

Article

Phenotypic Variability for Root Traits in Andean Common Beans Grown with and without Aluminum Stress Conditions

Daniel Ambachew¹, Asrat Asfaw² and Matthew W. Blair^{1,*} 

¹ Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN 37209, USA

² International Institute of Tropical Agriculture, Abuja 901101, Nigeria

* Correspondence: mblair@tnstate.edu

Abstract: Genetic variation in wild relatives, GenBank accessions, landraces, and cultivars can unlock key alleles for the traits of interest for breeding programs. Breeding programs often utilize different strategies to quantify the source of heritable variation for target traits. One neglected area of study is the root traits of diverse genotypes, and this is especially the case for aluminum toxicity effects on legumes such as the common bean, which is the most used pulse for direct human consumption. This study evaluated 267 genotypes of common bean that were part of the global Andean Diversity Panel (ADP), consisting mainly of genotypes assembled from public and private breeding programs in Africa and North America, as well as elite lines and land races from the USDA. The ADP was evaluated for root traits at the seedling stage in the Tennessee State University (TSU) greenhouse using a hydroponic system with a standard nutrient solution with and without aluminum (Al). The recorded data on the roots per trial were fit to a linear mixed model for the analysis of variance in order to test for the genotype differences. Adjusted means considered replication and blocks within replication as random effects and genotypes as fixed effects. These were then used for Pearson correlation tests and for principal component analysis (PCA), where the first two vectors accounted for 94.5% and 93% of the explained variation under the control and Al-treatment conditions, respectively. Genotypes were clustered based on the morphology of roots in response to Al-toxicity treatment using the Euclidean distance and Ward's hierarchical agglomerative clustering method, identifying four distinctive groups significant at $p < 0.01$. The intra-cluster distance was lower than the inter-cluster distances, which indicated a heterogeneous and homogeneous nature between and within clusters, respectively. The results suggest that crossing between accessions from two of the clusters would result in the maximum genetic segregation. One cluster was found to have a higher Al-toxicity tolerance than the others.

Keywords: aluminum toxicity; breeding for roots; genetic segregation; hierarchal clustering; pearson correlation; principal component; wide adaptability



Citation: Ambachew, D.; Asfaw, A.; Blair, M.W. Phenotypic Variability for Root Traits in Andean Common Beans Grown with and without Aluminum Stress Conditions.

Agronomy **2023**, *13*, 619. <https://doi.org/10.3390/agronomy13030619>

Academic Editor: Lixia Wang

Received: 13 January 2023

Revised: 17 February 2023

Accepted: 18 February 2023

Published: 22 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Sustainable crop production is key to the global food system. A tremendous challenge that we face today is satisfying the ever-growing demand for food, feed, and clothing under the current scenarios of climate change. The dramatic loss of arable land due to increasingly severe soil destruction by pollution and loss of crop yields due to biotic and abiotic stresses have exacerbated the challenge of mitigating climate caused loss to yield [1]. The common bean (*Phaseolus vulgaris* L.) is among the key crops in the global agri-food system. However, the production of common beans in many regions of the tropics and sub-tropics is being challenged by biotic and abiotic stresses, made worse by the calamities of climate change [2,3]. Aluminum (Al) toxicity [4,5] and salinity [6], with drought and heat stress, are the most important abiotic stresses limiting common bean production [7].

In this regard, common bean production is mainly constrained by Al toxicity in areas where there is acidic soil, namely those with a pH below 4.3 [8]. Al is the most abundant

metal in the Earth's crust [9] and the toxicity it causes is a significant problem for crop production in humid and semi-humid regions, representing 30–50% of the world's arable land [10–12]. Al toxicity can reduce common bean yields by up to 60% [13,14]. Although naturally occurring forms of Al are stable and do not interact with the biological processes of plants [15–17], Al is solubilized into toxic forms such as $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$ as the soil pH drops below 5 or further into the acidic soils range.

Most crop plants are sensitive to micro–molar concentrations of Al and once the solubilized forms enter into the roots, it interferes with various physiological and cellular processes. Toxic amounts of soluble Al inhibit the growth and function of roots by injuring the root apex [18–20]. These processes also affect the function of other plant parts, resulting in a high yield reduction [9]. Al toxicity can result in poor root growth and changes in root morphology, stunted plant growth, thicker lateral and taproots, browning of the roots, and inefficient water and nutrient uptake by the roots [21,22], which is also the case in *Phaseolus vulgaris* [23].

The development of new cultivars with stress resistance is the main objective of many common bean improvement programs, for example for drought [24–29], salinity [30,31], and Al toxicity [32,33]. Genetic variability for a trait of interest is a bedrock for any crop improvement effort through breeding. Genetic variation in wild relatives, GenBank accessions, landraces, and cultivars can unlock key alleles for the traits of interest for breeding programs. Breeding programs often utilize different strategies to quantify the source of heritable variation for target traits. To this end, numerous studies have been conducted to assess the available genetic diversity for abiotic stress resistance in common bean land races, commercial cultivars, and breeding lines. These studies were conducted under contrasting environments, with and without the stress conditions being studied. Most studies evaluated mainly the yield and yield components.

The study of root traits in diverse genotypes is one of the most important study areas, and it has not been given due attention. This is especially true for the effects of Al toxicity on legumes such as the common bean, which is the main pulse used for direct human consumption. Success with breeding is dependent on the genetic merits of the trait progenitors with all limits of fitness in order to obtain a higher amount of heterotic expression in progenies and a broad spectrum of variability in segregating populations. Therefore, the goal of this study was to evaluate the phenotypic variation for root attributes in a panel of Andean common bean gene pool grown under Al stress and under normal growth conditions, and to identify the association of traits and the identification of genotypes with better root ideotypes that could serve as parents in breeding programs aimed to improve Al-toxicity resistance in common beans. In the common bean, the Andean large-seeded genotypes coincide with areas of Al-toxicity stress, especially in Eastern and Southern Africa [4–7], and thus were the focus of this study.

2. Materials and Methods

2.1. Study Material and Treatment Design

The study material consisted of 262 genotypes that were part of an Andean bean diversity panel (ADP) from Cichy et al. [34]. The ADP consisted of genotypes mainly from Africa and North America collected from public and private breeding programs, elite lines, and land races by the United States Department of Agriculture (USDA). The ADP genotypes were evaluated for their root traits at the seedling stage in a greenhouse using a hydroponic system with a standard nutrient solution with two treatments: one with Al and one without Al (control). The seeds were surface sterilized for 1 min with 70% alcohol and 2% sodium hypochlorite (NaClO) and rinsed with deionized water and dried with a sterilized paper towel. To ensure the same time for the germination of genotypes, the seeds were scarified using a scalpel just on the opposite side of the seed micropyle. After scarification, the seeds were placed into a sterilized magenta box with a sterilized wet paper towel in it. Four days after germination, the seedlings were transplanted to a hydroponic system with a standard nutrient solution. The seedlings were placed in 30.2 cm deep,

65.4 cm long, 43.8 cm wide, and 50 L black tanks covered by 5 cm thick Styrofoam floating sheets that could carry 49 genotypes, two seedlings per genotype, and that were replicated three times over time.

