979; p. 449. ISBN 0471027049.
10. Wright, R.J. Soil aluminum toxicity and plant growth. Commun. Soil Sci. Plant Anal. 1989, 20, 1479–1497. [CrossRef]
11. University of Georgia, Agricultural and Environmental Services Laboratories. Available online: https://aesl.ces.uga.edu/publications/plant/Nutrient.html#:~:text=Aluminum%20is%20not%20considered%20a,is%20not%20required%20by%20plants.&text=Aluminum%20levels%20in%20excess%20of,is%20less%20than%2010%20ppm (accessed on 9 September 2021).
12. Jansen, S.; Broadley, M.R.; Robbrecht, E.; Smets, E. Aluminum hyperaccumulation in angiosperms: A review of if phylogenetic significance. *Bot. Rev. 2002*, 68, 235–269. [CrossRef]

13. Ma, J.F.; Ryan, P.R.; Delhaize, E. Aluminium tolerance in plants and the complexing role of organic acids. *Trends Plant Sci. 2001*, 6, 273–278. [CrossRef]

14. Vlamis, J.; Williams, D. Liming reduces aluminum and manganese toxicity in acid soils. *Calif. Agr. 1962*, 16, 6–7.

15. Cármino, M.P.; Reyes-Díaz, M.; Rengel, Z.; Alberdi, M.; Omen-Garcia, R.P.; Núñez-Nesi, A.; Inostroza-Blanchetteau, C. Aluminium stress differentially affects physiological performance and metabolic compounds in cultivars of highbush blueberry. *Sci. Rep. 2019*, 9, 11275.

16. Rodríguez, A.A.; Filho, S.C.V.; Müller, C.; Rodrigues, D.A.; Sales, J.F.; Zuchi, J.; Costa, A.C.; Rodrigues, C.L.; da Silva, A.A.; Barbosa, D.P. Tolerance of Eugenia dysenterica to aluminum: Germination and plant growth. *Plants 2019*, 8, 317. [CrossRef]

17. Ojeda-Rivera, J.O.; Oropeza-Aburto, A.; Herrera-Estrella, L. Dissection of root transcriptional responses to low pH, aluminum toxicity and iron excess under pi-liming conditions in *Arabidopsis* wild-type and stop1 seedlings. *Front. Plant Sci. 2020*, 11, 01200. [CrossRef]

18. Vance, W.; Pradeep, K.; Strachan, S.R.; Diffey, S.; Bell, R.W. Novel sources of tolerance of aluminium toxicity in wild cicer (*Cicer reticulatum* and *Cicer echinospermum*) collections. *Front. Plant Sci. 2021*, 12, 678211. [CrossRef]

19. Singh, C.K.; Singh, D.; Sharma, S.; Chandra, S.; Konjengbam, N.S.; Singh, D.; Kumar, A.; Upadhyaya, K.C.; Pal, M. Morpho-physiological characterization coupled with expression levels of a novel tolerance mechanism in wild and cultivated lentil under aluminum stress. *Protoplasma 2021*, 258, 1029–1045. [CrossRef]

20. Delhaize, E.; Ryan, P.R. Aluminum toxicity and tolerance in plants. *Plant Physiol. 1995*, 107, 315–321. [CrossRef]

21. Zhou, G.; Delhaize, E.; Zhou, M.; Ryan, P.R. Biotechnological solutions for enhancing the aluminium resistance of crop plants. In *Abiotic Stress in Plants-Mechanisms and Adaptations*; Shanker, A., Venkateswarlu, B., Eds.; *InTech: Rijeka, Croatia, 2011.* [CrossRef]

22. Kopittke, P.M.; Menzies, N.W.; Wang, P.; Blamey, F.P.C. Kinetic and nature of aluminium rhizotoxic effects: A review. *J. Exp. Bot. 2016*, 67, 4451–4467. [CrossRef]

23. Delhaize, E.; Ryan, P.R.; Randall, P. Aluminium tolerance in wheat (*Triticum aestivum* L.) II. Aluminium-stimulated excretion of malic acid from root apices. *Plant Physiol. 1993*, 103, 695–702. [CrossRef]

24. Kuo, M.C.; Kao, C.H. Aluminum effects on lipid peroxidation and antioxidative enzyme activities in rice leaves. *Biol. Plant. 2003*, 46, 149–152. [CrossRef]

