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Enhancing Maize Grain Yield in Acid Soils of Western Kenya Using Aluminium Tolerant Germplasm

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Abstract: Maize (*Zea mays* L.) is one of the world's most important cereals and is a staple food for many people in developing countries. However, in acid soils (pH < 5.5), its productivity is limited by aluminium (Al) toxicity, besides other factors. The objectives of this study were to: develop Al tolerant maize inbred lines for a maize breeding program in Kenya, develop single cross hybrids (SCHs) from some of the tolerant inbred lines and determine Al tolerance levels of the SCHs. One hundred and seventy five inbreds and 49 SCHs were developed and screened in nutrient culture containing 0 or 222 μM using Relative Net Root Growth (RNRG), hematoxylin staining (HS) and under Al saturated field conditions (44%-45.6%) at Sega and Chepkoilel. Seedling root growth was inhibited in 95% of the inbreds. F_1 hybrids obtained from inbreds varying in Al tolerance, exhibited tolerance equal to or greater than that of the more tolerant parent indicating a positive transgressive inheritance to Al toxicity. Fifty eight percent of the F_1 SCHs were heterotic for tolerance to Al toxicity. Al tolerance estimated by RNRG was well correlated to that of HS (r^2 = 0.88, P < 0.005) but minimally correlated with the field estimates (r^2 = 0.24-0.35), implying that RNRG can predict field selection under Al toxic soils by between 24% and 35%. Plant breeders should therefore employ both approaches in selecting cultivars under Al stress. This study has developed and identified Al tolerant inbreds and SCHs for use in the acid soils of Kenya and similar regions.

Key words: Maize, inbred lines, hybrids, heterosis, aluminium toxicity, acid soils.

1. Introduction

Aluminium (Al) toxicity and low available P are some of the most limiting plant growth factors on most acid soils worldwide [1]. Highly weathered acid soils occupy 40% of the world's arable soils [2]. They are found mainly in South America (26.7%), North America (19.4%), Africa (19.1%) and Asia (15.1%). The rest occur in Australia and New Zealand, Europe and Central America [3]. On highly acidic soils, (pH < 5.5), the rhizotoxic aluminum species, Al^{3+} is solubilized, inhibiting root growth and function in the majority of crops [4]. Al toxicity limits plant growth mainly through its adverse effects on root growth and development [5]. In addition, it increases drought susceptibility and limits plant access to subsoil nutrients, which restricts the full expression of the genetic potential of the plant [6]. According to Giller et al. [7], Al toxicity reduces the agronomic and recovery efficiencies of nutrients such as P by plants. As a result, crops grown in tropical acid soils with high Al toxicity can only recover and utilize between 10% and 25% of the P fertilizer applied due to its high fixations by Al and Fe oxides [8]. The level of Al saturation in Kenyan acid soils ranges between 20% and 45% which is too high for most crop species to tolerate [9]. According to these authors, most

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improved maize varieties and landraces grown by farmers are sensitive to high Al saturation (> 20%) commonly found in most maize growing areas in the region. This implies that such germplasm are unable to efficiently utilize the native soil phosphorus (P) or added P fertilizer as a result of reduction in root growth due to Al toxicity [10]. Moreover, these farmers incur up to 16.8% grain yield loss due to Al toxicity [11]. Acid soils cover over 13% of maize growing areas in Kenya [12]. In these areas (especially the marginal rainfall medium altitude areas), maize yields are very low, with averages of 1.0-1.5 t ha^{-1} compared to the research potential of over 5.0 t ha^{-1} in the same regions [13]. Al toxicity is partly responsible for the declining yields.

Conventionally, acid soils are mainly managed by liming the top soil layer to neutralize the exchangeable Al [14]. Besides, the use of lime is highly recommended for the management of acid soils in Kenya [12]. Lime reduces the levels of exchangeable Al^{3+}, Fe^{3+} and Mn^{4+} in acid soils and thus reduces P sorption. This makes both the native soil P and applied P fertilizers available for plant uptake [15]. Besides, lime is known to have longer residual effects on acid soils compared to other soil amendments such as organic and inorganic materials [16]. However, the adoption of such input technologies has largely been restricted to large scale farmers who can afford them despite the fact that such technologies would be best suitable for low input agriculture practiced by small scale farmers in the maize ecosystems of Kenya. For example, most resource-poor small holder farmers, who are also the majority in the acid soil areas of Kenya where maize is grown, have hardly adopted such technologies due to lack of credit and the relative high cost [17]. The two main sources of lime in Kenya (Homa and Athi lime) are located approximately 250 km away from the major maize growing regions in the country, where Al toxicity is a problem. This makes it expensive to transport the large tonnage of lime needed to mitigate Al toxicity in these regions. Furthermore, the few farmers who apply lime do not apply the recommended rates; hence this approach has been ineffective in managing Al toxicity in these regions [13].

