agriculture

MDPI

Article

Untapped Genetic Resources for Breeding Acidic Soil-Adapted Chickpea (*Cicer arietinum* L.) Cultivars

Hawi Negusse [1,2,*], Teklehaimanot Haileselassie [1], Mulatu Geleta [3] and Kassahun Tesfaye [1,2]

[1] Institute of Biotechnology, Addis Ababa University, Addis Ababa 1000, Ethiopia; teklehaimanot.hselassie@aau.edu.et or tekle1961@yahoo.com (T.H.); kassahun.tesfaye@aau.edu.et (K.T.)
[2] Bio and Emerging Technology Institute (BETin), Addis Ababa 1000, Ethiopia
[3] Department of Plant Breeding, Swedish University of Agricultural Sciences, 234 22 Lomma, Sweden; mulatu.geleta.dida@slu.se
* Correspondence: hawinegusse@gmail.com or hawi.negusse@betin.gov.et; Tel.: +251-(0)912-761085

Abstract: Globally, more than half of potentially arable land is acidic, and aluminum (Al) is the primary factor limiting plant growth and crop productivity on acidic soils worldwide. The development and utilization of Al-tolerant crops is a sustainable approach to enhancing crop production on acidic soils. For this purpose, screening available genetic resources under Al-stressed conditions is a crucial initial step. Hence, the present study aimed to evaluate the Al tolerance of 264 Ethiopian chickpea landraces under hydroponic conditions without Al (control) and with 120 µM Al (Al treatment). Significant ($p < 0.001$) variations were detected among the genotypes for all studied traits under control (0 µM Al) and 120 µM Al concentration. The relative growth values for the 120 µM Al/0 µM Al ratio was also significant, indicating the presence of a considerable amount of genetic variation in Ethiopian chickpea landraces in terms of Al tolerance. Based on relative root growth (RRG) as an Al-tolerance parameter, the genotypes were grouped into five distinct ($p < 0.001$) classes. The highest RRG value (1.59) was obtained for genotype ETC_209008, followed by ETC_41184 and ETC_212589, while ETC_208995 had the lowest RRG value of 0.27. Of the total landraces screened, 35% had higher RRG values than the tolerant genotype ETC_WL_1_2016 used as a reference, indicating the presence of adequate genotypes capable of outperforming the reference genotype on acidic soils. The genotypes identified in the present study may serve as sources of novel alleles in genes regulating Al tolerance in chickpea that can be utilized in breeding programs to improve the crop's adaptation to acidic soils, thus contributing to smallholder farmers' increased nutritional and food security.

Keywords: aluminum toxicity; *Cicer arietinum*; genetic diversity; hydroponic screening; landraces; relative root growth

Citation: Negusse, H.; Haileselassie, T.; Geleta, M.; Tesfaye, K. Untapped Genetic Resources for Breeding Acidic Soil-Adapted Chickpea (*Cicer arietinum* L.) Cultivars. *Agriculture* **2023**, *13*, 2127. https://doi.org/10.3390/agriculture13112127

Academic Editor: Jaime Prohens

Received: 13 October 2023
Revised: 7 November 2023
Accepted: 8 November 2023
Published: 10 November 2023

1. Introduction

Chickpea (*Cicer arietinum* L.) ranks as the world's second most important cool-season food legume after the common bean (*Phaseolus vulgaris* L.) [1]. It is a highly nutritious legume crop rich in protein, carbohydrates, essential minerals, and vitamins, while being low in fat and cholesterol [2]. It is cultivated globally on 15 million hectares of land, with an annual production of 15.87 million tons (MT) and an average productivity of 1.06 t ha^{-1} [1].

In Ethiopia, chickpea is an important multi-purpose crop that ranks third in area coverage and production volume after fava and haricot beans [3]. It is the primary source of protein in the diet and income of smallholder farmers [4]. Furthermore, it increases soil fertility by fixing atmospheric nitrogen, and facilitates efficient land management by being grown in rotation with cereals, including wheat and teff [4,5]. Ethiopia is the largest producer, consumer, and exporter of chickpea among African countries [1]. It accounts for more than 90% of Sub-Saharan Africa's chickpea production [5] and 60% of chickpea exports from Africa to global markets [6]. Ethiopia is also a secondary center of diversity for the cultivated chickpea and the center of origin for its wild relative *Cicer cuneatum* [7].

Despite its considerable economic significance, chickpea grain yield in Ethiopia is far below its potential yield (6 t ha^{-1}). Chickpea is commonly planted in the country close to the end of the main cropping season when the soil moisture is low, with the application of below-optimum agricultural inputs, subjecting the crop to a number of abiotic challenges during its life cycle. Globally, abiotic stresses account for the losses of 6.4 MT of chickpeas annually, with drought, heat, cold, soil salinity, waterlogging, soil acidity, nutrient toxicities, and deficiencies being the major ones [8].

More than 50% of the potentially arable lands of the world are acidic [9,10], and the majority (60%) of these soils exist in the tropics and sub-tropics, affecting crop productivity in developing nations, where food scarcity is still a critical problem [9,10]. In Ethiopia, nearly 43% of potentially cultivable lands are acidic [11,12], and most of these soils are found in the highlands where rainfall is high. Such acidic soils are more common in the northwestern, southwestern, southern, and central parts of the country than in other parts [12].

Acidic soils are often associated with nutrient deficiencies such as phosphorus and metal toxicities, including aluminum (Al), manganese (Mn), and iron (Fe) [9]. Al toxicity is the main factor affecting plant growth and productivity in acidic soils worldwide [13,14]. Al naturally exists in soil as insoluble and non-toxic alumino-silicates and oxides. However, as the soil pH drops below 5.0, these compounds undergo chemical decomposition and produce aluminum cations (Al^{3+}), the most important rhizo-toxic form of Al [10]. The initial symptom of Al toxicity is the inhibition of root growth [15–17], which occurs instantly within a few hours of exposure [16]. Hence, researchers commonly conduct root growth studies to assess Al tolerance in crop plants [18]. A nutrient solution assay is the most commonly used technique for screening Al tolerance in crops [17,19,20], as it is a rapid and non-destructive method [21–23] that provides easy access to the root system with precise control over nutrient composition and pH [23,24].

Soil acidity is commonly mitigated by applying lime and mineral fertilizers [18,25,26]. However, liming is only effective on surface soil, as liming the subsoils is technically demanding and expensive [27,28]. Hence, the development and use of crop plants tolerant to Al toxicity is an effective strategy, either alone or in conjunction with other acidic soil management practices, to develop sustainable solutions, thereby enhancing crop production on acidic soils [24,26]. To achieve this, the screening and identification of Al-tolerant germplasm under Al-stressed conditions is crucial.

Landraces are potential sources of genotypes with desirable traits, such as tolerance to biotic and abiotic stresses [29]. Due to their advantages, such as better adaptation to local environmental conditions, farmers in marginal areas prefer landraces to modern cultivars despite their lower yields. Hence, the systematic assessment of landraces may indicate variation patterns, making it easier to find alleles that can improve yield and resistance to abiotic stress, thus enhancing the yield and sustainability of staple crops in stressful environments [30,31].