2.2. Phenotyping System

Experiments were conducted at the Tennessee State University (TSU) main agricultural station, Nashville, TN. A hydroponic system was installed in the greenhouse at TSU. The system was equipped with two eight-channel air pumps to ensure a continuous aeration of the system. Submersible water pumps were used for continuous pumping of the nutrient solution. The purpose of continuous pumping of nutrients was to ensure continuous agitation and avoid sedimentation. The pH, electrical conductivity (EC), and temperature of the system around the roots, and the greenhouse temperature and relative humidity in the greenhouse were monitored in a real-time base using an automated remote-control system. The standard nutrient solution was composed of 286 μM $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 300 μM KNO_3 , 150 μM NH_4NO_3 , 2.5 μM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 150 μM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 14 μM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 5 μM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 5 μM $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 1 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1 μM ZnCl_2 , 0.2 μM $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 6 μM H_3BO_3 , 5 μM $\text{NaSiO}_3 \cdot 9\text{H}_2\text{O}$, 0.001 μM $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, and 57.5 μM NaCl , according to Rao et al. [35]. For the Al-toxicity treatment, 50 μM AlCl_3 was added upon seedling transfer. The pH was adjusted and maintained in the range of 5.6 to 6.0 and 4.0 to 4.5 for the control and Al treatments, respectively. Similarly, the electrical conductivity was maintained below 400 $\mu\text{S}/\text{cm}$ and above 250 $\mu\text{S}/\text{cm}$ for both treatments. The relative humidity of the greenhouse was maintained at 40–50%. The entire hydroponic system and greenhouse were maintained in a temperature range of 22–25 $^\circ\text{C}$, as described by Ambachew and Blair [32].

2.3. Root Trait Measurement

Phenotypic data on the (1) total root length (LEN), (2) average root diameter (AvRD), (3) number of root tips (NTIP), (4) root volume (RVOL), (5) number of forks (NFRK), (6) root surface area (RSA), (7) number of links (NLINK), and (8) number of crosses (NCRS) were recorded by scanning and analyzing the images of the harvested seedling roots using a software program WinRHIZO pro V 2008b (Regent Instruments, Inc., Quebec, Canada) and a flatbed EPSON perfection V850 pro scanner (Seiko EPSON Corporation, Owa, Suwa-shi, Nagano, Japan). The seedlings were harvested and stored in deionized water for image acquisition 24 h after transplanting to the hydroponic system. Then, the root images were acquired on a gray scale to a resolution of 800 dots per inch (dpi). The analysis was done on the root morphology by setting the rough edge and noise removal to a higher level, and the dark root on white background measurement option. The image acquisition took on average 30 s per sample.

2.4. Statistical Analysis

The recorded data on the roots per trial were first subjected to mixed model analysis using the PROC MIXED procedure in statistical analysis software SAS (V9.4) in order to obtain adjusted means by considering replication and blocks within replication as random effects and genotypes as fixed effects. The adjusted means were used for the subsequent analysis. Descriptive statistics was used to describe the data. Pearson correlation and variance inflation (VIF) tests were done to check for the multicollinearity of variables using the “cor” function and “vif” functions of the stats [36] and car [37] packages in R, respectively. The test for the correlation between the paired samples were calculated and the correlation matrix were generated using the “psych” [38] package. After removing the non-correlated and variables with a VIF threshold below 5, principal component analysis (PCA) and cluster analysis were conducted on scaled data to the mean of zero and variance of unity to avoid differences in the scales used for data recording. Euclidean distance was used to estimate the genotypic divergence. R packages “ecodist” [39] and the prcomp function of the “stats” package [36] were used to calculate the Mahalanobis distance, PCA, and clustering analysis. Genotypic divergence analysis was done using a cluster

validation package “clv” [40]. The PCA biplots were plotted using the “fviz” and “ggplot” functions of the “factoextra” [41] and “ggplot2” [42] packages, respectively. Hierarchical clustering of the genotypes was accomplished using the Ward.D2 [43] method and seven root traits (Len, RSA, RVOL, NTIP, NFRK, NCRS, and NLINK) were evaluated under Al treatment. The number of clusters was determined using the k-mean method. The average linkage method was used to estimate the inter and intra cluster genetic distances. The phylogram of hierarchical clustering was drawn using the “as.phylo” function of the “ape” package [44]. The percentage change in the total root length and average root diameter were calculated for each experimental unit as a ratio by taking the difference between the values in the Al and control treatments as numerators and the values in the control treatment as denominators, and the resultant change was multiplied by 100 to obtain the percent (%). Simple scattergram (x, y pair) between percentage change in the total root length and percentage change in average root diameter was made using the ggplot2 package in R [42].

3. Results

The mean performance of the genotypes for each treatment and root trait measured and the percent change calculated to index the treatment condition are presented in Supplemental Table S1. The results show the maximum reduction for all of the root traits (~64% average percentage reduction in root volume to 86% reduction in the number of root crosses). The relationships between the root traits under Al-treated and control conditions are shown with Pearson’s correlations in Table 1.

Table 1. Pearson correlation of seven root traits of 262 Andean common bean genotypes grown under control (above diagonal) and aluminum (below diagonal) treatment conditions.

Trait	LEN	RSA	Rvol	AvRD	NTIP	NFRK	NCRS	NLINK
LEN		0.93 ***	0.79 ***	−0.04	0.84 ***	0.92 ***	0.87 ***	0.92 ***
RSA	0.95 ***		0.95 ***	0.28 ***	0.75 ***	0.90 ***	0.74 ***	0.89 ***
Rvol	0.83 ***	0.96 ***		0.50 ***	0.59 ***	0.79 ***	0.57 ***	0.77 ***
AVRD	−0.05	0.20 ***	0.41 ***		−0.21 ***	0.06	−0.22 ***	0.03
NTIP	0.81 ***	0.77 ***	0.66 ***	−0.14 *		0.82 ***	0.8 ***	0.83 ***
NFRK	0.93 ***	0.89 ***	0.79 ***	−0.03	0.78 ***		0.92 **	1.00 ***
NCRS	0.83 ***	0.69 ***	0.53 ***	−0.22 ***	0.62 ***	0.88 ***		0.93 ***
NLINK	0.93 ***	0.89 ***	0.78 ***	−0.04	0.8 ***	1.00 ***	0.88 ***	

* significant at $p \leq 0.05$; * significant at $p \leq 0.01$; *** significant at $p \leq 0.001$; Abbreviations: LEN, total root length (cm); RSA, root surface area (cm²); RVOL, root volume (mm³); AVRD, average root diameter (mm); NTIP, number of root tips; NFRK, number of root forks; NCRS, number of root crosses; NLNK, number of links.