There is therefore a challenge and need for alternative, affordable and integrated approaches in the management of the problem of Al toxicity in order to increase maize productivity among the small holder farmers in the marginalised areas of Western Kenya. Selection, development and utilization of Al-tolerant maize genotypes, together with minimal inputs, are proposed as potentially sustainable and viable options for managing Al toxicity in such regions.

Screening of maize genotypes in nutrient solution using Relative Net Root Growth (RNRG) and hematoxylin staining (HS) has been successful over the past decade in selecting Al tolerant and sensitive genotypes [18-20]. Root staining with hematoxylin solution is a quick, rapid, efficient and reliable method of discerning among Al-tolerant and Al-sensitive maize genotypes since it is highly specific to Al accumulation [20]. The method allows for rapid evaluation of a large number of genotypes without destroying the root apical meristem [21]. Besides, field screening is one of the most direct screening methods for tolerance to Al toxicity in cereals as it allows a direct measurement of tolerance [22]. Accordingly, this study adopted these approaches in assessing various maize germplasm for tolerance to Al toxicity.

Genetic variation for aluminum (Al) tolerance in crop species can allow the development of cultivars that can give high yields when grown on acidic soils with high Al toxicity problems. In fact, such traits have been used to develop high-yielding, Al-tolerant maize hybrids for use in acid soils [23]. Kenyan farmers who grow maize on Al toxic soils do not yet have access to such cultivars. Earlier screening of Kenyan maize germplasm for Al toxicity showed that some of the Kenyan landraces are tolerant [24]. This study focussed on: developing maize inbred lines from

various sources including landraces and Brazilian introductions which contained CATETO (Al-tolerant Brazilian inbred line); selecting some of the inbred lines for tolerance to Al toxicity; using them to develop single crosses and testing the Al-tolerance in the single crosses.

2. Materials and Methods

2.1 Genetic Materials Used

Maize germplasm used in this study were developed from various sources: Kenya Agricultural Research Institute (KARI)-Kitale, KARI-Kakamega and KARI-Muguga. Others were Brazilian introductions to Kenya (single crosses) and derivatives of CATETO (Brazilian most Al tolerant inbred line) while the rest were local collections including Al tolerant 203B landrace, collected from Al toxic soils of Muranga county in central Kenya. All the sources were obtained in the year 2002 and were used to develop 175 inbred lines between the year 2003 and 2007 (Table 1). The inbred lines were either developed from single cross hybrids from the various sources or from topcrosses of these single cross hybrids crossed with the Kenyan testers for medium and high altitude. All the sources were individually selfed to F_6 to obtain the respective inbred lines which were screened for tolerance to Al toxicity in nutrient culture solution according to Magnavaca et al. [18] and also under field conditions (0 t ha^{-1} and 4 t ha^{-1} of lime).

Fourteen inbred lines were selected for tolerance to Al toxicity based on relative net root growth (RNRG), hematoxylin staining (HS) and grain yield at high Al saturation (43.1%-45%) (data not shown). The single cross hybrids were then generated in 2009 by crossing the selected Al tolerant inbred lines using North Caroline II mating design as described by Comstock and Robinson [25]. A total of 49 single crosses were developed. One of the single crosses, however, did not yield enough seeds and was therefore not included in the screening work. Forty-eight single cross hybrids and one commercial variety grown under Al toxic soils of Western Kenya (HD614) were therefore tested for tolerance to Al toxicity in nutrient solution culture. CON 5, 203B and K4 were used as Al tolerant checks while SCH 3 and REGNUR 0114 were used as susceptible checks [11].