25. Kochian, L.V. Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 1995*, 46, 237–260. [CrossRef]

26. Mukhopadhyay, M.; Bantawa, P.; Das, A.; Sarkar, B.; Bera, B.; Ghosh, P.; Mondal, T.K. Changes of growth, photosynthesis and alteration of leaf antioxidative defence system of tea (*Camellia sinensis* (L.) O. Kuntze) seedlings under aluminium stress. *Biometals 2012*, 25, 1141–1154. [CrossRef] [PubMed]

27. Kuo, M.C.; Kao, C.H. Effects of Aluminium on lipid peroxidation and antioxidative enzyme activities in rice leaves. *Biol. Plant. 2003*, 46, 149–152. [CrossRef]

28. Mukhopadhyay, M.; Bantawa, P.; Das, A.; Sarkar, B.; Bera, B.; Ghosh, P.; Mondal, T.K. Changes of growth, photosynthesis and alteration of leaf antioxidative defence system of tea (*Camellia sinensis* (L.) O. Kuntze) seedlings under aluminium stress. *Biometals 2012*, 25, 1141–1154. [CrossRef] [PubMed]

29. Pradhan, B.; Patra, S.; Dash, S.R.; Maharana, S.; Behera, C.; Jena, M. Antioxidant responses against aluminum metal stress in *Gleitlerinema amphibium*. *SN Appl. Sci. 2020*, 2, 800. [CrossRef]

30. Devi, S.S.; Saha, B.; Awasthi, J.P.; Regon, P.; Panda, S.K. Redox status and oxalate exudation determines the differential tolerance of two contrasting varieties of ‘Assam tea’ (*Camellia sinensis* (L.) O. Kuntze) in response to aluminium toxicity. *Hortic. Environ. Biotechnol. 2020*, 61, 485–499. [CrossRef]

31. Silva, S. Aluminium toxicity targets in plants. *J. Bot. 2012*, 2012, 219462. [CrossRef]

32. Ribeiro, C.; Cambraia, J.; Peixoto, P.H.P.; da Fonseca, E.M., Jr. Antioxidant system response induced by aluminum in two rice cultivars. *Braz. Soc. Plant Physiol. 2012*, 24, 107–116. [CrossRef]

33. You, J.; Chan, Z. ROS regulation during abiotic stress responses in crop plants. *Front. Plant Sci. 2015*, 6, 1092. [CrossRef]

34. Ou-Yang, C.; Gao, S.; Mei, L.J.; Chung, T.W.; Tang, L.; Wang, S.H.; Chen, F. Effects of aluminum toxicity on the growth and antioxidative status of *Jatropha curcas* seedlings. *J. Med. Plant Res. 2013*, 8, 178–185. Available online: https://academicjournals.org/article/article1390386071_Ou-yang%20et%20al.pdf (accessed on 12 July 2021).

35. Nasr, N.; Carapetian, J.; Heidari, R.; Asri Rezaei, S.; Abbaspour, N.; Darvishzadeh, R.; Ghezelbash, F. The effect of aluminum on enzymes activities in two wheat cultivars. *Afr. J. Biotechnol. 2011*, 10, 3534–3544.

36. Nogueirol, R.C.; Monteiro, F.A.; Azevedo, R.A. Tropical soils cultivated with tomato: Fractionation and speciation of Al. *J. Environ. Monit. Assess. 2015*, 187, 160. [CrossRef] [PubMed]

37. Du, B.; Nian, H.; Zhang, Z.; Yang, C. Effects of aluminum on superoxide dismutase and peroxide activities, and lipid peroxidation in the roots and calluses of soybeans differing in aluminum tolerance. *Acta Physiol. Plant. 2010*, 32, 883–890. [CrossRef]

38. Saxena, S.C.; Joshi, P.; Grimm, B.; Arora, S. Alleviation of ultraviolet C induced oxidative through overexpression of cytosolic ascorbate peroxidase. *Biologia 2011*, 66, 1052–1059. [CrossRef]

39. Rajput, P.; Chaudhary, M.; Sharma, R.A. Comparative non-enzymatic and enzymatic antioxidant potential screening in species of genus *Urtica*. *IJPSR 2021*, 12, 2876–2883. [CrossRef]
40. Jiang, M.; Zhang, J. Effect of ascorbic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol. 2001, 42, 1265–1273. [CrossRef]

41. Sairam, R.K.; Deshmukh, P.S.; Saxena, D.C. Role of antioxidant systems in wheat genotypes tolerance to water stress. Biol. Plant. 1998, 41, 387–394. [CrossRef]

42. Mittler, R.; Zilinskas, B.A. Regulation of pea cystolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant J. 1994, 5, 397–405. [CrossRef]