Pearson correlation indicated the presence of a significant correlation among some root traits. There was a significant positive correlation ($r > 0.79$, $p < 0.001$) between total root length and other traits, except for the average root diameter. The average root diameter had significant negative correlations with the number of root tips ($r = -0.21$, and $r = -0.14$ at $p < 0.001$ under the control and Al treatment, respectively) and the number of root crosses ($r = -0.22$, $p < 0.001$) under both treatment conditions. It also showed a significant positive correlation with root surface area ($r = 0.2$ under the control treatment and $r = 0.2$ under the Al treatment) and root volume ($r = 0.5$ under the control and $r = 0.41$ under the Al treatment). A perfect highly significant positive correlation was observed between the number of root tips and the number of root links.

The principal components of control and Al treatments are presented in Tables 2 and 3, respectively. We also assessed the multicollinearity of traits using the variance inflation factor and removed traits with values below a threshold level of 5 from the PCA. After removing the average root diameter, the final PCA was done using the remaining root traits.

Table 2. Eigenvalues and percentage of total variation and component matrix for the first four principal component axes of seven root traits of seedlings of 267 Andean common bean genotypes grown under the control treatment.

Principal Components	1	2	3	4
Eigenvalues	6.019	0.598	0.241	0.103
Standard Deviation	2.453	0.773	0.490	0.320
Proportion of Variance	86.0	8.50	3.40	1.50
Cumulative Proportion	86.0	94.5	98.0	99.42
Component Matrix				
LEN	0.394	−0.014	−0.088	0.767
RSA	0.387	0.389	−0.070	0.166
RVOL	0.343	0.688	−0.045	−0.182
NTIP	0.353	− 0.372	− 0.817	− 0.230
NFRK	0.399	−0.078	0.280	− 0.364
NCRS	0.367	− 0.466	0.419	0.184
NLNK	0.399	−0.119	0.252	−0.363

Abbreviations: LEN, total root length (cm); RSA, root surface area (cm²); RVOL, root volume (mm³); NTIP, number of root tips; NFRK, number of root forks; NCRS, number of root crosses; NLNK, number of links. Numbers in bold are significant ($p < 0.001$).

Table 3. Eigenvalues and percentage of total variation and component matrix for the principal component axes of seven root traits of seedlings for 267 Andean common bean genotypes grown under AI stress treatment.

Principal Components	1	2	3	4
Eigenvalues	5.934	0.577	0.347	0.099
Standard Deviation	2.436	0.760	0.589	0.314
Proportion of Variance (%)	84.75	8.20	5.00	1.40
Cumulative Proportion (%)	84.75	93.0	98.0	99.0
Component Matrix				
LEN	− 0.400	−0.001	−0.028	0.584
RSA	− 0.393	− 0.346	−0.176	0.223
RVOL	− 0.355	− 0.608	− 0.296	−0.107
NTIP	− 0.346	−0.071	0.905	0.039
NFRK	− 0.400	0.185	−0.079	−0.512
NCRS	− 0.345	0.662	− 0.233	0.303
NLNK	− 0.401	0.184	−0.040	− 0.491

Abbreviations: LEN, total root length (cm); RSA, root surface area (cm²); RVOL, root volume (mm³); NTIP, number of root tips; NFRK, number of root forks; NCRS, number of root crosses; NLNK, number of links. Numbers in bold are significant ($p < 0.001$).

Under the control treatment, the first four principal components accounted for 99.42% of the total variation of roots that existed in the ADP. The analysis of eigenvectors indicated that the first principal component alone accounted for 86% of the total variation. The observed variation for the second, third, and fourth components were 8.5%, 3.4%, and 1.5%, respectively. All of the root traits considered had positive correlations and a large contribution to the first principal component.

In the second component, the root surface area (0.389), root volume (0.688), number of root tips (−0.372), and number of root crosses (−0.466) were the most contributing traits to the second component (Table 2). The Cos² of the variables indicated that the root surface area and root volume in the positive direction and the number of root tips and number of root crosses in the negative direction were the major contributing traits. Similarly, on the bases of Cos² value for individuals, ADP-0554, ADP-0624, ADP0775, and ADP0645 in the negative direction and ADP-0575 in the positive direction, were the top five genotypes contributing towards the PCA (Figure 1).

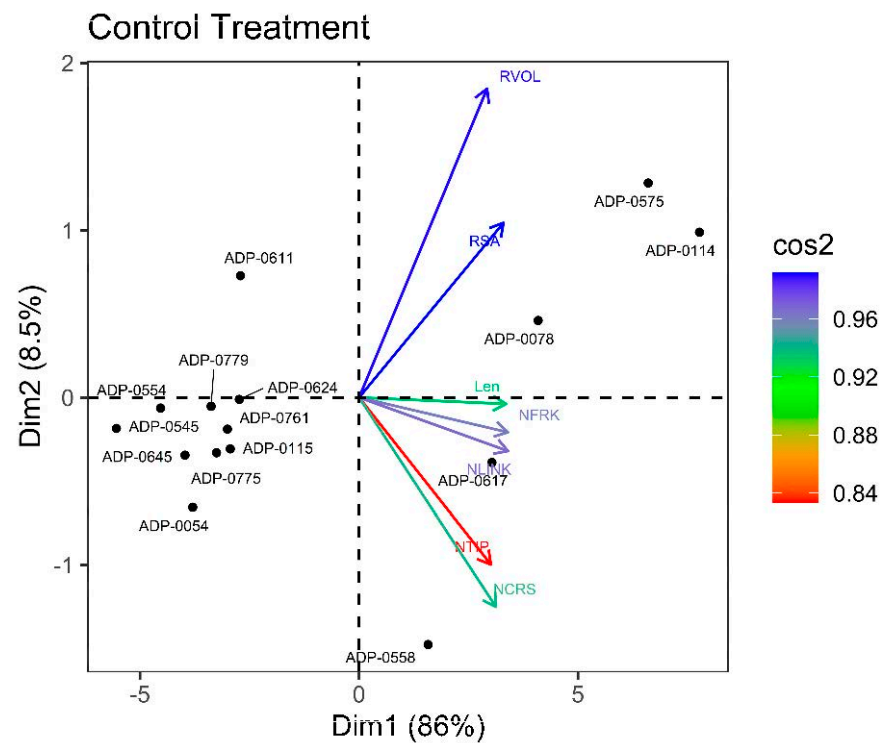


Figure 1. Genotype \times trait biplot displaying trait associations across seven root traits of Andean common bean genotypes grown under control treatment of non-stress hydroponics and the top 15 genotypes contributing towards PCA based on Cos^2 Value. Abbreviations as in the previous tables.