2.2 Description of Experimental Sites

Chepkoilel site is located at 0°34′37.24″N; 35°15′10.04″E, 2,143 m above sea level (a.s.l), and has between 900 and 1,100 mm rainfall with a 10-26 °C temperature range. The soils are chromic ferralsols characterized by low pH 4.8, and Al saturation of 45.6% with P levels of 4.4 mg P kg^{-1} of soil [13]. Sega site is located at 0°15′N and 34°20′E. It has an elevation of between 1,140 and 1,400 m (a.s.l) with a bimodal annual average rainfall pattern of between 800 and 1,200 mm. The mean minimum temperature ranges between 15 and 17 °C, while the mean maximum range is 27-30 °C. The soils are OrthicAcrisols characterized by low pH 4.5 and a mean Al saturation of 43.1% and 2.2 mg P kg^{-1} of soil [13].

2.3 Experimental Design and Procedures

Seeds of each line were surface sterilized in 1% sodium hypochlorite and rinsed thoroughly with sterile

Table 1 Description of maize inbred lines used as parents of the single cross hybrids.

Original source of germplasm	No. of inbred lines developed from various sources
Brazilian single crosses	95
Landrace (203B)	34
KARI-Muguga lines	18
KARI-Kakamega lines	14
KARI-Kitale lines	14
Al standards from Kenya and Brazil	5

distilled water to remove all traces of the hypochlorite. The seeds were set to germinate inside paper rolls moistened with aerated distilled water. These were placed vertically on plastic trays covered with aluminium foil, which were incubated in darkness for three days in a growth chamber set at 26 ± 3 °C. The experiment was conducted at the Botany laboratory in Chepkoilel University College. The setup was a completely randomized design (CRD) replicated three times. Treatments consisted of single cross maize hybrids (49) or inbred lines (175) and two levels of Al (0 µM or 222 µM Al). Eight litre trays were used to hold nutrient solution under continuous aeration.

The nutrient solution was prepared according to Magnavaca et al. [18]. Three days old uniform-sized seedlings with no visible injury or damage on their roots were transferred to the cups on a perforated styrofoam sheet and stabilized for 24 h in nutrient solution without added Al at pH 4.0 after which the Initial root length (IRL) was measured. The seedlings were then transferred to fresh nutrient solution where Al was added to the trays as Al K $(SO_4)_2$ $12H_2O$ to attain the stated concentration which corresponds to free Al^{3+} µM activities of (0) and (39) respectively [26]. The seedlings were then grown in a growth chamber at a photoperiod of 14 h of light and 10 h of darkness. The day length growth room conditions were approximately 340 µmol photons m^{-2} s^{-1} of light intensity, 30 ± 2 °C and 70% relative humidity; the dark conditions were 22 ± 2 °C and 90% relative air humidity.

Seventy two hours after transplanting, final seminal root length (FSRL) was measured and the net seminal root length (NSRL) calculated from the difference between FSRL and initial seminal root length ISRL [18]. The tolerance level was assessed using relative net root growth (RNRG), where,

$$\text{RNRG} = \text{NSRL under Al treatment/NSRL under control} \times 100 \quad (1)$$

The heterosis for the F1 single crosses was calculated using both mid-parent heterosis (MPh) and high parent heterosis (HPh) for comparison [27]. The two indices were expressed in percentages as:

$$MP\% = \frac{F1 - M}{MP} \times 100 \quad (2)$$

$$HP = \frac{F1 - HP}{HP} \times 100 \quad (3)$$

Where, F1 = performance of hybrid, MP = average performance of both parents and HP = performance of high parent.

Hematoxylin staining was used as a confirmatory test for tolerance to Al toxicity in selecting the Al tolerant inbred lines. The seedlings of 20 selected (tolerant, moderately tolerant and sensitive) maize inbred lines were subjected to hematoxylin staining as described by Cancado et al. [20]. Visual scores for root staining intensity were made on a scale of 1-5, as follows: non-stained roots were classified as very tolerant (Scale 1), faintly stained roots as tolerant (Scale 2), moderately stained roots as moderately tolerant (Scale 3), well stained roots as sensitive (Scale 4) and those with deeply stained roots as very sensitive (Scale 5) [20].