Chickpea is generally considered as sensitive to low pH and Al toxicity. Physiological and metabolic studies of chickpea revealed that Al stress significantly impacts the root growth of chickpea and results in losses of plasma membrane integrity and membrane damage [32]. However, previous reports also indicated considerable genetic variation in Al tolerance within chickpea germplasm [28,33–35]. Significant differences within a given crop for Al tolerance have been reported in different pulse crops, including the common bean [36], cowpea [37], pigeon pea [19], barrel medic [38], and soybean [39], and in the oil-seed crop sunflower [40].

Al tolerance is not prioritized as a breeding target in Ethiopia. Due to this, few and insufficient studies exist, with Alemu and Lule [41] and Negusse et al. [35] being the only reports so far. Therefore, the present study aimed to (i) characterize the Al tolerances of 264 Ethiopian chickpea landraces and (ii) identify genotypes tolerant to Al-toxicity that can serve as new sources of Al tolerance for use in chickpea breeding programs.

2. Materials and Methods

2.1. Plant Materials

A total of 262 chickpea landraces, viz. 209 chickpea accessions collected from major chickpea growing regions received from the Ethiopian Biodiversity Institute (EBI), and 53 landraces collected by targeted own collection from different parts of the country, were evaluated for Al tolerance. In addition, based on a previous screening study, local landrace ETC_WL_1_2016 and cultivar Akaki were included in the experiment as tolerant and susceptible references, respectively [35]. The seeds of Akaki were obtained from the Debrezeit Agricultural Research Center (DZARC), Ethiopia, and those of ETC_WL_1_2016 were collected from a farmer field in Oromia Regional State, Kelem Wollega zone, Seyo Wereda (Table S1; Figure 1). A map depicting the extent and distribution of acidic soils in Ethiopia, adopted from [11], is included in Figure S1. For the sake of simplicity, hereafter, the term 'genotype' will be used to represent all 264 chickpea germplasms.

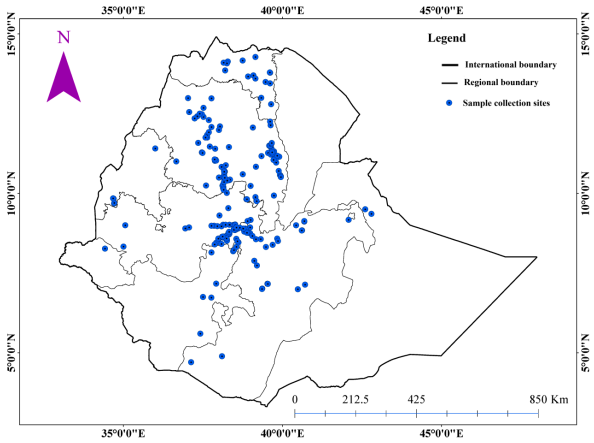

Figure 1. Map of Ethiopia illustrating the collection sites of the 264 chickpea germplasms used in the present study.

2.2. Nutrient Medium Composition

Seeds of each genotype were surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 5 min, thoroughly rinsed 3–5 times with distilled water, and germinated in the dark at room temperature for 72 h on a moistened paper towel in a Petri dish. Then, the initial root and shoot lengths of each seedling were measured, and seedlings of each genotype with similar root length and shoot length were transferred into a plastic mesh fixed on a plastic tray containing 8 L of low ionic strength hydroponic medium. The nutrient medium was prepared as described in [35] and consisted of 500 µM KNO_3; 500 µM $CaCl_2$; 500 µM NH_4NO_3; 200 µM $MgSO_47H_2O$; 100 µM KH_2PO_4; 46 µM H_3BO_3; 2 µM $MnCl_24H_2O$; 1 µM $ZnSO_47H_2O$; 0.5 µM $NaMoO_42H_2O$; and 0.3 µM $CuSO_45H_2O$. Iron (20 µM) was supplied as Fe: EDTA prepared from equimolar amounts of $FeCl_3 \cdot 6H_2O$ and Na_2EDTA.

2.3. Experimental Setup and Aluminum Treatment

The study was set up in a randomized complete block (RCB) design with two replications and two Al concentrations, 0 µM Al (control) and 120 µM Al, and Al was added in the form Al_2 $(SO_4)_3 \cdot 18H_2O$. The 120 µM Al concentration was selected based on its discrimination power in the dose–response study and Al tolerance screening study conducted on chickpea [35].

Five seedlings were planted per Al X genotype combination in each replicate, and data from the two replicates were combined to determine the mean performance of each genotype. The pH of both solutions was adjusted to 4.5 using 1 M HCl and/or 1 M NaOH solutions. The solutions were continuously aerated with an air pump connected to an air stone, and were replaced every 72 h to minimize pH and Al fluctuations.

2.4. Measured Phenotypic Traits

After continuous growth for six days, intact seedlings were removed from the nutrient solutions to record root and shoot traits. The root length (RL) and shoot length (SL) were measured with a centimeter-graded ruler, and the root fresh weight (RFW) and shoot fresh weight (SFW) were measured with an analytical balance. The root dry weight (RDW) and shoot dry weight (SDW) were also recorded after drying the samples in an oven at 70 °C for 72 h [35]. The relative root growth was calculated according to the formula below and was used as a measure of Al tolerance [42].

$$\text{Relative root growth} = \left(\frac{\text{root length at 120 } \mu\text{M Al}}{\text{root length at 0 } \mu\text{M Al}}\right) \times 100 \quad (1)$$

2.5. Statistical Analysis

An analysis of variance (ANOVA) was performed using the generalized linear model (GLM) implemented in R [43] with the '*aov*' function. The Ryan–Einot–Gabriel—Welsch (REGW) multiple-range test was used to compare genotype means and rank them accordingly. Pearson's correlation analysis between the measured traits was computed using the R package 4.1.1 '*corrplot*' [44]. The R packages "*Factoextra*" [45] and "*FactoMineR*" [46] were used to perform principal component analysis (PCA).

3. Results

3.1. Variation in Response of Chickpea Genotypes to Aluminum Stress

The analysis of variance (ANOVA) revealed significant ($p < 0.001$) differences among chickpea genotypes for all measured traits under control (0 µM Al), Al stress (120 µM Al), and for the ratios of the measurements under the two conditions (120 µM Al/0 µM Al) (Table 1). All root and shoot traits of the chickpea genotypes revealed growth reduction at 120 µM Al concentration compared to the control (Figure 2). Particularly, Al stress had a substantial ($p < 0.001$) negative impact on the root length of the chickpea genotypes, which resulted in a 29% growth reduction compared to the control. However, the Al-induced reduction in root growth differed among the chickpea genotypes evaluated.

In the nutrient solution without Al, the root length (RL) of the chickpea genotypes ranged from 3.80 cm (ETC_GN_1_2016) to 20.71 cm (ETC_K_1_2016), with an overall mean of 13.6 cm, whereas at 120 µM Al concentration, it ranged from 3.67 cm (ETC_223064) to 16.70 cm (ETC_41249), with an overall mean of 9.64 cm. Likewise, the shoot length (SL) of the chickpea genotypes ranged from 6.25 cm (ETC_H_7_2016) to 24.32 cm (ETC_209021) in the nutrient solution without Al, while it ranged from 5.77 cm (ETC_241126) to 22.73 cm (ETC_209021) at 120 µM Al concentration, with an average of 13.76 cm and 12.22 cm, respectively.

