

Aluminium Tolerance Assessment of 141 Maize Germplasms in a Solution Culture

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Abstract Aluminum (Al) toxicity usually occurs in acidic soils with a pH of 5.5 or lower. The selection and breeding of Al-tolerant cultivars is a useful approach for protecting maize from Al toxicity. Rapid and reliable screening methods must be developed to discriminate Al-tolerant and Al-sensitive maize genotypes. The relative root growth (RRG) of the longest root in a toxic Al solution was used to classify 141 maize germplasm lines into three groups with varied Al sensitivity: Al-sensitive, moderately Al-tolerant, and Al-tolerant. Among these lines, the cultivars HZ85 and 178 had the highest RRG values and therefore the highest Al tolerance. Further root assessment of six representative lines using other methods, such as digital imaging analysis of total root length and superficial area or volume, biomass measurement, and hematoxylin staining, was roughly consistent with the classification based on RRG. These results indicated that the RRG of the longest root could be used as a reliable and reproducible phenotypic index for the evaluation of Al tolerance in maize genotypes. Cultivars with different Al tolerances can be used to improve breeding and explore the mechanism of Al tolerance in maize.

Keywords Maize, Aluminum Toxicity, Phenotypic Index, Digital Imaging, Hematoxylin Staining

1. Introduction

Acidic soils, with a pH of 5.5 or lower, are distributed extensively across the world. Approximately 30% of the world's total land area and over 50% of potentially arable lands are acidic, with the majority (60%) of this area in the tropics and subtropics [1]. China also has a large amount of acidic soils. Xiong and Li [2] reported that acidic soils are distributed in 14 provinces and occupy up to 2030 thousand km², or 21% of cultivated land, in China. Soils acidify very

slowly, over hundreds to millions of years, under natural conditions. However, the anthropogenic acidification of soils has received more attention. The overuse of N fertilizer contributes substantially to regional soil acidification both directly and indirectly [3]. Moreover, acid rain, which contains nitric and sulfuric acids, is increasing the rate of soil acidification in many locations in China [4].

A number of factors contribute to acidic soil toxicity depending on soil composition. In acidic soils with a high mineral nutrition content, the primary factor limiting plant growth is Al toxicity, which is most serious when soil acidity is below pH 4.0 [5-7]. At low pH values, toxic forms of Al, such as Al(OH)₂⁺, Al(OH)₂²⁺, and Al³⁺, are released into the soil solution. For most crops, free Al³⁺ is the main toxic Al ion that actively damages root growth, thus affecting the development of the whole plant [5, 8]. Roots exposed to toxic Al tend to become short, thick, and underdeveloped, thereby reducing nutrient uptake and increasing susceptibility to environmental stresses [9]. The application of lime in acidic soils can increase the soil pH and hence reduce the Al toxicity. However, acidity cannot always be corrected in this way due to economic limitations and labor requirements [10]. Furthermore, the heavy application of lime may adversely affect some crops in the rotation or cause deficiencies of certain nutrients.

To relieve the toxicity of Al stress and improve the crop yield in acidic soils, the breeding and cultivation of Al-tolerant crops is a useful alternative approach. The first step in this approach is to select a reliable method for screening numerous crop lines that possess Al resistance at various levels.

Much effort has been devoted to improving screening methods for Al tolerance to better identify cultivar differences [11]. The primary screening methods for Al tolerance include field evaluations, soil or pot assays, and solution culture assays [4, 12, 13]. Molecular marker-assisted selection has also been developed to screen Al-tolerant genotypes in barley [14]. Field-based screening

methods are generally more laborious, time consuming, and expensive, whereas laboratory- and greenhouse-based techniques are becoming more widely employed due to their rapidity, nondestructive nature, and high accuracy. Among these methods, the nutrient solution technique is the preferred choice for most research because it provides easy access to root systems, tight control over nutrient availability and pH, and nondestructive measurement of tolerance [15]. Moreover, the nutrient solution technique is most suitable for determining Al activity and avoids other soil-associated stresses [16].