The table above presents the eigenvalues for the principal components for the traits, pattern, and magnitude of the association of traits with each principal component under the AI-treatment condition. Similar to the control treatment, the first four principal components explained 99.36% of the observed total variation, with the first principal component being the major component accounting for 84.8% of the total variation. The observed variation for the second, third, and fourth components were 8.2%, 5%, and 1.4%, respectively. As opposed to the control treatment, all of the root traits considered had a large and negative contribution to the first principal component. The root surface area (-0.34), root volume (-0.60), and number of root crosses (0.66) were the most contributing root traits for the second component.

According to the Cos^2 of variables, the root surface area and root volume in the negative direction and the number of root crosses in the negative direction were the major contributing traits. Cos^2 also identified ADP-0605, ADP-0667, ADP-0726, ADP-0592, and ADP-0561, all in the positive direction, as the most top five contributing genotypes towards PCA (Figure 2). In general, despite the treatment condition, and regardless of the direction of contribution, all the root traits considered contributed to the first principal component which could be regarded as one for root system architecture. Similarly, as the root volume and root surface area were the major contributors to the second principal component, this could be regarded as a contributor of root volume.

The pattern of divergence between the 15 genotypes with larger contributions to the first two principal components is given in Figures 1 and 2. Under the control treatment, it can be discerned from Figure 1 that the genotype diversity in all of the root traits considered was large. Three genotypes ADP-0575, ADP-0078 and ADP-0114 in the first quadrant of the biplot had a large and positive contribution towards both principal components, while ADP-0617 and ADP-0611 had a large positive and negative contribution to the first principal component, and vice versa to the second component. The majority of the 15 genotypes were populated in the third quadrant of the biplot and their contribution towards the principal components were in the negative direction.

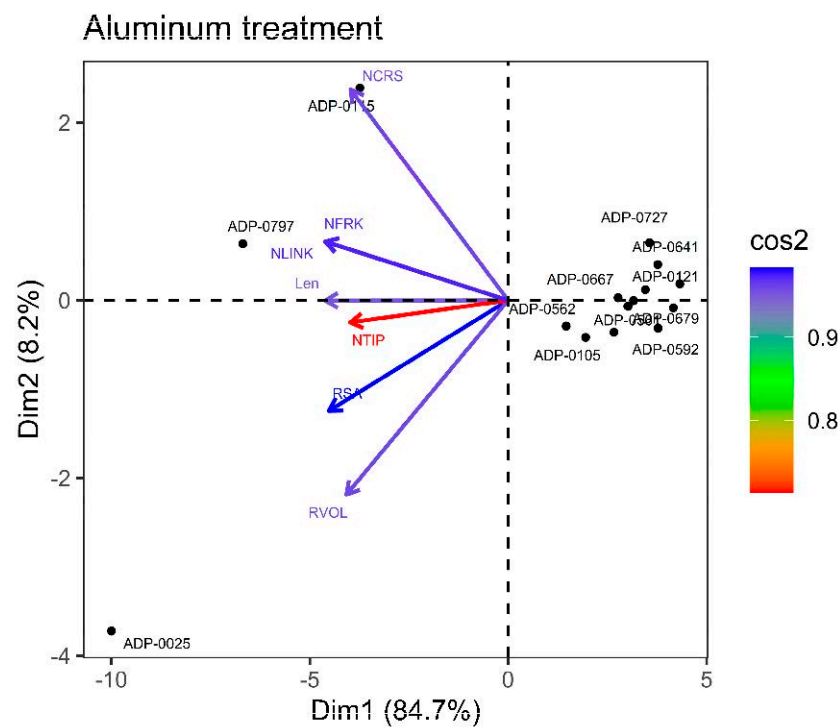


Figure 2. Genotype \times trait biplot displaying trait association across seven root traits of Andean common bean genotypes grown under Al stress treatment and top 15 genotypes contributing towards PCA based on Cos^2 value. Abbreviations are the same as in previous tables and the ADP number is as found in the panel description [34].

Under Al treatment (Figure 2), ADP-0025 had a large negative contribution to the first and second components. In the second quadrant, two genotypes (ADP-0797 and ADP-0115) had a large positive contribution to the second component, but a negative contribution to the first component. As most of the genotypes occupied the first and fourth quadrants, they had the largest positive effect on the first component and a very small contribution to the second component in both positive and negative directions.

The clustering of genotypes based on the morphology of roots in response to Al-stress treatment using the Euclidean distance and Ward's hierarchical agglomerative clustering method identified four distinctive groups (Figure 3). Cluster III was the largest group, and it accommodated 53.05% of the genotypes, followed by the second largest group of Cluster IV, which consisted of 25.19% of the genotypes. Cluster II and Cluster I consisted of 15.27% and 6.49% of the genotypes, respectively (Supplementary Table S2, Figure 3).

The estimated intra- and inter-cluster distances values are presented in Table 4. The differences between clusters were significant at $p < 0.01$. The intra-cluster distance was lower than the inter-cluster distances, which indicated a heterogeneous and homogeneous nature between and within clusters, respectively. The highest intra-cluster distance (92.32) was observed in Cluster I, followed by Clusters II (58.11) and VI (54.02). The minimum intra cluster distance was observed for cluster III (42.94).

Although Cluster III contained the largest number of genotypes (58.11%), it had the shortest intra-cluster distance. This indicates that the genotypes grouped in this cluster were more similar compared with the rest of the genotypes grouped in different clusters. Cluster I was more heterogeneous (Table 4 and Figure 4) compared with the other clusters. The differences between clusters in terms of a mean group performance for the genotypes in each cluster for each root trait are shown in Figure 4. The first cluster had greater mean values for each trait (total root length, root surface area, root volume, number of root tips, number of root crosses, and number of root links and number of root links) compared with the other clusters, followed by the second, third, and fourth clusters. The Al-tolerant

genotypes of each cluster were based of the indices calculated from the two experiments and are listed in the Discussion section. These were genotypes in the first and second clusters of our clustering results.