Al tolerance, expressed as relative root growth (RRG) and relative shoot growth (RSG), revealed significant ($p < 0.001$) genotypic variations among the chickpea genotypes, and ranged from 0.27 (ETC_208995) to 1.59 (ETC_209008) and from 0.43 (ETC_41322) to 1.31 (ETC_215667), with an average of 0.74 and 0.89, respectively.

Table 1. A summary of the variation in the root and shoot traits of the chickpea genotypes in a control (without Al) and 120 µM Al solution as well as their relative performance under the ratio of the two conditions.

Treatment	Traits	Minimum	Maximum	Mean	CV %	R^2	MSE
Control	RL	3.80	20.71	13.6 ***	12.62	0.85	2.95
	SL	6.25	24.32	13.76 ***	8.35	0.92	1.32
	RFW	0.08	0.48	0.21 ***	10.76	0.90	0.00
	SFW	0.15	0.43	0.24 ***	11.67	0.87	0.00
	RDW	0.04	0.16	0.06 ***	9.13	0.92	0.00
	SDW	0.06	0.15	0.09 ***	9.78	0.88	0.00

Table 1. *Cont.*

Treatment	Traits	Minimum	Maximum	Mean	CV %	R^2	MSE
Al treatment	RL	3.67	16.70	9.64 ***	13.51	0.90	1.70
	SL	5.77	22.73	12.22 ***	13.15	0.86	2.58
	RFW	0.09	0.34	0.16 ***	11.99	0.87	0.00
	SFW	0.10	0.37	0.20 ***	13.07	0.86	0.00
	RDW	0.03	0.12	0.06 ***	10.48	0.90	0.00
	SDW	0.04	0.14	0.08 ***	12.08	0.87	0.00
Relative performance	RRG	0.27	1.59	0.74 ***	21.25	0.78	0.02
	RSG	0.43	1.31	0.89 ***	15.38	0.62	0.02
	RRFW	0.43	1.36	0.79 ***	15.40	0.73	0.01
	RSFW	0.53	1.41	0.85 **	17.42	0.57	0.02
	RRDW	0.50	1.45	0.88 ***	14.94	0.66	0.02
	RSDW	0.46	1.39	0.91 *	16.67	0.55	0.02

RL, root length; SL, shoot length; RFW, root fresh weight; SFW, shoot fresh weight; RDW, root dry weight; SDW, shoot dry weight; RRG, relative root growth; RSG, relative shoot growth; RRFW, relative root fresh weight; RSFW, relative shoot fresh weight; RRDW, relative root dry weight; RSDW, relative shoot dry weight; CV, coefficient of variation; R^2, coefficient of determination; MSE, mean square error. Significance codes: '***' 0.001; '**' 0.01; '*' 0.05.

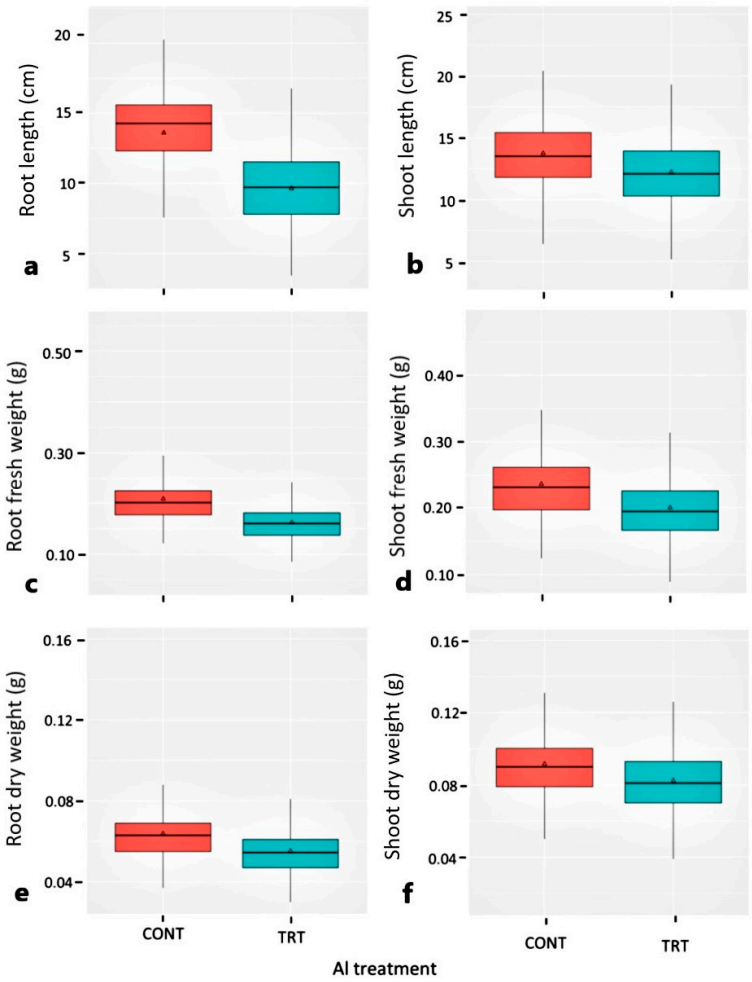

Figure 2. Box-and-whisker plots for the root and shoot traits of the chickpea genotypes, elucidating the overall responses of the genotypes under the control (0 µM Al) and Al treatment (120 µM Al) conditions, exhibiting the effects of Al stress on measured traits: (**a**) root length (cm), (**b**) shoot length (cm), (**c**) root fresh weight (g), (**d**) shoot fresh weight (g), (**e**) root dry weight (g), and (**f**) shoot dry weight (g). CONT: the overall responses of the chickpea genotypes for each trait in the control (0 µM Al) solution; TRT: the overall responses of the chickpea genotypes for each trait at 120 µM Al concentration.

3.2. Variation in the Performance of Chickpea Genotypes

Overall, the mean performance of the chickpea genotypes at 120 µM Al concentration revealed a wide range of variations to Al stress (Table 1). Although Al toxicity significantly impacted the growth and biomass of the chickpea genotypes, some genotypes performed well despite the high concentration of Al applied, indicating that they have the genetic potential to withstand Al stress.

Genotype ETC_41249 revealed the highest mean RL at 120 µM Al concentration, followed by ETC_41224 and ETC_209000, whereas genotype ETC_K_1_2016 recorded the highest RL at 0 µM Al, followed by ETC_209021 and ETC_A_2_2016. Genotypes ETC_41249, ETC_209000, and ETC_41224 had the longest root in the absence of Al (0 µM Al). Moreover, genotypes ETC_41046, ETC_41191, ETC_216853, ETC_41271, ETC_41086, and ETC_30288 showed increased growth under the Al treatment compared to the control.

The highest mean RRG value was recorded in genotype ETC_209008, followed by ETC_41184 and ETC_212589, while the lowest was recorded in genotype ETC_208995 (Table S2). The tolerant and susceptible references, viz., ETC_WL_1_2016 and Akaki, exhibited a RRG of 0.77 and 0.49, respectively. Of the total genotypes evaluated, 35.23% had an RRG higher than ETC_WL_1_2016, while 89.77% of the genotypes had an RRG higher than Akaki.