Several screening criteria have been applied for the evaluation of Al tolerance in nutrient solution culture. Root growth parameters, including root growth length, volume, superficial area, and biomass, have been used as root tolerance indexes to evaluate genotypes for Al tolerance [17-19]. Comparing Al tolerance levels in wheat, triticale, and rye by measuring root elongation in Al-containing hydroponic solutions, [20] found that rye had a higher degree of Al tolerance than did the other cereals. Dai et al. [10] evaluated the tolerance of 173 Tibetan wheat landraces to Al stress using root regeneration length measurements in hydroponic solutions containing Al. Fortunately, tedious manual measurements of root growth length have been replaced by automated methods of digital image analysis; for example, these methods were effective for evaluating the Al tolerance of sorghum plants after a four-day treatment in nutrient solutions [21]. The volume and superficial area of root systems, which describe the dynamic state of root growth based on digital imaging analysis, are reasonable criteria for measuring the Al tolerance of genotypes in nutrient solution culture [22]. Other phenotypic parameters, such as the fresh and dry weights of the whole root system used as indexes of root biomass, also have been used for the assessment of germplasm for Al tolerance [23].

In addition, hematoxylin staining of roots, which obtains results quickly and efficiently, is suitable for screening a large segregating population. This method, as originally described by Berezovskii et al. [24], has long been used in applications as diverse as genotypic classification [25, 26], genetic characterization [27, 28], and selection [29, 30].

The above approaches provide many options for evaluating maize genotypes for Al tolerance. Several studies have evaluated Al tolerance in maize cultivars from distinct ecotypes using the relative root growth (RRG) of the longest root [31, 32] and hematoxylin staining [33]. In the current study, we performed Al tolerance screening in solution for 141 maize genotypes and classified them into three different groups according to the RRG of the longest root. These results were further confirmed by other methods such as root growth measurement based on digital imaging analysis, whole root biomass measurement, and hematoxylin staining. Finally, we identified maize genotypes with different Al tolerances that may be used in future Al-tolerant breeding strategies.

2. Materials and Methods

2.1. Plant Materials and Al Treatment

The 144 maize germplasm lines used in this experiment were collected from China. The seeds were surface sterilized with 75% alcohol for 1 min and then with 0.1% NaClO (v/v) for 20 min. After being completely washed with flowing water, the seeds were placed on filter papers saturated with distilled water and germinated at 26°C for 3 days in the dark. The seedlings were then transferred to a growth chamber and grown at 26°C/24°C (light/dark, 16:8 h) under a light intensity of 500 mmol photons m⁻² s⁻¹. Before the Al treatment, the seedlings were first cultured in an aerated 200 μmol CaCl₂ (pH = 4.0) solution for 24 h. Subsequently, these seedlings were further cultured in an aerated solution containing 60 μmol AlCl₃ and 200 μmol CaCl₂ (pH = 4.0) for 3 days. Seedlings treated in the same way without the addition of AlCl₃ to the solution were used as controls.

2.2. RRG Measurement

The measurement of root growth length was performed using at least 20 uniform seedlings of each maize line: 10 for the Al treatment and 10 as controls. The longest root length before (initial root growth (IRG)) and after treatment (final root growth (FRG)) were measured by a flexible ruler. Net root growth (NRG) was calculated as FRG – IRG, and the RRG of each line was determined as RRG = NRG of Al-treated seedlings/NRG of control seedlings. Three replicates were performed for each line for both the Al-treated and control seedlings.

2.3. Digital Scanning and Dynamic Growth Analysis of Root Systems

After Al treatment, the seedling root systems of six representative maize lines, 178, HZ85, CA339, 5022(B), B73, and Mo17, were measured using a scanner (Epson Expression 10000 XL). Data on the total root length, superficial area, and volume were then quantified and analyzed using the digital scanning system. The same measurements were performed on the control seedlings.

2.4. Measuring the Biomass of Root Systems

The total root systems of lines 178, HZ85, CA339, 5022(B), B73, and Mo17 were collected individually after treatment with or without Al for 3 days, as described above. These fresh roots were dried in an oven at 60°C for 2 days and weighed before and after drying using a microgram balance.

2.5 Hematoxylin Staining

Roots of 3-day-old treated and control seedlings of the six maize lines (178, HZ85, CA339, 5022(B), B73, and Mo17)

were stained using a modification of the method described by [24]. Roots were exposed to AlCl₃ solution for 3 days and then washed for 30 min in distilled water. After being stained in hematoxylin solution for 20 min, the roots were washed again for another 30 min in distilled water and then photographed using a digital camera. The stain solution was prepared before the experiment by placing 0.2 g of hematoxylin and 0.02 g of KIO₃ in 100 ml of water and stirring the solution overnight to dissolve the hematoxylin.