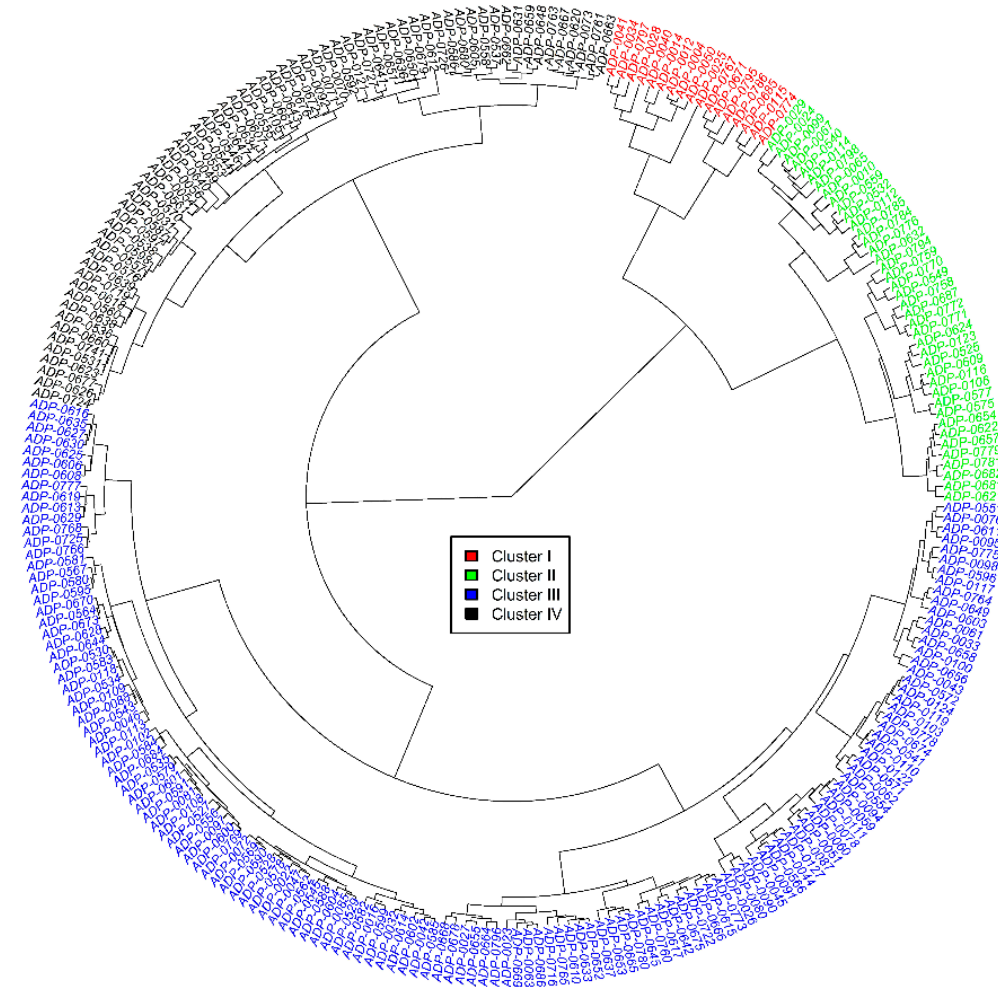


Figure 3. Phylogram of hierarchal clustering of 262 common bean genotypes grouped into four clusters based on response to AI-toxicity treatment. Euclidean distance was used for clustering based on seven quantitative root trait variables.

Table 4. Intra- (diagonal values) and inter-cluster divergence (off-diagonal values) in 262 genotypes from the Andean common bean diversity panel (ADP) evaluated under AI-toxicity treatment based on pairwise generalized squared distance.

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	92.32	446.22	720.64	955.82
Cluster II		58.11	277.38	511.94
Cluster III			42.94	236.80
Cluster IV				54.02

$\chi^2 = 15.09$ at $p < 0.01$

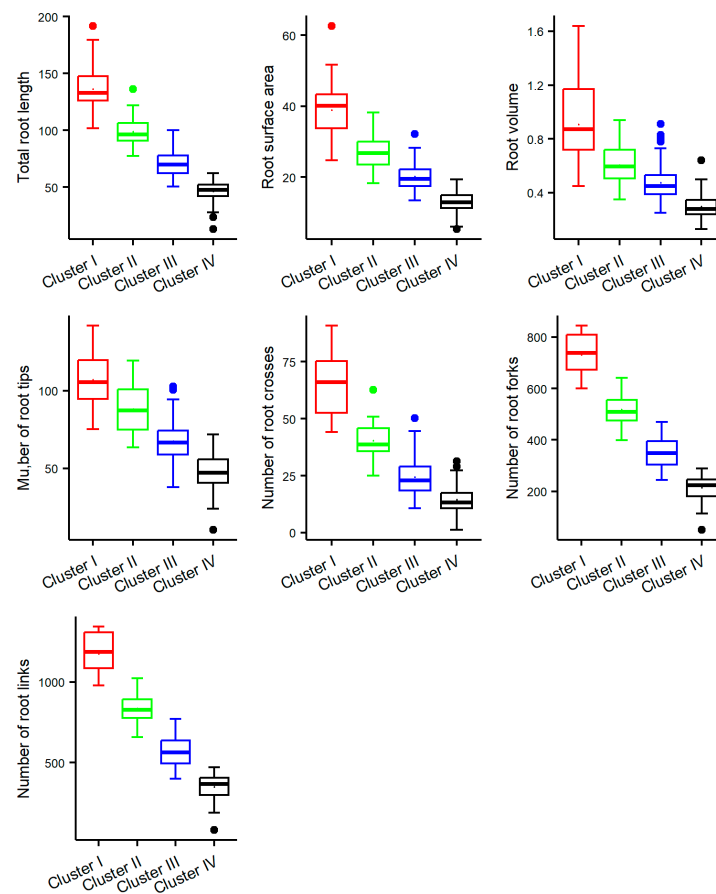


Figure 4. Boxplot for each of the variables with the average and standard difference of error values for each bean genotype cluster from the root phenotyping of ADP entries [34].

4. Discussion

The root system is the major set of organs through which plants interact with numerous biotic and abiotic factors of soil. Root architecture and physiology enables plants to avoid most environmental challenges by recognizing and reacting to them. Roots are used by plants to uptake water and nutrients from the soil, to store food and nutrients, and they are used to anchor them to the ground. The structure and function of root systems are greatly impacted by genetic and environmental factors. Rhizo-toxicity of Al inhibits root growth and function. Understanding the Al responses, their components, and the pattern and magnitude of correlations between traits is valuable in order to specify breeding objectives. Assessing the available genetic diversity for root traits is an important task of common bean breeding programs interested in improving root systems, but is difficult to carry out “below ground”. A hydroponic system such as the one used here helps to provide root trait data without digging up plants.