3.3. Grouping of Chickpea Genotypes Based on Their Relative Root Growth Values

Based on their RRG values, the genotypes were divided into five Al tolerance groups, namely, highly sensitive (HS) (RRG < 0.4), sensitive (S) (0.4–0.59), moderately tolerant (MT) (0.6–0.79), tolerant (T) (0.8–0.99), and highly tolerant (HT) (RRG > 1.0). Among the 264 chickpea genotypes, 45.4% (120 genotypes) were classified under the MT class. The T and HT classes contained 56 (21.2%) and 28 (10.6%) genotypes, respectively, whereas 52 (19.7%) and 8 (3%) genotypes were assigned to the S and HS classes, respectively (Figure 3). In a subsequent analysis performed using the mean RRG of each genotype, significant differences ($p < 0.001$) were observed among the five Al tolerance classes (Figure 4).

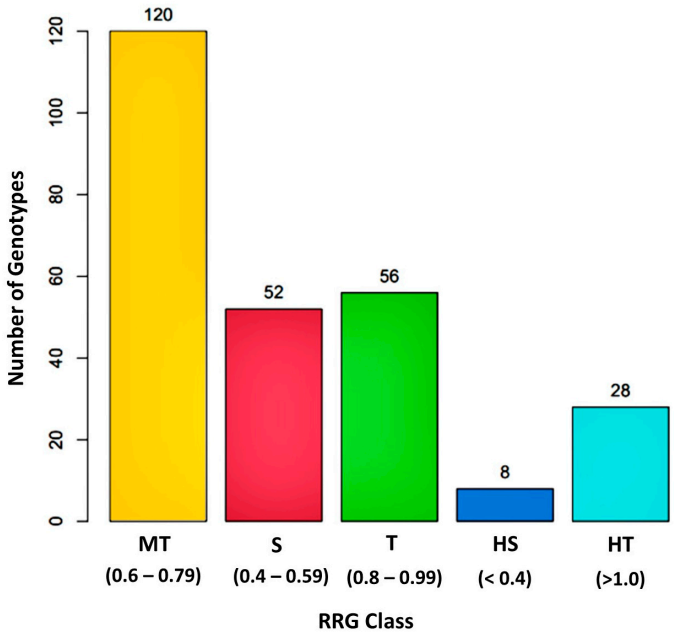

Figure 3. Bar graphs showing the proportions of the 264 chickpea genotypes categorized under different Al tolerance classes based on their relative root growth (RRG) values. Tolerance classes are HS: highly sensitive; S: sensitive; MT: moderately tolerant; T: tolerant; and HT: highly tolerant. Values in brackets are ranges of RRG values. Each bar represents the total number of chickpea genotypes categorized under each tolerance class.

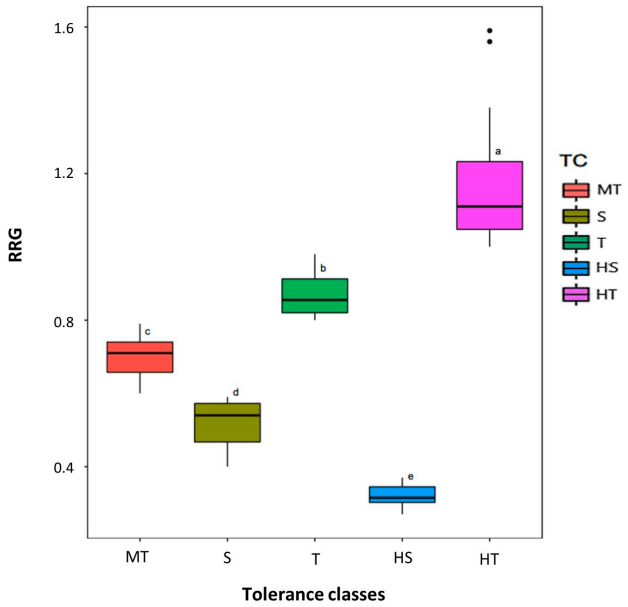

Figure 4. Box-and-whisker plots based on mean relative root growth (RRG) values depicting the variations within and among genotypes in different Al-tolerance classes. The mean separation was computed using Tukey's HSD test. Box plots with different letters are significantly different from each other. Vertical lines extending from each plot represents ranges of values within each tolerance class. HS: highly sensitive; S: sensitive; MT: moderately tolerant; T: tolerant; and HT: highly tolerant.

3.4. Correlation between Root and Shoot Traits

The results of Pearson's correlation analysis revealed significant positive correlations between the root and shoot traits of the chickpea landraces at 120 µM Al concentration (Figure 5). Similarly, all traits showed significantly positive correlations with each other in the 0 µM Al solution, with the exception of SL and RFW. A strong positive correlation was found between SL and shoot biomasses traits (SFW and SDW) at 0 and 120 µM Al concentrations (Figure 5). Additionally, RL at 120 µM Al concentration exhibited a significantly positive but moderate correlation (r = 0.49, $p < 0.001$) with RL at 0 µM Al concentration and RRG (r = 0.48, $p < 0.001$) (Figure 6).

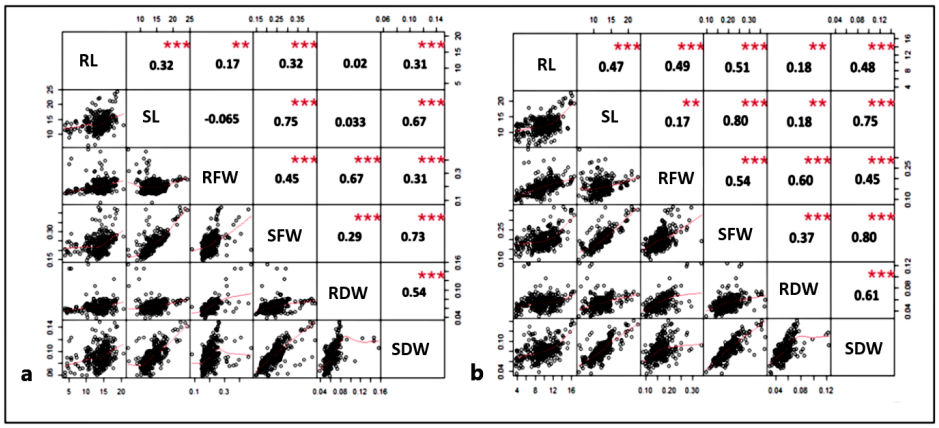

Figure 5. Graphical and numerical representation of the correlation between the root and shoot traits of the chickpea genotypes when treated at (**a**) 0 µM Al concentration and (**b**) 120 µM Al concentration. RL, root length; SL, shoot length; RFW, root fresh weight; SFW, shoot fresh weight; RDW, root dry weight; SDW, shoot dry weight. Significance codes: '***' 0.001; '**' 0.01.