43. Dárko, É.; Ambrus, H.; Stefanovits-Bányaí, É.; Fodor, J.; Bakos, F.; Barnabás, B. Aluminium toxicity, Al tolerance and oxidative stress in an Al-sensitive wheat genotype and in Al-tolerant lines developed by in vitro micropropagation selection. Plant Sci. 2004, 166, 583–591. [CrossRef]

44. Shigeoka, S.; Ishikawa, T.; Tami, M.; Miyagawa, Y.; Takeda, T.; Yabuta, Y.; Yoshimura, K. Regulation and function of ascorbate peroxidase isoenzymes. J. Exp. Bot. 2002, 53, 1305–1319. [CrossRef]

45. Boscolo, P.R.S.; Menossi, M.; Jorge, R.A. Aluminium-induced oxidative stress in maize. Phytochemistry 2003, 62, 181–189. [CrossRef]

46. Li, W.; Mo, W.; Ashraf, U.; Li, G.; Wen, T.; Abrar, M.; Gao, L.; Liu, J.; Hu, J. Evaluation of physiological indices of waterlogging tolerance of different maize varieties in South China. Ecol. Environ. Res. 2018, 16, 2059–2072. [CrossRef]

47. Alzahrani, S.M.; Alaraidh, I.A.; Migdadi, H.; Alghamdi, S.; Khan, M.A.; Ahmad, P. Physiological, biochemical, and antioxidant properties of two genotypes of Vicia faba grown under salinity stress. Pak. J. Bot. 2019, 51, 786–798. [CrossRef]

48. Hussain, H.A.; Men, S.; Hussain, S.; Chen, Y.; Ali, S.; Zhang, S.; Zhang, K.; Li, Y.; Xu, Q.; Liao, C. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Sci. Rep. 2019, 9, 1–12. [CrossRef] [PubMed]

49. Zeeshan, M.; Lu, M.; Sehar, S.; Holford, P.; Wu, F. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. Agronomy 2020, 10, 127. [CrossRef]

50. Jiang, M.; Zhang, J. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol. 2001, 42, 1265–1273. [CrossRef]

51. Chahardoli, A.; Karimi, N.; Ma, X.; Qalekhani, F. Effects of engineered aluminum and nickel oxide nanoparticles on the growth of common bean cultivars and lines to aluminum toxicity. Agronomy 2020, 10, 296. [CrossRef]

52. FREDDI, O.S.; TAVANTI, R.F.R.; CARVALHO, M.P.; MONTANARI, R.; ANDREOTTI, M. Restrictions of the common bean productivity in direct seedling system in the Brazilian cerrado. Nativa 2017, 5, 237–243. [CrossRef]

53. Birachi, E.A.; Ochieng, J.; Wozemba, D.; Ruraduma, C.; Niyuhire, M.C.; Ochieng, D. Factors influencing smallholder farmers’ bean production and supply to market in Burundi. Afr. Crop Sci. J. 2011, 19, 335–342. Available online: https://www.ajol.info/index.php/acsj/article/view/74193 (accessed on 12 July 2021).

54. Zhao, J.; Li, H.; Luo, Z.; Huang, D.; Yang, L.; Zou, L.; Li, Z.; Shi, J.; Zhang, X.; Xi, L.; et al. Advances in developing screening methods and improving aluminum resistance in common bean and Bracharia. Rev. Bras. Agric. 2008, 14, 1–7. [CrossRef]

55. Blair, M.W.; López-Marin, H.D.; Rao, I.M. Role of aluminum resistant Andean common bean (Phaseolus vulgaris L.) genotypes for acidic soil tolerance. Int. J. Adv. Res. Publ. 2017, 1, 39–46. Available online: http://www.ijarp.org/published-research-papers/sep2017/Review-Paper-On-Breeding-Common-Bean-phaseolus vulgaris L-Genotypes-For-Acidic-Soil-Tolerance.pdf (accessed on 12 July 2021).

56. Freddi, O.S.; Tavanti, R.F.R.; Carvalho, M.P.; Montanari, R.; Andreotti, M. Restrictions of the common bean productivity in direct seedling system in the Brazilian cerrado. Nativa 2017, 5, 237–243. [CrossRef]

57. Freddi, O.S.; Tavanti, R.F.R.; Carvalho, M.P.; Montanari, R.; Andreotti, M. Restrictions of the common bean productivity in direct seedling system in the Brazilian cerrado. Nativa 2017, 5, 237–243. [CrossRef]