3. Results

3.1. Classification of 141 Maize Genotypes According to RRG

RRG of the longest root is the most commonly used

parameter for estimating Al tolerance in cereals [31]. According to the RRG of the longest root, all of the tested maize lines were classified into three categories: Al-tolerant (RRG > 30%), moderately Al-tolerant (15% < RRG ≤ 30%), and Al-sensitive (RRG ≤ 15%). Among the 141 maize germplasm lines, 24 were scored as Al-tolerant, 63 as moderately Al-tolerant, and 54 as Al-sensitive (Table 1). The highest Al tolerance was exhibited by line 178, with an RRG > 40%, while 22 lines had an RRG < 10% and demonstrated the highest sensitivity to Al. Subsequently, two standard lines from each of the three groups were further examined using other methods: the Al-tolerant lines 178 (RRG = 0.4502) and HZ85 (RRG = 0.3833), moderately Al-tolerant lines CA339 (RRG = 0.2915) and 5022(B) (RRG = 0.2938), and Al-sensitive lines B73 (RRG = 0.1329) and Mo17 (RRG = 0.1206).

Table 1. Aluminium-tolerant evaluation of 141 maize seedlings based on relative root growth (RRG) of the longest root

Maize germplasm	RRG	Sensitivity to Al	Maize germplasm	RRG	Sensitivity to Al	Maize germplasm	RRG	Sensitivity to Al
Jin Huang 31	0.118		Wen Huang	0.1399		Jin Huang 48	0.1503	
Jin Huang 55	0.0644		U8112	0.0923		Jin Huang 49	0.1517	
Jin Huang 76	0.1382		Duo Huang 29	0.0277		Jin Huang 61	0.1508	
P127	0.0746		Jin Huang 96B	0.0941		Jin Huang 63	0.1909	
P137	0.096		CN962	0.0842		P140	0.1641	
R3	0.0974		CN165	0.1379		Shan Dong 8129	0.1522	
Zhong Zong	0.107		CN117	0.0828		Zhong Zong	0.2657	
Wei Er 157	0.1301		7063	0.1405		P141	0.2297	
CA8917 (sticky)	0.0641		08W-SC16	0.1294		91041-2	0.1807	
91041-2	0.0996		08W-SC20-4	0.1346		R18	0.1693	
Za C546	0.1286		08W-SC36	0.1144		CA091	0.2716	
Shen 5003	0.0652		CN4397	0.0824		CA23	0.2189	
K22	0.1172		8065	0.1458		H18	0.2755	
Cai 11-8	0.0917	Al sensitive	Chuan 219-1	0.1293	Al sensitive	Duo 16	0.2486	moderately Al tolerant
H6	0.1428		Chuan 273	0.1471		A513	0.2077	
Ji 846	0.1048		Qi 319	0.1385		8129	0.1719	
374	0.1079		3189	0.1007		H10	0.252	
CA181	0.1316		81565	0.0967		Jan-87	0.2142	
Liao Ju 311	0.0652		196	0.1013		Ji 818	0.2974	
Tie 9010	0.0934		CAL58	0.1111		C8605-2	0.2857	
706 Fu	0.1277		653	0.1329		Shen 5003	0.2895	
7884	0.1184		K10	0.1094		Ji 53	0.1546	
8723	0.0764		698-4	0.123		4379	0.228	
Ye 52106	0.1357		M14	0.1137		200B	0.24	
Q66	0.099		K12	0.0227		CA042	0.1587	
Drought 21	0.096		Mo17	0.1206		Feb-48	0.1659	
Ji (03)	0.064		B73	0.1329		K12	0.2254	