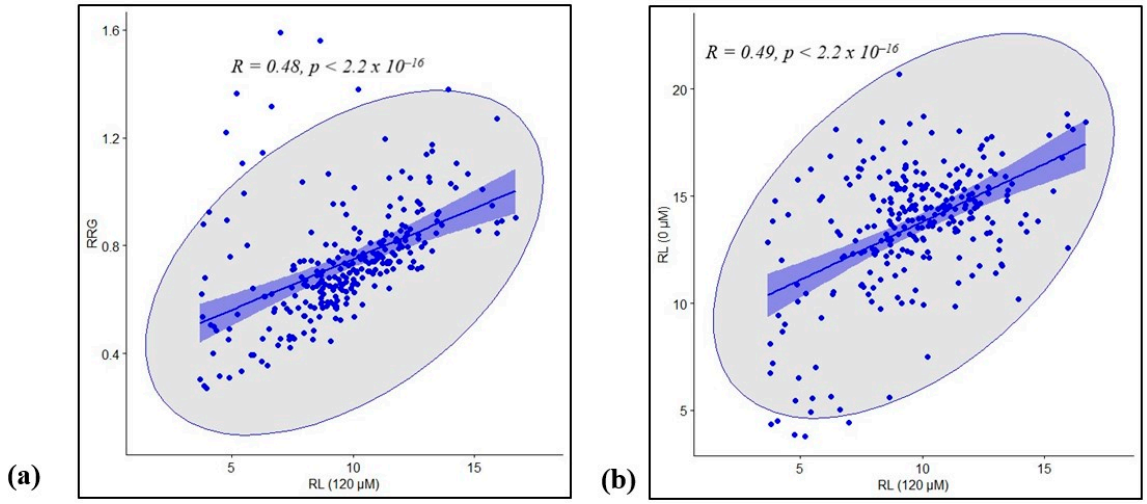

Figure 6. Relationship between (**a**) RL at 120 µM Al concentration and RRG, and (**b**) RL at 120 µM Al concentration with RL at 0 µM Al concentration. RL, root length; RRG, relative root growth.

3.5. Principal Component Analysis (PCA)

A principal component analysis based on six direct and six relative phenotypic traits revealed that the first four principal components (PCs) explained 78.78% (PC1 = 46.96%, PC2 = 13.91%, PC3 = 9.79%, and PC4 = 8.12%) of the total variation (Table 2, Figure 7a). Hence, the original variables were transformed into four PCs to represent over three quarters of the variations contributed by the traits.

Table 2. Eigenvalues and percentage of variance explained individually and cumulatively by the first four principal components, and the contribution of each trait to the variance explained by each principal component.

PCs	Eigen Value	Percentage of Explained Variance	Cumulative Percentage of Variance
PC1	5.6	46.96	46.96
PC2	1.7	13.91	60.87
PC3	1.2	9.79	70.66
PC4	1.0	8.12	78.78
Trait contribution to the first four PCs			
Traits	PC1	PC2	PC3	PC4
RL	0.62	−0.25	−0.05	−0.57
SL	0.66	−0.45	−0.51	0.09
RFW	0.63	0.20	0.57	−0.25
SFW	0.76	−0.50	−0.18	0.01
RDW	0.54	−0.25	0.67	0.36
SDW	0.77	−0.50	−0.03	0.24
RRG	0.68	0.21	0.02	−0.36
RSG	0.74	0.30	−0.22	0.20
RRFW	0.67	0.40	0.06	−0.29
RSFW	0.76	0.46	−0.08	0.03
RRDW	0.62	0.40	0.16	0.23
RSDW	0.74	0.34	−0.16	0.27

PC1: principal component 1; PC2: principal component 2; PC3: principal component 3; PC4: principal component 4; RL: root length; SL: shoot length; RFW: root fresh weight; SFW: shoot fresh weight; RDW: root dry weight; SDW: shoot dry weight; RRG: relative root growth; RSG: relative shoot growth; RRFW: relative root fresh weight; RSFW: relative shoot fresh weight; RRDW: relative root dry weight; RSDW: relative shoot dry weight.

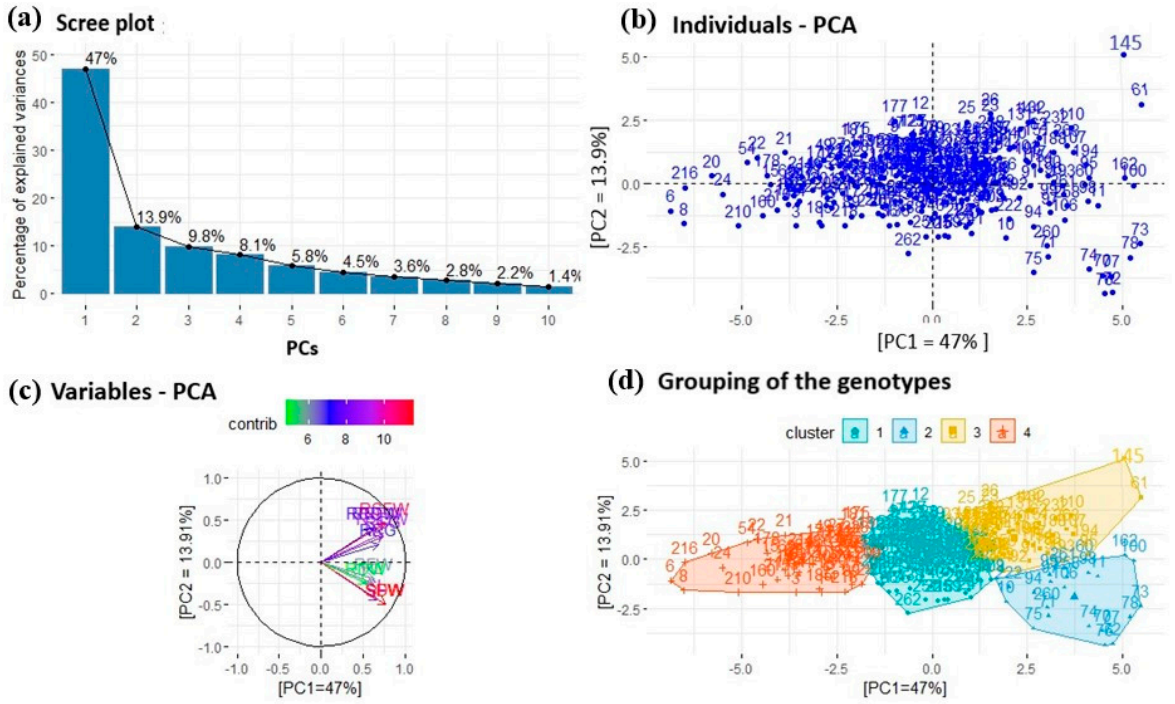

Figure 7. (**a**) Scree plot illustrating the percentage of total phenotypic variance explained by each PC, (**b**) PCA bi-plot, (**c**) variable contribution to the first two PCs, and (**d**) PCA grouping of the 264 chickpea genotypes tested at 120 µM Al concentration.

The analysis also indicated positive correlations between the measured phenotypic traits (Figure 7c). All traits positively contributed to the variance explained by the first principal component (PC1), with the shoot traits SFW, SDW, RSG, RSFW, and RSDW making the highest contribution (Table 2, Figure 7c).

The PCA grouped the genotypes into four main clusters. The first cluster contained the largest proportion of genotypes (124 genotypes, 47%), whereas the second cluster had the lowest proportion (24 genotypes, 9%). The third and fourth clusters contained 56 (21%) and 60 (23%) genotypes, respectively (Figure 7d). Notably, clusters 2 and 3 had only moderately tolerant, tolerant, and highly tolerant genotypes, whereas cluster 1 comprised genotypes from all five Al tolerance classes (Figure 7d). However, all the highly susceptible and most of the susceptible genotypes were grouped together in cluster 4.