Our study evaluated the association of root traits and the genetic divergence of 262 Andean common bean genotypes under contrasting Al-toxicity treatments (control with no Al-toxicity treatment and Al stress with 50 μ M Al treatment). Our experiments generated useful insights regarding how Al toxicity differentially changes the root response in different genotypes. This in turn will help with the development of new varieties with enhanced adaptation to difficult soils related to Al toxicity. We found that 50 μ M Al-stress treatment was enough to uncover genotypic differences for the root traits studied. A higher percentage of reduction was observed in all of the root traits measured under Al-toxicity treatment compared with the control, except for the average root diameter, which showed a 21% increase (Supplemental Table S2). Previous studies on common bean screening for Al stress

tolerance also created a consistent level of Al stress under hydroponic growing conditions, but with different Al levels [19,35,45,46].

More specifically, the correlation results indicated that a reduction in root elongation, lateral root initiation and outgrowth, and increased root diameter are important effects of Al toxicity in common beans. A higher percentage decrease in the total root length, number of root tips, number of root forks, number of root links, and number of root crosses had a negative correlation with the average root diameter. The negative correlation between total root length and average root diameter under control and Al-toxicity treatments was also reported by previous authors [4,35,46,47]. Four genotypes, ADP-0010, ADP-0025, ADP-770, and ADP-0786 (Figure 5), had the lowest percentage change in their total root length and average root diameter under Al-toxicity treatment and were considered as Al-resistant genotypes. Genotypes populated around the origin of the scattergram could also be considered as Al stress tolerant. Similar studies have suggested that genotypes with lower values of percentage inhibition for total root length and percentage increase for average root diameter could be identified as Al resistant [48].

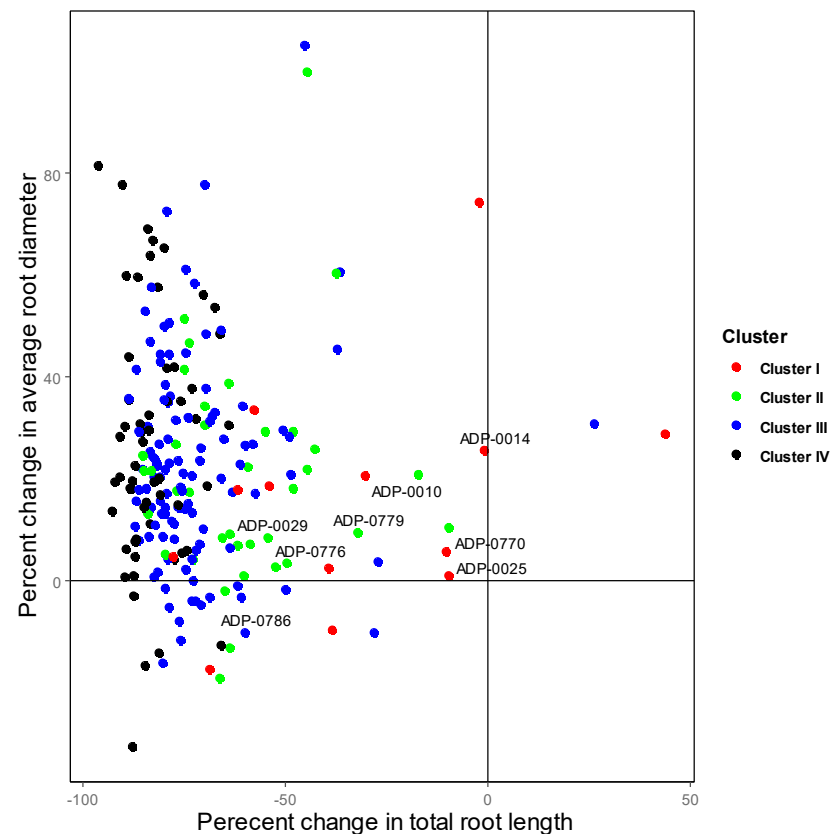


Figure 5. Scattergram identifying Andean bean genotypes from ADP with a low percentage total reduction in root length and low percentage increase in average root diameter. Colors as in Figure 4 for the four clusters of genotypes.

Most of the genotypes exhibited the largest percentage inhibition for total root length and percentage increase for average root diameter, and were susceptible to Al toxicity. Genotypes, namely, ADP-0014, ADP-0028, and ADP-0545, had an increased percentage of total root length and average root diameter (up to 40%), and were considered to be moderately resistant to Al toxicity. Blair, López-Marín, and Rao [4] identified common bean genotypes from the Andean gene pool of a Latin American origin for Al tolerance. Meanwhile, Butare et al. [33,46] showed that interspecific inbred lines combining multiple *Phaseolus* species are sometimes tolerant to a higher level of Al concentration.

The Al resistance we reported in this study could be due to different reasons. Resistant plants developed two different resistance mechanisms to Al toxicity. The mechanisms of toxicity resistance studied in many plant species identified both external and internal resistance mechanisms [13]. The external resistance mechanism is that through which plants exclude Al^{3+} ions from the root apex using the selective permeability of the plasma membrane, exudation of organic acids, producing root mucilage and exudation of the root phosphate. The internal resistance mechanisms that conferred the ability to tolerate toxicity in the plant symplasm were Al binding proteins [9]. These mechanisms are related to mitochondrial metabolism and acid transport [49].

Generally, the common bean is relatively poor when adapting to Al stress conditions [23]. However, studies have reported that some Al-resistant common bean genotypes display Al-activated exudation of citrate and other ion chelating organic compounds [11,21,45]. Organic acid exudation was found to be reduced in Al sensitive genotypes [50]. The exudation of citrate and other organic acids help the plant to exclude this ion from their root system. Recent advances in physiological, biochemical, and molecular studies have also revealed that modification of the binding properties of the root apoplast contributes to Al resistance [51].

The cluster analysis clearly separated the genotypes and grouped them into four groups. Genotypes grouped into the same cluster presumably diverge little from one another as the aggregate characters are measured. Generally, maximum genetic segregation and genetic recombination are expected from crosses that involve parents from the clusters characterized by significant distances. The current study, therefore, suggests that a crossing between accessions from Clusters I and IV will give rise to maximum genetic segregation. Cluster I is characterized by higher values for all of the traits and can be considered as an Al-toxicity tolerance gene source. The genotypes that were identified as tolerant (ADP-0010, ADP-025, and ADP-786) and moderately tolerant (ADP-0014 and ADP-0028) to Al toxicity are also members of this group (Figure 3 and Table S2). In conclusion, the genotypes listed above could be candidates for root crossing in order to enhance Al-toxicity tolerance in Andean common beans, and we found Cluster I to be most appropriate.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy13030619/s1>. Table S1: Cluster groups and mean values of genotypes under Al treatment. Table S2: Descriptive statistics of root traits of 262 Andean common bean genotypes evaluated under the control and Al treatment.