Maize germplasm	RRG	Sensitivity to Al	Maize germplasm	RRG	Sensitivity to Al	Maize germplasm	RRG	Sensitivity to Al
832	0.1724		Qi 205	0.1818		08W-SC88-1	0.318	
485	0.2734		Lu 2548	0.2827		08W-SC91-1	0.32381	
3141	0.1718		X178	0.1611		08W-SC93-1	0.3412	
Apr-29	0.2466		Shen 137	0.1602		08W-SC95-4	0.32	
Zheng 22	0.2872		803	0.2638		08W-SC119-1	0.3771	
Zhong 17	0.2524		Duo Huang 212	0.25	moderately Al tolerant	08W-SC127-3	0.3439	Al tolerant
Shen 136	0.2256		44	0.2601		08W-SC128-2	0.3571	
Wu 314	0.2916		Lin Xi 020	0.2027		08W-SC136-1	0.3636	
Hua 160	0.2061		B84	0.3		515	0.3041	
C103	0.1994		WF9	0.2282		Ji 853	0.3238	
Nov-38	0.1738		CA339	0.2915		HZ85	0.3833	
Mo Qun 17	0.1971		5022 (B)	0.2938		178	0.4502	
Zhong Huang 2041220	0.2538	moderately Al tolerant	314	0.359				
Zhong Zi 01	0.1697		643	0.3381				
CN563	0.2296		2548	0.3056				
08W-SC17-1	0.1852		Chuan 1073-7	0.3643				
08W-SC-23-2	0.2572		Qi 318	0.3585				
08W-SC35-1	0.2104		OH-43	0.3372	Al tolerant			
08W-SC37	0.1674		Ji 495	0.3369				
Zhong Huang 68	0.2445		Lin Xi 2	0.3059				
141	0.1538		7922	0.3006				
Lin Xi 004102	0.1502		Drought 23	0.3121				
08W-SC110-1	0.2875		Mixture 517	0.3455		CV%	46.8391	
08W-SC130-1	0.2608		598	0.3163		LSD (P=0.05)	0.0213	

Note: The six representative lines for the three classes were highlighted in black bold.

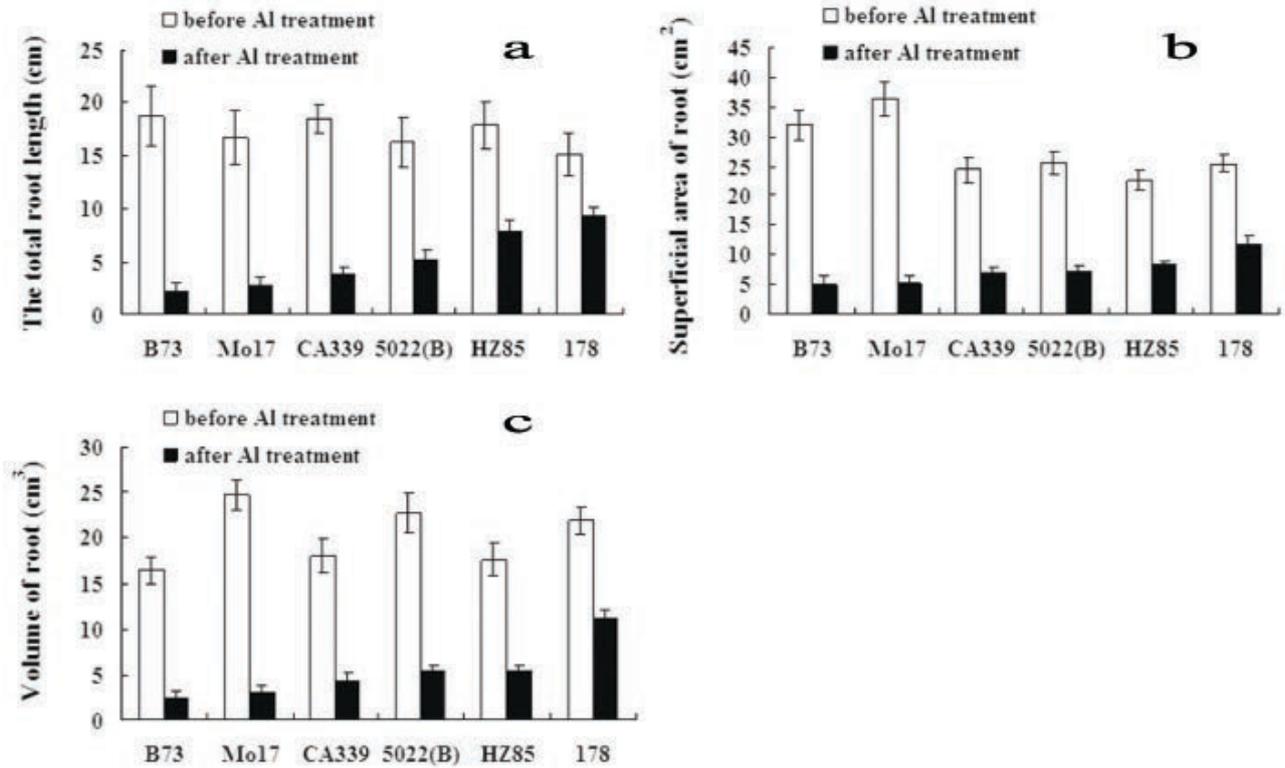
3.2. Al Responses of Six Standard Maize Lines Assessed by Digital Imaging Analysis