The experiment for screening inbred lines for tolerance to Al toxicity under field conditions was set up in a randomized complete block design (RCBD) with 4 treatments in 3 replications at 2 sites. Some plots received phosphorus (P) and lime (L) (P + L); while others received either P (+P) or L (+L). The control plot received neither P nor L. Phosphorus was applied as triple super phosphate (TSP) at the rate of 26 kg P ha^{-1}. Agricultural lime from Koru liming company in Kisumu containing approximately 21% CaO was applied 2 months before planting at the rate of 4 t ha^{-1}. CaO in the plots was to receive lime at each site as recommended by Kisinyo et al. [13]. Planting was done in March 2010 at Chepkoilel and Sega sites at a spacing of 0.75 m between the rows and 0.3 m within the row in a 3 m long plot comprising 2 rows each. Nitrogen was used in top dressing six weeks after planting on all the plots in the form of calcium ammonium nitrate (CAN) at the rate of 75 kg N ha^{-1}. Weeding was done manually thrice and the crop

protected from stalk borer (*Buseola fusca* L.) damage using 2-3 granules of Beta-cyhalothrin (Bulldock GR 0.05) at a rate of 6 kg ha^{-1} applied in the whorl of each plant after thinning. Data was recorded on grain yield (t ha^{-1}) plant height (cm), ear height (cm), days to 50% tasseling and days to 50% silking.

2.4 Statistical Analysis

The RNRG and hematoxylin staining data was subjected to 1-way analysis of variance using the General Linear Models procedure of Genstat and means compared using Tukey's range test using the following model:

$$X_{ijk} = \mu + \alpha_i + \Sigma_{ij} \quad (4)$$

Where, X_{ijk}: plot observation, μ: overall mean; α_i: treatment effect; Σi: experimental error due to treatments [28, 29]. Grain yield and yield component data were subjected to 2-way analysis of variance by fitting the following model:

$$X_{ijk} = \mu + \alpha_i + \beta_j + \Sigma_{ij} \quad (29)$$

Where, X_{ijk}: plot observation, μ: overall mean; αi: treatment effect; β_j: block effect; Σ_{ij}: experimental error due to treatments and blocks [30].

Phenotypic correlation between RNRG and hematoxylin staining and between RNRG and grain yield were computed by regression and correlation analysis, using Genstat software (Payne et al., 2009). The regression and correlation were analyzed based on the model:

$$Y_i = \beta_o + \beta_i X_i + \Sigma_i \quad (6)$$

where, Y_i: *the ith observation of the response Y;* β_o: *population parameter giving the intercept;* β_1: population parameter giving the slope; Σ_i: error term. Correlation coefficient *r* was calculated using the equation:

$$r = \frac{COV(X,Y)}{S_X\, S_Y} \quad (7)$$

where, COV (*X*, *Y*): Covariance *X* (predictor) and *Y* = predicted parameter, S_x: standard deviation of the predictor parameter; S_y: standard deviation of the predicted parameter [31].

3. Results and Discussion

3.1 Phenotypic Variation for Tolerance to Aluminium Toxicity among the Inbred Lines

Significant phenotypic variation ($P > 0.05$) in tolerance to Al toxicity was observed among the inbred lines based on an Al tolerance threshold of 50% RNRG (Figs. 1 and 2). Root growth inhibition occurred in 95% of the inbred lines. However, root growth in nine tolerant inbred lines (203B, 203B-14, CATAL 237/67X63-5, CON 5, HASR, 203B-30, HS 53x280-16, HS 26x294-6 and 203B-15) remained unaffected after exposure to 39 μM Al^{3+} (Fig. 2). Similar observations were reported in *Sesbania* (*Sesbania sesban* (L.) *Merr, sorghum* (*Sorghum bicolor* (L.) Moench and in maize but at lower concentrations of between 148 and 200 μM [32, 33]. Such resistance is partly a result of maintaining cell wall and plasma membrane integrity [34]. Landrace 203B which was used as one of the tolerant standards (Fig. 3a) had the highest root growth followed by some of its derivatives, such as 203B-14 and 203B-39. However, other inbred lines derived from the same landrace (203B-25 and 203B-28) were among the most Al sensitive lines. These results imply that these lines could have initially received pollen from other Al sensitive lines owing to the out crossing nature of maize since the starting material was an open pollinated variety (OPV) and hence such segregants could have emerged. The 203B landrace and its inbred lines remains an invaluable source of Al tolerance which can be exploited in production of acid tolerant maize varieties.