4. Discussion

In order to develop genotypes tolerant to Al toxicity, a detailed evaluation of the genetic variability within a crop species for Al tolerance is crucial. The present study evaluated the Al tolerances of 264 Ethiopian chickpea genotypes under hydroponic conditions using an optimal concentration of 120 µM Al. The 120 µM Al concentration was selected based on a previous Al tolerance screening study conducted on chickpea [35].

All studied phenotypic traits revealed significant ($p < 0.001$) variations among the genotypes, indicating the presence of substantial genetic variation within the Ethiopian chickpea gene pool, signifying the possibility of enhancing the productivity of chickpea under Al stress conditions. Variability in Al tolerance has also been reported earlier in chickpea [28,34,35] as well as other pulse legumes, including pigeon pea [19], common bean [47], and lentil [48], and, in a recent report, in the oil-seed crop sunflower [40].

In this study, the 120 µM Al treatment impeded the performance of the root and shoot traits of the chickpea genotypes with a substantial effect on root length (up to 29% reduction in root growth) and a more significant impact on sensitive and highly sensitive genotypes. Negusse et al. [36] reported a similar result in chickpea cultivars, revealing root length as

the most negatively affected trait, with an average reduction rate of 64%. This provides an insight into the characteristic effects of Al toxicity on chickpea.

The primary and immediate symptom of Al toxicity is the inhibition of root growth [15–17], which begins within minutes of exposure by disrupting root cell elongation [10]. This ultimately results in an impaired root system deficient in proper water and nutrient uptake. Hence, root traits are of paramount interest and extensively used as a parameter of choice by researchers working on Al tolerance screening of crop plants [18]. Moreover, among root traits, relative root growth (RRG) or root tolerance index (RTI) has been the most widely used trait, as it excludes underlying genotype-specific variations (size and vigor) and provides a reliable ranking of genotypes for Al tolerance [49,50].

In the present study, both RL and RRG showed significant variations among the chickpea genotypes. RRG as an Al tolerance parameter classified the 264 Ethiopian chickpea genotypes into five groups: highly sensitive (3.0%), sensitive (20%), moderately tolerant (45.45%), tolerant (15.15%), and highly tolerant (16.67%). Overall, 204 genotypes could be regarded as tolerant (moderate to high), while the remaining 60 were susceptible, including those highly susceptible. In contrast to our result, Sledge et al. [38] found that *M. truncatula* accessions were typically sensitive to Al toxicity. Out of the 321 *M. truncatula* accessions evaluated, 198 (61.88%) were highly sensitive, 109 (33.96%) were sensitive, and only 14 (4.36%) accessions were Al-tolerant.

Among the genotypes screened, 35% had a higher RRG than ETC_WL_1_2016, indicating that a great number of landraces superior in Al tolerance to the tolerant reference genotype exist in the Ethiopian chickpea gene pool. The use of RRG as an Al tolerance parameter was reported in maize [49] and lentil [50], where in maize, the RRG of the longest root was used to classify 141 germplasms into three tolerance classes [49]. In lentil, RRG estimates were used to classify 111 accessions into five Al tolerance groups [50].

The highest RRG value was obtained for genotype ETC_209008, followed by ETC_41184 and ETC_212589, while ETC_208995 had the lowest RRG value. It is noteworthy that genotypes ETC_41249, ETC_41224, ETC_209000, ETC_209021, and ETC_41054 had the longest roots under both control and Al treatment conditions, indicating the high root vigor of the genotypes. Moreover, all genotypes listed above were Al-tolerant, with an RRG value ranging from 0.85 to 0.91. Vigorous roots are beneficial for the efficient utilization of nutrients and water in the soil stratum, as plant growth is mainly dependent on the capacity of roots to explore and utilize these resources [50]. Hence, genotypes that grow vigorously in the control and Al treatment solutions have a greater capacity of growing in acidic soils with high Al saturation [47]. In contrast, genotypes with low root vigor in the two nutrient solutions would be less able to explore the soil area for water and minerals, and hence have a smaller capacity for growth and increased sensitivity to Al in the soil.

The highly tolerant (HT) genotypes identified in this study showed an RRG value above 1.0, indicating a slight increase in root growth under the Al treatment compared to the control. In wheat, the highly tolerant cultivar Atlas-66 exhibited a relative root length or RRG of 119 or 1.19 at 148 µM Al concentration compared to the control [51]. In cowpea cultivars, Paliwal [37] reported that the most Al-tolerant genotype (Co 3) performed extremely well in the Al-containing solution, and showed a 20.4% increase in root growth with exposure to Al as compared to the control. Hence, he concluded that even at relatively toxic Al concentrations, Al can be beneficial by stimulating the growth of some cowpea genotypes. Thus, genotypes that perform well in the presence of Al are regarded as Al-tolerant genotypes. Root growth stimulation in response to Al has also been reported in fava bean [52] and barrel medic genotypes [42]. However, the exact mechanism of stimulation is not well understood.

The Al-tolerant genotypes identified in the present study trace back their collection to areas marked by ATA as regions with acidic soil prevalence, viz. North, South, and West Shewa, West Wello, East and West Gojjam, North Gondar, as well as the Southern region (Semen Omo zones). The dominant soil types in these zones are moderately to strongly acidic [11]. In particular, genotypes ETC_209008 and ETC_41184 were originally collected

by EBI from the Southern region, Hadiya zone, and the Oromia region, West Shewa zone, respectively, which are both identified by ATA as having a serious acidic soil problem. Hence, these genotypes could serve as sources of Al tolerance genes. In barley, landraces obtained from acidic volcanic soils in Japan and Korea were suggested as sources of the Al resistance gene due to their Al tolerance in both the solution culture and acidic soil conditions [26].

The present hydroponic study identified Al-tolerant chickpea genotypes with high root vigor. The Al-tolerant genetic materials identified through this study could be utilized in chickpea breeding programs as sources of favorable alleles for Al tolerance, which will eventually lead to the development of Al-tolerant cultivars with enhanced productivity and ultimately contribute to sustainable food security.

5. Conclusions

The present study evaluated the Al tolerances of 264 Ethiopian chickpea genotypes. The study revealed the presence of considerable genetic variation in the Ethiopian chickpea gene pool. The tolerant and highly tolerant genotypes identified in the present study, including ETC_209008, ETC_41184, ETC_212589, and others not included in this list, could be considered as new sources of genes/alleles for Al-tolerant chickpea breeding. Furthermore, these genotypes could also be utilized to map genes that confer Al tolerance to chickpea, as well as to investigate the genetic basis of chickpea Al tolerance. Future studies should consider the evaluation of chickpea landraces on acidic soils at multi-location field trials, as this provides a realistic environment for assessing chickpea genetic materials. Furthermore, advanced physiological and genetic studies should be carried out to shed light on the possible mechanisms and genes involved in chickpea Al tolerance. In conclusion, the tolerant genetic materials identified in this study may serve as sources of novel genes or alleles that could be utilized to develop aluminum-tolerant cultivars using advanced breeding techniques, allowing for the expansion of cultivation areas and increasing chickpea productivity on acidic soils. Thus, this enhances food and nutritional security and supports small-scale farmers in acidic areas of the world to practice sustainable agriculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13112127/s1, Figure S1: The extent and Distribution of Acidic Soils in Ethiopia, Table S1: Passport data for 264 Ethiopian chickpea genotypes used in the study, Table S2: Mean performances of chickpea genotypes for all root and shoot traits evaluated at 0 and 120 μM Al solutions, as well as relative performance at 120 μM/0 μM Al.