58. Zeeshan, M.; Lu, M.; Sehar, S.; Holford, P.; Wu, F. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. Agronomy 2020, 10, 127. [CrossRef]
66. Llugany, M.; Poschenrieder, C.; Barceló, J. Monitoring of aluminum-induced inhibition of root elongation in four maize cultivars differing in tolerance to aluminum and proton toxicity. *Physiol. Plant.* 1995, 93, 265–277. [CrossRef]

67. Yang, Z.B.; Eticha, D.; Albacete, A.; Rao, I.M.; Roitsch, T.; Horst, W.J. Physiological and molecular interaction between aluminum toxicity and drought stress in common bean (*Phaseolus vulgaris*). *J. Exp. Bot.* 2012, 63, 3109–3125. [CrossRef]

68. Massot, N.; Llugany, M.; Poschenrieder, C.; Barceló, J. Callose production as indicator of aluminum toxicity in bean cultivars. *J. Plant Nutr.* 1999, 22, 1–10. [CrossRef]

69. Ma, J.F.; Nagao, S.; Sato, K.; Ito, H.; Furukawa, J.; Takeda, K. Molecular mapping of a gene responsible for Al-activated secretion of citrate in barley. *J. Exp. Bot.* 2004, 55, 1335–1341. [CrossRef]

70. Rhind, S.M. Anthropogenic pollutants: A threat to ecosystem sustainability? *Philos. Trans. R. Soc. B.* 2009, 364, 1534. [CrossRef] [PubMed]

71. Godfray, H.C.J.; Garnett, T. Food security and sustainable intensification. *Philos. Trans. R. Soc. B.* 2014, 369, 1639. [CrossRef] [PubMed]

72. Kochian, L.V.; Piñeros, M.A.; Hoekenga, O.A. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil* 2005, 274, 175–195. [CrossRef]

73. Zhang, J.; He, Z.; Tian, H.; Zhu, G.; Peng, X. Identification of aluminium-responsive genes in rice cultivars with different aluminium resistances. *J. Exp. Bot.* 2007, 58, 2269–2278. [CrossRef]

74. Kochian, L.V.; Piñeros, M.A.; Liu, J.; Magalhaes, J.V. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. *Annu. Rev. Plant Biol.* 2015, 66, 571–598. [CrossRef] [PubMed]

75. Liu, Q.; Yang, J.L.; He, L.S.; Li, Y.Y.; Zheng, S.J. Effect of aluminum on cell wall, plasma membrane, antioxidants and root elongation in triticale. *Biol. Plant.* 2008, 52, 87–92. [CrossRef] [PubMed]

76. Jones, D.L.; Blancoflor, E.B.; Kochian, L.V.; Gilroy, S. Spatial coordinatation of aluminum uptake, production of reactive oxygen species and root elongation in bean. *Plant Physiol.* 2006, 140, 174–178. [CrossRef]

77. Haug, A.; Weis, C. Aluminum-induced changes in calmodulin. In *Molecular and Cellular Aspects of Calcium in Plant Development*; Trewavas, A.J., Ed.; Springer: Boston, MA, USA. [CrossRef]

78. Rahman, M.A.; Lee, S.-H.; Ji, H.C.; Kabir, A.H.; Jones, C.S.; Lee, K.W. Importance of Mineral Nutrition for Mitigating Aluminum Toxicity in Plants on Acidic Soils: Current Status and Opportunities. *Int. J. Mol. Sci.* 2018, 19, 3073. [CrossRef]

79. Rangel, A.F.; Rao, I.M.; Horst, W.J. Spatial aluminium sensitivity of root apices of two common bean (*Phaseolus vulgaris*) genotypes with contrasting aluminium resistance. *J. Exp. Bot.* 2007, 58, 3895–3904. [CrossRef]

80. Rangel, A.F.; Mobin, M.; Rao, I.M.; Horst, W.J. Proton toxicity interferes with the screening of common bean (*Phaseolus vulgaris*) genotypes for aluminum resistance in nutrient solution. *J. Plant Nutr. Soil Sci.* 2005, 168, 607–616. [CrossRef]

81. Zheng, S.J.; Yang, J.L. Target sites of aluminum phytotoxicity. *Biol. Plant.* 2005, 49, 321–331. [CrossRef]

82. Huang, H.; Ullah, F.; Zhou, D.X.; Yi, M.; Zhao, Y. Mechanisms of ROS regulation of plant development and stress responses. *Front. Plant Sci.* 2019, 10, 800. [CrossRef] [PubMed]

83. Nahar, K.; Hasanuzzaman, M.; Suzuki, T.; Fujita, M. Polyamines-induced aluminum tolerance in mung bean: A study on antioxidant defence and methylglyoxal detoxification systems. *Ecotoxicology* 2017, 26, 58–73. [CrossRef]