CON 5, which was used as another Al tolerant standard, expressed a RNRG of 105% under similar conditions compared to 203B, 203B-14 and others. CON 5 is an elite homogenous population from KARI which has been classified as Al tolerant [23]. A study by this author indicated that 55% of tolerance to Al toxicity in CON 5 is attributed to exclusion of Al from the root tips owing to the activity of ZmMATE1 gene. The highly tolerant CATAL 237/67XL3-5 is a

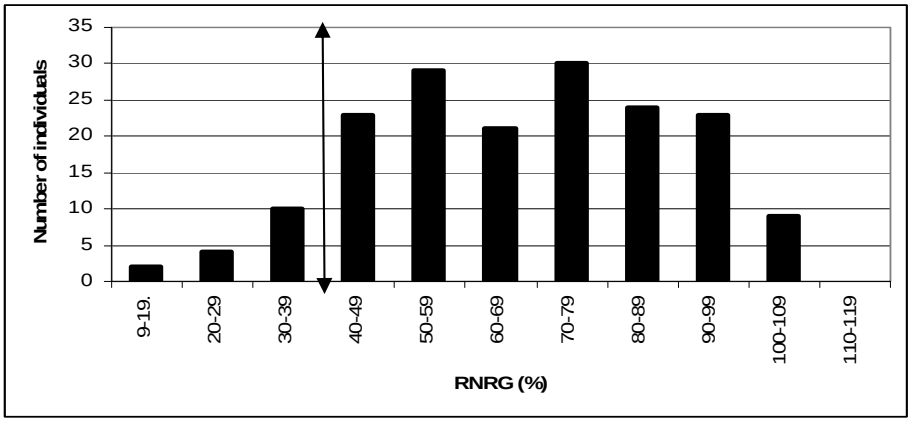


Fig. 1 Percent of relative net root growth (RNRG) frequency distribution for maize inbred lines. The double arrowed line depicts the threshold for Al sensitivity (RNRG < 50%) and tolerance (RNRG > 50%). 175 maize inbred lines were grown in nutrient solution containing µM Al^{3+} for three days.

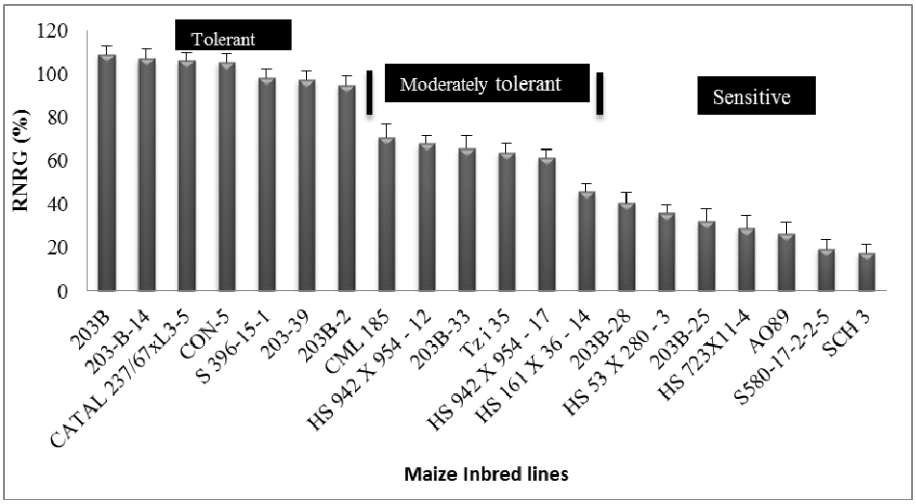


Fig. 2 Relative net root growth of selected 20 inbred lines after 3 days of exposure to Al treatment. Percent of relative net root growth (RNRG) values are the means of three replications (seven plants per replication). The error bars are standard error bars (SE). Selection was based on clustering of the means of 175 inbred lines into three homogenous categories; the inbreds therefore represented each of the categories.