Within the first few days of germination, the root systems of maize seedlings are fibrous and produce multiple primary, secondary, and tertiary roots. A root digital imaging system can quantify the total root length, superficial area, and volume of the entire maize root structure. As expected, all of the six maize genotypes showed some degree of root growth inhibition when grown in the solution containing 60 $\mu\text{mol Al}^{3+}$ (Fig. 1). For the Al-tolerant genotypes HZ85 and 178, the total root length after Al exposure decreased from 17.86 cm to 7.89 cm (55.81%) and from 15.12 cm to 9.42 cm (37.7%), respectively (Fig. 1a). The moderately Al-tolerant cultivars CA339 and 5022(B) demonstrated significant root growth disturbance, with inhibition levels of 78.95% and 68.12%, respectively. In the Al-sensitive cultivars Mo17 and B73, the mean total root lengths were severely inhibited, reaching only 12.5% those of the control plants. These results showed that total root length inhibition generally agreed with the classification of the three maize groups according to RRG data.

The superficial area and volume of the whole root system were effective indexes of the growth and development of the root system as impacted by abiotic stress in the soil.

Exposure of the maize cultivars to a toxic Al solution decreased both superficial area and volume of the whole root system to different degrees (Fig. 1b, c). When subjected to Al, the Al-tolerant cultivars HZ85 and 178 presented only 37.28% and 46.14% of the superficial area and 31.28% and 41% of the volume, respectively, of the control plants. For the moderately Al-tolerant cultivars CA339 and 5022(B), both the superficial area and volume of whole root system were dramatically reduced, reaching less than 20% those of the control plants. Similar results were also observed for the Al-sensitive cultivars B73 and Mo17, which exhibited even more severe Al inhibition of the superficial area and volume of the whole root system. As can also be seen in the chart (Fig. 1b, c), the data obtained for the superficial area and volume of the whole root systems again indicated that the Al responses of the six maize cultivars were in accordance with the classifications based on the RRG of the longest root measurement.

Further evaluation revealed that the RRG values positively correlated with the total root length, superficial area, and volume of the entire maize root. The correlation relation analysis shows that the correlation coefficients between the RRG of the seminal root and the root length was moderate ($r=0.53$) while that between the RRG and the root superficial area ($r=0.12$) or root volume ($r=0.21$) was weak.