Author Contributions: Conceptualization, H.N., K.T. and T.H.; methodology, H.N. and K.T.; software, H.N.; validation, H.N., K.T., T.H. and M.G.; formal analysis, H.N. and K.T.; investigation, H.N. and K.T.; resources, K.T. and T.H.; data curation, H.N., K.T. and M.G.; writing—original draft preparation, H.N.; writing—review and editing, H.N., K.T., T.H. and M.G.; visualization, H.N., K.T. and M.G.; supervision, K.T., T.H. and M.G.; project administration, K.T. and T.H.; funding acquisition, K.T. and T.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was part of a collaborative project entitled 'Enhancing Food Security through Improved Productivity, Nutrition and Marketing of Chickpea in Central and Western Ethiopia'. The study was supported by the USAID-PEER Program Cycle-4, administered by the U.S. National Academies of Sciences, Engineering and Medicine (NAS), and by the USAID Award AID-OAA-A-14-00005 to D.R.C. and K.T.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: The authors would like to acknowledge the Institute of Biotechnology, Addis Ababa University (AAU), for the assistance and facilities provided during the collection of chickpea germplasm. We would like to acknowledge the Ethiopian Biodiversity Institute (EBI) and the Debre Zeit Agricultural Research Center (DZARC) for providing seeds of chickpea landraces and improved

chickpea varieties, respectively. The authors are grateful to Douglas Cook, University of California, Davis, for the material support provided during the study.

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

References

1. FAOSTAT. FAO (Food and Agriculture Organization of the United Nations). Available online: https://www.fao.org/faostat/en/ (accessed on 20 October 2022).
2. Muehlbauer, F.J.; Sarker, A. Economic importance of chickpea: Production, value, and world trade. In *The Chickpea Genome*; Varshney, R.K., Thudi, M.M.F., Eds.; Springer: Cham, Switzerland, 2017; pp. 5–12. ISBN 9783319661179.
3. CSA (Central Statistical Agency). *The Federal Democratic Republic of Ethiopia Central Statistical Agency Report on Area, Production and Farm Management Practice of Belg Season Crops for Private Peasant Holdings*; Ethiopia Statistics Service: Addis Ababa, Ethiopia, 2021; pp. 1–142.
4. Kassie, M.; Shiferaw, B.; Asfaw, S.; Abate, T.; Muricho, G.; Ferede, S.; Eshete, M.; Assefa, K. Current Situation and Future Outlooks of the Chickpea Sub-sector in Ethiopia. *ICRISAT EIAR* **2009**, 1–39.
5. Verkaart, S.; Munyua, B.G.; Mausch, K.; Michler, J.D. Welfare impacts of improved chickpea adoption: A pathway for rural development in Ethiopia? *Food Policy* **2017**, *66*, 50–61. [CrossRef]
6. Damte, T.; Ojiewo, C.O. Current status of wilt/root rot diseases in major chickpea growing areas of Ethiopia. *Arch. Phytopathol. Plant Prot.* **2016**, *49*, 222–238. [CrossRef]
7. Fikre, A.; Desmae, H.; Ahmed, S. Tapping the economic potential of chickpea in sub-saharan africa. *Agronomy* **2020**, *10*, 1707. [CrossRef]
8. Toker, C.; Lluch, C.; Tejera, N.A.; Serraj, R.; Siddique, K.H.M. Abiotic Stresses. In *Chickpea Breeding and Management*; Yadav, S.S., Redden, R.J., Chen, W.S.B., Eds.; Centre for Agriculture and Bioscience International (CABI): Wallingford, UK, 2007; pp. 474–496. ISBN 9781845932138.
9. Kochian, L.V.; Hoekenga, O.A.; Piñeros, M.A. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.* **2004**, *55*, 459–493. [CrossRef]
10. 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]
11. Kassahun, B. *Soil Fertility Mapping and Fertilizer Blending*; Agricultural Transformation Agency (ATA): Addis Ababa, Ethiopia, 2015.
12. Mosissa, F. Progress of Soil Acidity Management Research in Ethiopia. *Adv. Crop Sci. Technol.* **2018**, *6*, 1–8. [CrossRef]
13. Foy, C.D.; Chaney, R.L.; White, M.C. The Physiology of Metal Toxicity in Plants. *Annu. Rev. Plant Physiol.* **1978**, *29*, 511–566. [CrossRef]
14. Ryan, P.R.; Tyerman, S.D.; Sasaki, T.; Furuichi, T.; Yamamoto, Y.; Zhang, W.H.; Delhaize, E. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. *J. Exp. Bot.* **2011**, *62*, 9–20. [CrossRef]
15. Delhaize, E.; Ryan, P.R.; Randall, P.J. Aluminum Tolerance in Wheat (*Triticum aestivum* L.) II. Aluminum-Stimulated Excretion of Malic Acid from Root Apices. *Plant Physiol.* **1993**, *103*, 695–702. [CrossRef]
16. Kochian, L.V. Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1995**, *46*, 237–260. [CrossRef]
17. 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.E., Eds.; CIMMYT: Mexico City, Mexico, 2001; ISBN 970-648-077-3.
18. Fageria, N.K.; Wright, R.J.; Baligar, V.C. Rice cultivar response to aluminum in nutrient solution. *Commun. Soil Sci. Plant Anal.* **1988**, *19*, 1133–1142. [CrossRef]
19. Choudhary, A.K.; Singh, D. Screening of pigeonpea genotypes for nutrient uptake efficiency under aluminium toxicity. *Physiol. Mol. Biol. Plants* **2011**, *17*, 145–152. [CrossRef]
20. Wayima, E.F.; Ligaba-Osena, A.; Dagne, K.; Tesfaye, K.; Machuka, E.M.; Mutiga, S.K.; Delhaize, E. Screening of diverse Ethiopian durum wheat accessions for aluminum tolerance. *Agronomy* **2019**, *9*, 440. [CrossRef]
21. Giaveno, C.D.; Miranda Filho, J.B. Rapid screening for aluminum tolerance in maize (*Zea mays* L.). *Genet. Mol. Biol.* **2000**, *23*, 847–850. [CrossRef]
22. Dai, S.F.; Yan, Z.H.; Liu, D.C.; Zhang, L.Q.; Wei, Y.M.; Zheng, Y.L. Evaluation on Chinese Bread Wheat Landraces for Low pH and Aluminum Tolerance Using Hydroponic Screening. *Agric. Sci. China* **2009**, *8*, 285–292. [CrossRef]
23. Shavrukov, Y.; Genc, Y.; Hayes, J. The Use of Hydroponics in Abiotic Stress Tolerance Research. In *Hydroponics—A Standard Methodology for Plant Biological Researches*; Asao, T., Ed.; InTech: Rijeka, Croatia, 2012; pp. 3–66.
24. Hede, A.R.; Skovmand, B.; Ribaut, J.M.; González-De-León, D.; Stølen, O. Evaluation of aluminium tolerance in a spring rye collection by hydroponic screening. *Plant Breed.* **2002**, *121*, 241–248. [CrossRef]
25. Delhaize, E.; Ryan, P.R. Aluminum toxicity and tolerance in plants. *Plant Physiol.* **1995**, *107*, 315–321. [CrossRef]
26. Moroni, J.S.; Sato, K.; Scott, B.J.; Conyers, M.; Read, B.J.; Fisher, R.; Poile, G. Novel barley (*Hordeum vulgare* L.) germplasm resistant to acidic soil. *Crop Pasture Sci.* **2010**, *61*, 540–553. [CrossRef]
27. Tesfaye, M. Overexpression of Malate Dehydrogenase in Transgenic Alfalfa Enhances Organic Acid Synthesis and Confers Tolerance to Aluminum. *Plant Physiol.* **2001**, *127*, 1836–1844. [CrossRef]