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

Funding: This study was supported by the Evans Allen fund from the USDA under the project TENX-007 and 1890 Capacity Building Grants for Biotechnology teaching and professional development (2018-98821-27731) at the Tennessee State University.

Data Availability Statement: Data available by request from senior authors.

Acknowledgments: The authors wish to thank USDA personnel for the ADP seed supply.

Conflicts of Interest: The authors declare no conflict of interest and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Porch, T.; Beaver, J.; Debouck, D.; Jackson, S.; Kelly, J.; Dempewolf, H. Use of wild relatives and closely related species to adapt common bean to climate change. *Agronomy* **2013**, *3*, 433–461. [CrossRef]
2. Ambachew, D.; Mekbib, F.; Asfaw, A.; Beebe, S.E.; Blair, M.W. Trait associations in common bean genotypes grown under drought stress and field infestation by BSM bean fly. *Crop J.* **2015**, *3*, 305–316. [CrossRef]

3. Omae, H.; Kumar, A.; Shono, M. Adaptation to high temperature and water deficit in the common bean (*Phaseolus vulgaris* L.) during the Reproductive Period. *J. Bot.* **2012**, *2012*, 1–6. [[CrossRef](#)]
4. Blair, M.W.; López-Marín, H.D.; Rao, I.M. Identification of aluminum resistant Andean common bean (*Phaseolus vulgaris* L.) genotypes. *Brazil Plant Physiol.* **2009**, *21*, 291–300. [[CrossRef](#)]
5. Graham, P.H. Some problems and potentials of field beans (*Phaseolus vulgaris* L.) in Latin America. *F Crop Res.* **1978**, *1*, 295–317. [[CrossRef](#)]
6. Lone, A.A.; Khan, M.N.; Gul, A.; Dar, Z.A.; Iqbal, A.M.; Lone, B.A.; Ahangar, A.; Rasool, F.U.; Ali, G.; Nisar, F.; et al. Common beans and abiotic stress challenges. *Curr. J. Appl. Sci. Technol.* **2021**, *40*, 41–53. [[CrossRef](#)]
7. Rao, I.M. Role of Physiology in Improving Crop Adaptation to Abiotic Stresses in the Tropics: The Case of Common Bean and Tropical Forages. In *Handbook of Plant and Crop Physiology*; CRC Press: Boca Raton, FL, USA, 2001.
8. Rangel, A.F.; Mobin, M.; Rao, I.M.; Horst, W.J. Proton toxicity interferes with the screening of common bean (*Phaseolus vulgaris* L.) genotypes for aluminium resistance in nutrient solution. *J. Plant Nutr. Soil Sci.* **2005**, *168*, 607–616. [[CrossRef](#)]
9. Gupta, N.; Gaurav, S.S.; Kumar, A. Molecular basis of Aluminium toxicity in plants: A Review. *Am. J. Plant Sci.* **2013**, *4*, 21–37. [[CrossRef](#)]
10. Bojórquez-Quintal, E.; Escalante-Magaña, 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](#)]
11. Rangel, A.; 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](#)]
12. 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](#)]
13. Hede, A.R.; Skovmand, B.; López-Cesati, J. Acid soils and Aluminum toxicity. In *Application of Physiology in Wheat Breeding*; Reynolds, M.P., Ortiz-Monasterio, J.I., McNab, A., Eds.; CIMMYT: Mexico City, Mexico, 2001; pp. 172–182.
14. Horst, W.J.; Rangel, A.F.A.F.; Eticha, D.; Ischitani, M.; Rao, I.M. Aluminum toxicity and resistance in *Phaseolus vulgaris* physiology drives molecular biology. In Proceedings of the International Symposium on Plant-Soil Interactions at Low pH, Guangzhou, China, 17–21 May 2009.
15. Delhaize, E.; Ryan, P.R.; Randall, P.J. Aluminum tolerance in wheat (*Triticum aestivum* L.). *Plant Physiol.* **1993**, *103*, 695–702. [[CrossRef](#)]
16. Delhaize, E.; Ryan, P.R. Aluminum toxicity and tolerance in plants. *Plant Physiol.* **1995**, *107*, 315–321. [[CrossRef](#)] [[PubMed](#)]
17. Delhaize, E.; Ma, J.F.; Ryan, P.R. Transcriptional regulation of aluminium tolerance genes. *Trends Plant Sci.* **2012**, *17*, 341–348. [[CrossRef](#)]
18. Rangel, A.F.; Rao, I.M.; Horst, W.J. Spatial aluminium sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes with contrasting aluminium resistance. *J. Exp. Bot.* **2007**, *58*, 3895–3904. [[CrossRef](#)]
19. Yang, Z.B.; Eticha, D.; Rotter, B.; Rao, I.M.; Horst, W.J. Physiological and molecular analysis of polyethylene glycol-induced reduction of aluminium accumulation in the root tips of common bean (*Phaseolus vulgaris*). *New Phytol.* **2011**, *192*, 99–113. [[CrossRef](#)]
20. Yang, Z.B.; Rao, I.M.; Horst, W.J. Interaction of aluminium and drought stress on root growth and crop yield on acid soils. *Plant Soil.* **2013**, *372*, 3–25. [[CrossRef](#)]
21. Mugai, E.N.; Agong, S.G.; Matsumoto, H. Aluminium tolerance mechanisms in *Phaseolus vulgaris* L.: Citrate synthase activity and TTC reduction are well correlated with citrate secretion. *Soil Sci. Plant Nutr.* **2000**, *46*, 939–950. [[CrossRef](#)]
22. Bartoli, G.; Sanità di Toppi, L.; Andreucci, A.; Ruffini Castiglione, M. Aluminum effects on embryo suspensor polytene chromosomes of *Phaseolus coccineus* L. *Plant Biosys.* **2017**, *3504*, 880–888.
23. Beebe, S.E.; Rao, I.M.; Blair, M.W.; Butare, L. Breeding for abiotic stress tolerance in common bean: Present and future challenges. *SABRAO J. Breed. Genet.* **2009**, *41*, 1–10.
24. Miklas, P.N.; Kelly, J.D.; Beebe, S.E.; Blair, M.W. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica* **2006**, *147*, 105–131. [[CrossRef](#)]
25. Darkwa, K.; Ambachew, D.; Mohammed, H.; Asfaw, A.; Blair, M.W. Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. *Crop J.* **2016**, *4*, 367–376. [[CrossRef](#)]
26. Dramadri, I.O.; Nkalubo, S.T.; Kramer, D.M.; Kelly, J.D. Genome-wide association analysis of drought adaptive traits in common bean. *Crop Sci.* **2021**, *61*, 3232–3253. [[CrossRef](#)]
27. Papathanasiou, F.; Ninou, E.; Mylonas, I.; Baxevanos, D.; Papadopoulou, F.; Avdikos, I.; Sistanis, I.; Koskosidis, A.; Vlachostergios, D.N.; Stefanou, S.; et al. The Evaluation of Common Bean (*Phaseolus vulgaris* L.) Genotypes under Water Stress Based on Physiological and Agronomic Parameters. *Plants* **2022**, *11*, 2432. [[CrossRef](#)]
28. Cortés, A.J.; Monserrate, F.A.; Ramírez-Villegas, J.; Madriñán, S.; Blair, M.W. Drought tolerance in wild plant populations: The case of common beans (*Phaseolus vulgaris* L.). *PLoS ONE* **2013**, *8*, e62898. [[CrossRef](#)]
29. Asfaw, A.; Ambachew, D.; Shah, T.; Blair, M.W. Trait associations in diversity panels of the two common bean (*Phaseolus vulgaris* L.) gene pools grown under well-watered and water-stress conditions. *Front. Plant Sci.* **2017**, *8*, 733. [[CrossRef](#)]
30. Kouam, E.B.; Ndo, S.M.; Mandou, M.S.; Chotangui, A.H.; Tankou, C.M. Genotypic variation in tolerance to salinity of common beans cultivated in western Cameroon as assessed at germination and during early seedling growth. *Open Agric.* **2017**, *2*, 600–610. [[CrossRef](#)]