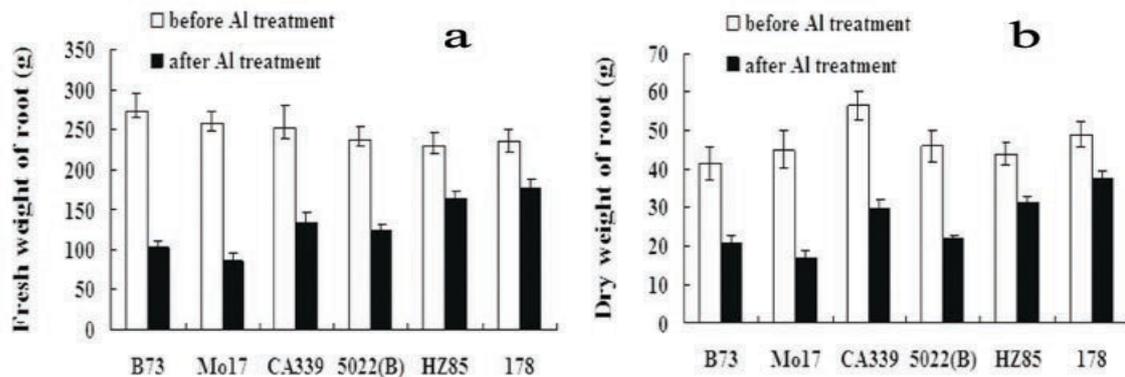


The six maize cultivars 178, HZ85, CA339, 5022(B), B73, and Mo17 were assessed by digital scanning of the total root length (A), superficial area (B), and volume (C) after treatment with 60 μmol aluminum for three days. Vertical bars indicate the SD of the mean values.

Figure 1. Digital scanning analysis of the total root system

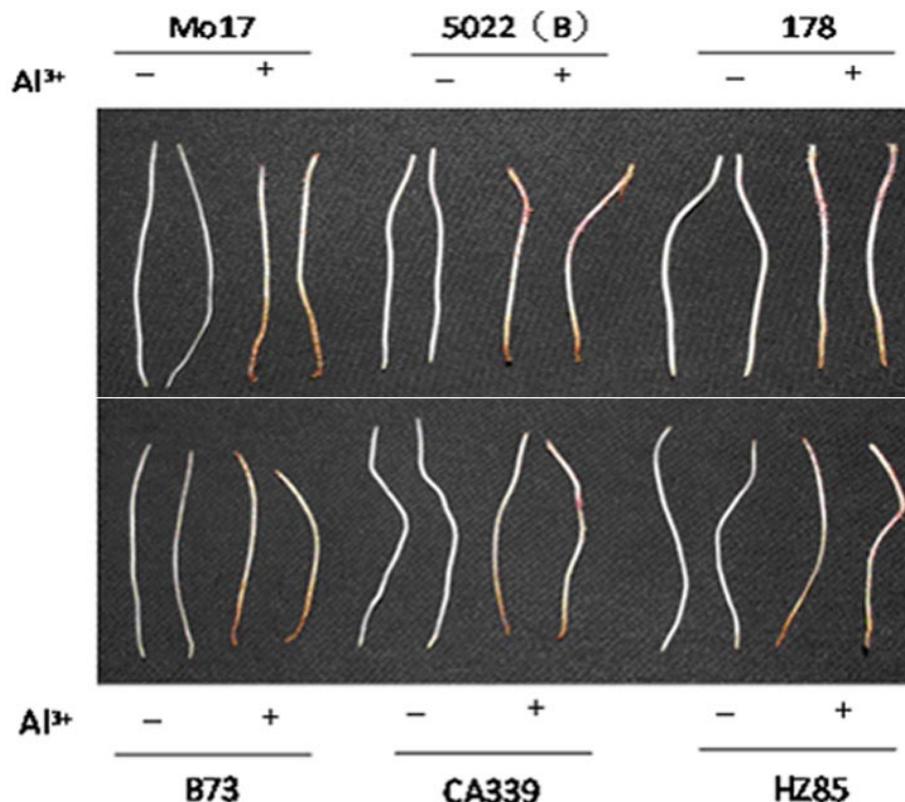
3.3. Evaluation of the Root Biomass of Six Maize Cultivars Exposed to Al

The biomass of the root can reflect its development and growth. When subjected to Al stress, Al-tolerant cultivars proliferate more roots than do Al-sensitive cultivars [34]. Root biomass can be described by the dry and fresh weights of whole root systems. After the Al stress in this experiment, both the dry and fresh weights of all six cultivars significantly decreased to different extents (Fig. 2a, b). For the Al-tolerant cultivars HZ85 and 178, the fresh root weights shrank to 71.76% and 75.07% and the coherent dry weights decreased to 71.77% and 76.63% of the control, respectively. In contrast, fresh weight decreased in the Al-sensitive cultivars B73 and Mo17 by 62.19% and 66.71%, respectively, and dry weight by 49.59% and 62.35%, respectively, due to Al stress. For the moderately Al-tolerant cultivars CA339 and 5022(B), Al stress inhibited dry weight by roughly 45% and fresh weight by approximately 55% (Fig. 2). The correlation coefficient (*r*) between the RRG and the fresh root and dry root was up to 0.84 and 0.61 respectively. This result also suggested that root biomass was suitable for rating the Al sensitivity of genotypes and was in good agreement with the RRG measurements.