28. Singh, D.; Raje, R.S. Genetics of aluminium tolerance in chickpea (*Cicer arietinum*). *Plant Breed.* **2011**, *130*, 563–568. [CrossRef]
29. Zeven, A.C. Landraces: A review of definitions and classifications. *Euphytica* **1998**, *104*, 127–139. [CrossRef]
30. Azeez, M.A.; Adubi, A.O.; Durodola, F.A. Landraces and Crop Genetic Improvement. In *Rediscovery of Landraces as a Resource for the Future*; IntechOpen: Rijeka, Croatia, 2018; pp. 1–20. [CrossRef]
31. Akter, M.B.; Mosab-Bin, A.; Kamruzzaman, M.; Reflinur, R.; Nahar, N.; Rana, M.S.; Hoque, M.I.; Islam, M.S. Morpho-molecular diversity study of rice cultivars in Bangladesh. *Czech J. Genet. Plant Breed.* **2022**, *58*, 64–72. [CrossRef]
32. Choudhury, S.; Sharma, P. Aluminum stress inhibits root growth and alters physiological and metabolic responses in chickpea (*Cicer arietinum* L.). *Plant Physiol. Biochem.* **2014**, *85*, 63–70. [CrossRef]
33. Rai, R. Effects of soil acidity factors on interaction of chickpea (*Cicer arietinum* L.) genotypes and Rhizobium strains: Symbiotic N-fixation, grain quality. In *Plant-Soil Interactions at Low pH, Proceedings of the Second International Symposium on Plant-Soil Interactions at Low pH, Beckley, WV, USA, 24–29 June 1990*; Springer: Berlin/Heidelberg, Germany, 1991; pp. 619–631.
34. Vance, W.; Pradeep, K.; Strachan, S.R.; Diffey, S.; Bell, R.W. Novel Sources of Tolerance to Aluminium Toxicity in Wild Cicer (*Cicer reticulatum* and *Cicer echinospermum*) Collections. *Front. Plant Sci.* **2021**, *12*, 678211. [CrossRef]
35. Negusse, H.; Cook, D.R.; Haileselassie, T.; Tesfaye, K. Identification of Aluminum Tolerance in Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm Identification of Aluminum Tolerance in Ethiopian Chickpea. *Agronomy* **2022**, *12*, 948. [CrossRef]
36. 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]
37. Paliwal, K.; Sivaguru, M.; Thiruselvi, A. Identification of an aluminum tolerant tropical cowpea cultivar by growth and biomass accumu-lation parameters. *J. Plant Nutr.* **1994**, *17*, 367–376. [CrossRef]
38. Sledge, M.K.; Pechter, P.; Payton, M.E. Aluminum tolerance in Medicago truncatula germplasm. *Crop Sci.* **2005**, *45*, 2001–2004. [CrossRef]
39. Villagarcia, M.R.; Carter, T.E.; Rufty, T.W.; Niewoehner, A.S.; Jennette, M.W.; Arrellano, C. Genotypic rankings for aluminum tolerance of soybean roots grown in hydroponics and sand culture. *Crop Sci.* **2001**, *41*, 1499–1507. [CrossRef]
40. Singh, V.K.; Chander, S.; Sheoran, R.K.; Anu; Sheoran, O.P.; Garcia-Oliveira, A.L. Genetic variability for aluminium tolerance in sunflower (*Helianthus annuus* L.). *Czech J. Genet. Plant Breed.* **2022**, *58*, 201–209. [CrossRef]
41. Alemu, B.; Lule, D. Yield and agronomic performances of desi type chickpea genotypes against acidic soil of Western Ethiopia. *J. Agric. Biotechnol. Sustain. Dev.* **2018**, *10*, 116–121. [CrossRef]
42. Narasimhamoorthy, B.; Bouton, J.H.; Olsen, K.M.; Sledge, M.K. Quantitative trait loci and candidate gene mapping of aluminum tolerance in diploid alfalfa. *Theor. Appl. Genet.* **2007**, *114*, 901–913. [CrossRef] [PubMed]
43. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021.
44. Wei, T.; Simko, V.; Levy, M.; Xie, Y.; Jin, Y.; Zemla, J.; Freidank, M.; Cai, J.; Protivinsky, T. Package 'corrplot'. *Statistician* **2021**, *56*, 316–324.
45. Kassambara, A.; Mundt, F. Package 'Factoextra'. Extract and Visualize the Results of Multivariate Data Analyses. 2017, 76. R Packages. 2020. Available online: https://rpkgsdatanovia.com/factoextra/index (accessed on 20 April 2023).
46. Lê, S.; Josse, J.; Husson, F. FactoMineR: An R package for multivariate analysis. *J. Stat. Softw.* **2008**, *25*, 1–18. [CrossRef]
47. Blair, M.W.; López-Marín, H.D.; Rao, I.M. Identification of aluminum resistant Andean common bean (*Phaseolus vulgaris* L.) genotypes. *Brazilian J. Plant Physiol.* **2009**, *21*, 291–300. [CrossRef]
48. Singh, D.; Dikshit, H.K.; Singh, R. Variation of aluminium tolerance in lentil (*Lens culinaris* Medik.). *Plant Breed.* **2012**, *131*, 751–761. [CrossRef]
49. Xu, L.M.; Liu, W.; Cui, B.M.; Wang, N.; Ding, J.Z.; Liu, C.; Gao, S.B.; Zhang, S.Z. Aluminum tolerance assessment of 141 maize germplasms in solution cultures. *Univers. J. Agric. Res.* **2017**, *5*, 1–9.
50. Kulkarni, V.; Sawbridge, T.; Kaur, S.; Hayden, M.; Slater, A.T.; Norton, S.L. New sources of lentil germplasm for aluminium toxicity tolerance identified by high throughput hydroponic screening. *Physiol. Mol. Biol. Plants* **2021**, *27*, 563–576. [CrossRef]
51. Cosic, T.; Poljak, M.; Custic, M.; Rengel, Z. Aiuminium tolerance of durum wheat germplasm. *Euphytica* **1994**, *78*, 239–243. [CrossRef]
52. Belachew, K.Y. *Root and Shoot Traits Associated with Acidity and Drought Tolerance in Vicia faba L. Plants*; University of Helsinki: Helsinki, Finland, 2019.