31. Çiftçi, V.; Türkmen, Ö.; Do, Y.; Erdinç, Ç.; Suat, B. Variation of salinity tolerance in bean genotypes. *Afr. J. Agric. Econ. Rural Dev.* **2014**, *2*, 1–9.
32. Ambachew, D.; Blair, M.W. Genome wide association mapping of root traits in the andean genepool of common bean (*Phaseolus vulgaris* L.) grown with and without aluminum toxicity. *Front. Plant Sci.* **2021**, *12*, 628687. [[CrossRef](#)]
33. Butare, L.; Rao, I.M.; Lepoivre, P.; Cajiao, C.; Polania, J.; Cuasquer, J.; Beebe, S. Phenotypic evaluation of interspecific recombinant inbred lines (RILs) of *Phaseolus* species for aluminium resistance and shoot and root growth response to aluminium-toxic acid soil. *Euphytica* **2012**, *186*, 715–730. [[CrossRef](#)]
34. Cichy, K.A.; Poch, T.G.; Beaver, J.S.; Cregan, P.; Fourie, D.; Glahn, R.P.; Grusak, M.A.; Kamfwa, K.; Katuuramu, D.N.; McClean, P.; et al. A *Phaseolus vulgaris* diversity panel for andean bean improvement. *Crop Sci.* **2015**, *55*, 2149–2160. [[CrossRef](#)]
35. Rao, I.M.; Wenzl, P.; Arango Vélez, A.; Miles, J.W.; Watanabe, T.; Shinano, T.; Osaki, M.; Wagatsuma, T.; Manrique, G.; Beebe, S.E.; et al. Advances in developing screening methods and improving aluminum resistance in common bean and brachiaria. *Rev. Bras. Agrociencia* **2008**, *14*, 1–7.
36. R Core Team. A language and environment for statistical computing. R Foundation for Statistical Computing. 2020. Available online: <https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing> (accessed on 7 February 2023).
37. Fox, J.; Weisberg, S. An R Companion to Applied Regression: Appendices. Robust Regres R. 2019. Available online: <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion> (accessed on 17 February 2023).
38. Revelle, W.R. *psych: Procedures for Personality and Psychological Research*; Northwestern University: Evanston, IL, USA, 2020. Available online: <https://cran.r-project.org/package=psych> (accessed on 17 February 2023).
39. Goslee, S.C.; Urban, D.L. The ecodist package for dissimilarity-based analysis of ecological data. *J. Stat Softw.* **2007**, *22*, 1–19. [[CrossRef](#)]
40. Nieweglowski, L. *clv: Cluster Validation Techniques*, R package version 0.3–2.1. 2010. Available online: <https://cran.r-project.org/web/packages/clv/index.html> (accessed on 17 February 2023).
41. Kassambara, A.; Mundt, F. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses; 2020; R Package Version 1.0.7. Available online: <https://CRAN.R-project.org/package=factoextra> (accessed on 17 February 2023).
42. Wickham, H. *ggplot2 Elegant Graphics for Data Analysis Second Edition*. Robert, G., Kurt, H., Giovanni, P., Eds.; 2016, p. 268. Available online: <https://ggplot2.tidyverse.org> (accessed on 17 February 2023).
43. Murtagh, F.; Legendre, P. Ward’s Hierarchical Agglomerative Clustering Method: Which Algorithms Implement Ward’s Criterion? *J. Classif.* **2014**, *6*, 4–6. [[CrossRef](#)]
44. Paradis, E.; Schliep, K. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **2019**, *35*, 526–528. [[CrossRef](#)]
45. Eticha, D.; Zahn, M.; Bremer, M.; Yang, Z.; Rangel, A.F.; Rao, I.M.; Horst, W.J. Transcriptomic analysis reveals differential gene expression in response to aluminium in common bean (*Phaseolus vulgaris*) genotypes. *Ann. Bot.* **2010**, *105*, 1119–1128. [[CrossRef](#)]
46. Butare, L.; Rao, I.M.; Lepoivre, P.; Polania, J.; Cajiao, C.; Cuasquer, J.; Beebe, S. New genetic sources of resistance in the genus *Phaseolus* to individual and combined aluminium toxicity and progressive soil drying stresses. *Euphytica* **2011**, *181*, 385–404. [[CrossRef](#)]
47. López-Marín, H.D.; Rao, I.M.; Blair, M.W. Quantitative trait loci for root morphology traits under aluminum stress in common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* **2009**, *119*, 449–458. [[CrossRef](#)]
48. Rao, I.M.; Miles, J.W.; Beebe, S.E.; Horst, W.J. Root adaptations to soils with low fertility and aluminium toxicity. *Ann. Bot.* **2016**, *118*, 593–605. [[CrossRef](#)]
49. Nunes-Nesi, A.; Brito, D.S.; Inostroza-Blancheteau, C.; Fernie, A.R.; Araújo, W.L. The complex role of mitochondrial metabolism in plant aluminum resistance. *Trends Plant Sci.* **2014**, *19*, 399–407. [[CrossRef](#)]
50. Miyasaka, S.C.; George Buta, J.; Howell, R.K.; Foy, C.D. Mechanism of aluminum tolerance in snapbeans: Root exudation of citric acid. *Plant Physiol.* **1991**, *96*, 737–743. [[CrossRef](#)]
51. Horst, W.J.; Wang, Y.; Eticha, D. The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: A review. *Ann. Bot.* **2010**, *106*, 185–197. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.