The fresh (A) and dry (B) weights of the six maize cultivars 178, HZ85, CA339, 5022(B), B73, and Mo17 were measured after treatment with 60 μmol aluminum for three days. Vertical bars indicate the SD of the mean values.

Figure 2. Measurement of the root biomass



Root fragments of the six maize cultivars 178, HZ85, CA339, 5022(B), B73, and Mo17 were stained in hematoxylin solution after three days of exposure to 60 μmol aluminum.

Figure 3. Hematoxylin staining patterns and staining intensity

3.4. Estimation of Al Tolerance of Maize Cultivars by Hematoxylin Staining

A rapid method for evaluating Al tolerance using hematoxylin staining has been developed based on the formation of a colored complex between hematoxylin and root-bound Al. The color intensity of stained root apices subjected to Al stress can be directly measured, as susceptible genotypes tend to accumulate higher amounts of Al in their root tissues. These variations have been assessed as stained, unstained, and partially stained to represent Al-sensitive, tolerant, and intermediate genotypes, respectively [24]. Hematoxylin staining analysis showed that B73 and Mo17 exhibited the most intense root staining, whereas HZ85 and 178 showed the least intense staining. CA339 and 5022(B) were moderately stained compared with the other lines (Fig. 3). These results clearly indicated that hematoxylin staining was indeed able to discriminate the Al sensitivity of maize genotypes early in root development.

4. Discussion

A rapid and reliable screening method is necessary for crop breeders to effectively select Al-tolerant germplasm. Numerous methods and phenotypic indexes have been developed based on the physiological responses of plants subjected to Al [12, 13, 35]. Among these methods, RRG is

the most widely used phenotypic index for rating Al tolerance in cereals [19, 31, 32]. For example, Khatiwada [18] classified 62 traditional rice cultivars grown on lowland acidic sulfate soils in Asia and West Africa into three different groups according to the RRG of the longest root when exposed to a nutrient solution containing 30 ppm Al for 14 days. Using a similar approach, Piñeros [32] ranked six maize lines into three main categories according to the RRG of the seminal root after growth in a full nutrient solution containing an effective 40 μmol Al^{3+} for 3 days: highly Al-tolerant ($\text{RRG} > 80\%$), moderately Al-tolerant ($55\% < \text{RRG} \leq 80\%$), and Al sensitive ($\text{RRG} \leq 55\%$). In the current study, we classified 141 maize germplasm lines into three groups according to the RRG of the longest root after exposure to 60 μmol Al for 3 days (Table 1). The RRG levels used to classify the maize lines were significantly higher than those used for the corresponding categories in the previous study. Furthermore, among the 22 Al-sensitive maize lines with $\text{RRG} < 10\%$, Duo Huang 29 and K12 had the lowest values ($\text{RRG} = 0.0227$) and exhibited the highest sensitivity to Al toxicity. This result showed that the root growth of some maize lines was seriously inhibited in 60 μmol Al solution and those roots could not further develop beyond this threshold of Al concentration.

Multiple factors may have caused the low RRG values in the current study. Generally, different genotypes vary in tolerance to certain stress environments. The six maize lines in Piñeros's study were chosen based on their different

genotypic origins and ecological zones, while most of the 141 maize germplasm lines in this study were collected from the main maize production regions of China, which have no acidic soils or are not typically acidic. Therefore, the most tolerant cultivars in this study, such as 178, had relatively lower RRG values, while Cateto-Colombia, which originated from a tropical zone rich in acidic soil, exhibited the strongest Al tolerance and the highest RRG value. The optimal Al concentration employed in any given study depends on the general tolerance level of the genotypes screened and whether complete growth inhibition is desired [9]. In this study, although some sensitive maize lines possessed low RRG levels, 60 $\mu\text{mol Al}^{3+}$ did not completely inhibit root growth and could be used to screen and classify the 141 maize genotypes for Al tolerance. The low RRG values could have been increased by reducing Al^{3+} concentration, but the evaluation of maize genotypes would have been unaffected. In addition, the seedlings subjected to Al stress were supplied only with 60 $\mu\text{mol AlCl}_3$ together with 200 $\mu\text{mol CaCl}_2$ in the current study, whereas in Piñeros's experiment, the treated seedlings were grown in a full nutrition solution with 40 $\mu\text{mol effective Al}^{3+}$. The extra nutrition in Piñeros's test may have caused more vigorous root growth even under Al stress, as reflected by the higher RRG values in that study. Moreover, some ions in a full nutrition solution, such as phosphate, calcium, and magnesium, can interact with Al to alleviate Al toxicity [7, 36]. A simple solution only containing Al may somehow affect plant development even if the nutrition satisfied the seedlings' developmental requirements, thereby making the seedlings more sensitive to Al toxicity.