84. Yamamoto, Y.; Kobayashi, Y.; Devi, S.R.; Rikiishi, S.; Matsumoto, H. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. *Plant Physiol.* 2002, 128, 63–72. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC148944/ (accessed on 12 July 2021). [CrossRef] [PubMed]

85. Hossain, M.A.; Bhattacharjee, S.; Armin, S.M.; Qian, P.; Xin, W.; Li, H.Y.; Burritt, D.J.; Fujita, M.; Tran, L.S.P. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: Insights from ROS detoxification and scavenging. *Front. Plant Sci.* 2015, 6, 1–19. [CrossRef] [PubMed]

86. Zheng, H.; Zhang, S.; Meng, Q.; Zou, J.; Jiang, W.; Liu, D. Effects of aluminum on nucleoli in root tip cells, root growth and the antioxidant defence system in *Vicia faba* L. *Acta Biol. Crac. Ser. Bot.* 2009, 51, 99–106.

87. Chen, Q.; Wu, K.H.; Zhang, Y.N.; Phan, X.H.; Li, K.Z.; Yu, Y.X.; Chen, L.M. Physiological and molecular responses of broad bean (*Vicia faba* L.) to aluminum stress. *Acta Physiol. Plant.* 2012, 34, 2251–2263. [CrossRef]

88. Rangel, A.F.; Rao, I.M.; Braun, H.P.; Horst, W.J. Aluminum resistance in common bean (*Phaseolus vulgaris*) involves induction and maintenance of citrate exudation from root apices. *Physiol. Plant.* 2010, 138, 176–190. [CrossRef]

89. Yamamoto, Y.; Kobayashi, Y.; Matsumoto, H. Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiol.* 2001, 125, 199–208. [CrossRef]

90. Horst, W.J.; Asher, C.J.; Cakmak, I.; Szulkiewica, P.; Wissemeyer, A.H. Short-term response of soybean roots to aluminum. *J. Plants Physiol.* 1992, 140, 174–178. [CrossRef]

91. Kinraide, T.B. Aluminum enhancement of plant growth in acid rooting media. A case of reciprocal alleviation of toxicity by two toxic cations. *Physiol. Plant.* 1993, 88, 619–625. [CrossRef]

92. Birouste, M.; Zamora-Ledezma, E.; Bossard, C.; Pérez-Ramos, I.M.; Roumet, C. Measurement of fine root tissue density: A comparison of three methods reveals the potential of root dry matter content. *Plant Soil* 2014, 374, 299–313. [CrossRef]

93. Giannopolities, C.H.; Ries, S.K. Superoxide Dismutase I. Occurrence in Higher Plant. *Plant Physiol.* 1977, 59, 309–314. Available online: https://shibbolethps.jstor.org/start/entitityID=https%3A%2F%2Fpid.unideb.hu%2Fsamolis2%2Fpmid%2Fmetadata_data.php&ddest=https://www.jstor.org/stable/4264724&site=jstor (accessed on 12 July 2021). [CrossRef] [PubMed]
94. Beyer, W.F.; Fridovich, I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal. Biochem.* 1987, 161, 559–566. [CrossRef]

95. Zeislin, N.; Ben-Zaken, R. Peroxidases, phenylalanine ammonia-lyase and lignification in peduncles of rose flowers. *Plant Physiol. Biochem.* 1991, 29, 147–151.

96. Keller, A.; Mohamed, A.; Drose, S.; Brandt, U.; Fleming, I.; Brandes, R.P. Analysis of dichlorodihydrofluorescein and dihydrocalcein as probes for the detection of intracellular reactive oxygen species. *Free Radic. Res.* 2004, 38, 1257–1267. [CrossRef]

97. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 1968, 125, 189–198. [CrossRef]

98. Ghasemi, A.; Zahediasl, S. Normality Tests for Statistical Analysis: A Guide for Non-Statisticians. *Int. J. Endocrinol. Metab.* 2012, 10, 486–489. [CrossRef] [PubMed]

99. Nachar, N. The Mann-Whitney U: A Test for Assessing whether Two Independent Samples Come from the Same Distribution. *Tutor. Quant. Methods Psychol.* 2008, 4, 13–30. Available online: https://www.tqmp.org/RegularArticles/vol04-1/p013/p013.pdf (accessed on 12 July 2021). [CrossRef]

100. Schober, P.; Boer, C.; Schwarte, L.A. Correlation coefficient: Appropriate use and interpretation. *Anesth. Analg.* 2018, 12, 1763–1768. [CrossRef]