The RRG of the longest root has been widely used as a suitable phenotypic index for assessing Al tolerance in cereal seedlings cultivated in nutrient solution. Nevertheless, this value should not be used as the single assessment criterion, as it may be misleading in genotypes that accumulate high amounts of Al in the aerial part of the plant or manifest Al tolerance at the adult stage [6, 36, 37]. Famoso et al. [31], for example, found that RRG of the longest root was not the best predictor of Al tolerance in rice. Therefore, the six representative maize lines HZ85, 178, CA339, 5022 (B), B73, and Mo17, which belonged to different Al tolerance groups, were used to further investigate the sensitivity to Al stress with other methods.

Previous reports in maize, wheat, sorghum, soybean, sugarcane, and tobacco have indicated that more severe inhibition occurs in lateral roots than in the longest root in Al-sensitive genotypes [22, 23, 38, 39]. Furthermore, Famoso et al. [31] found that the RRG of the longest root was not a good proxy for the total root system in evaluating the Al tolerance of genotypes. A genotype may therefore appear tolerant based on the longest root measurement while actually exhibiting overall root growth inhibition. Intriguingly, based on the digital scanning data of the total root length, superficial area, and volume, the six representative cultivars in this study were assigned into the

same groups as those previously assigned by the RRG of the longest root (Fig. 1, Table 1).

In addition to the proxy mentioned above, comparing the root biomass of plants in the presence or absence of Al is another common criterion used to measure Al toxicity [23]. Root biomass (fresh and dry weights) measurements of the six representative maize cultivars after short-term culturing again classified the genotypes into the previously assigned groups (Fig. 2).

Hematoxylin staining, a rapid and simple method, was also used to evaluate the Al stress phenotypes of maize seedlings in the current study. Hematoxylin, a dye commonly used in cytogenetic studies, has also been employed as a quick, nondestructive way of assessing plant genotypes for Al tolerance [24, 25, 40-42]. Berezovskii et al. [24] recommended a qualitative scale to rate different genotypes for Al tolerance based on how completely their roots were stained. The classification of the six maize lines according to hematoxylin staining was in consensus with the results for the other methods (Figs. 1-3, Table 1). Similar results have also been reported for Canadian cultivars by Zale and Briggs [43]. These results showed a potential relationship between hematoxylin ratings and root growth measurements in cereals.

A long evolutionary history of exposure to Al is likely to favor the development of highly adapted genotypes with polygenic control of resistance, because Al resistance can be modified by a considerable number of genes [44, 45]. The molecular mechanisms of Al tolerance have been gradually unveiled by the great advances recently made in research on higher plants [46, 47]. However, reports regarding Al-tolerant maize germplasm and molecular explorations of Al tolerance have been comparatively limited. Due to the rapid spread of acidic soils, the exploration and application of Al tolerance genes in crop breeding, molecular marker-assisted selection, and genetic engineering are ongoing.

5. Conclusions

Al toxicity is the main limiting factor for maize root elongation in acidic soils. Several methods have been developed to classify differences in the sensitivity to Al exposure of plant germplasm. The current study classified maize germplasm lines using four approaches based on Al stress responses. A total of 141 maize germplasm lines were classified into three groups based on the RRG of the longest root. Among these lines, 178 and HZ85 were the most tolerant maize cultivars. Two representative lines from each of the three different groups were then further evaluated. Similar group rankings were obtained by measuring the total root length, volume, and superficial area, taking fresh and dry weights, and hematoxylin staining. In conclusion, multiple criteria may be flexibly combined to rationally classify the Al tolerance of different maize genotypes.

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