(a) 203B inbred line (b) SCH3 inbred line

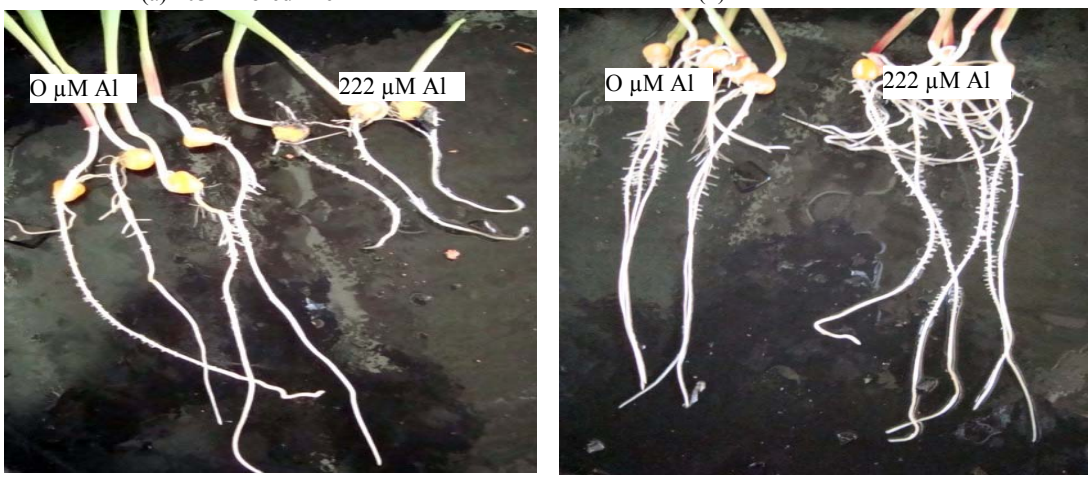


Fig. 3 a, b: Root growth response to Al stress by inbred line 203B and sensitive inbred line SCH3.

derivative of CATETO, the Brazilian Al tolerant standard. Studies have shown that CATETO has high expression of ZmMATE1, the Al tolerance gene [23]. This suggests that CATAL 237/67XL3-5 may be using a similar Al tolerance mechanism as CATETO. Studies on CATETO have indicated that two genes (ZmMATE1 and ZmMATE2) co-localize to major Al tolerance Quantitative Trait Loci (QTLs) in maize [23]. As to whether the Al tolerance in 203B, CON5 and K4 is as a result of ZmMATE allele or a separate gene is yet to be determined. Interestingly, studies by Matonyei [23] showed that CON 5, 203B and some of its derivatives were apparently more tolerant than CATETO, even though, they expressed lower ZmMATE1 activity than the latter. These findings clearly point to the possibility that the Kenyan sources could have a different gene in play. The least root growth response (17%) was observed in inbred line SCH3 (Al sensitive line from Brazil) (Fig. 3b) as expected.

3.2 Variations in Staining Rate of Hematoxylin in Maize Inbred Lines

The inbred lines differed significantly with regard to hematoxylin staining adsorption when subjected to Al stress. Al tolerant lines had lower adsorption rate (< 3) compared to the sensitive ones (≥ 4). The very sensitive line, A089, showed an intense dark-blue coloration indicating deeply stained roots, the sensitive line REGNUR 00114 showed blue coloration in the roots indicating well stained roots, while the tolerant line CATAL 237/67XL3-5 showed clear root apices, i.e., non-stained roots (Figs. 4a and b). These findings compare well with previous observations in pea roots [36], maize roots [20, 21] and in rice [37]. According to these authors, the sensitive lines tend to accumulate more Al in their root tips, hence adsorbing more hematoxylin stain. These results into the blue coloration compared to the tolerant lines which do not bind the hematoxylin stain and exclude Al from the cells.

The correlation between RNRG and hematoxylin staining showed a negative trend (Fig. 5) probably because sensitive seedlings have low RNRG as a result of high quantities of accumulated aluminium in the root cap and, therefore, they normally show high hematoxylin adsorption rate. The tolerant genotypes have some mechanisms to avoid aluminium toxicity, therefore, they express higher RNRG and lower hematoxylin absorption rate. These findings are in agreement with those of Cancado et al. [20] who reported a strong negative correlation (r = -0.693 and -0.816) between hematoxylin absorption rate (HS), NSRL and RNRG, respectively.

A regression analysis of RNRG on the hematoxylin adsorption rate indicated that 88% of all the observed variance in tolerance could be explained by hematoxylin adsorption rate. Therefore, the colouration of the root apices with hematoxylin can be employed, without restriction as an informative index of Al tolerance.

3.3 Performance of Inbred Lines under Field Condition and Correlations with Al Screening Data

At Sega, under control (No P, No L), the inbred lines produced grain yields of between 0 and 2.4 t ha^{-1}. However, with the addition of lime (4 t ha^{-1}), the grain yield increased to between 0.4 and 3.9 t ha^{-1}. Under control (no P, no L), majority of the inbred lines (70%) expressed grain yields of between 0.0 and 0.9 t ha^{-1} while the rest yielded between 1.0 and 2.4 t ha^{-1} (Figs. 6 and 7).

Regression of grain yield under additional phosphorus in Al toxic soils on percent RNRG showed positive, but non-significant trend $P \leq 0.05$ with coefficient of determination (R^2 = 0.24 and 0.35) for Sega and Chepkoilel sites, respectively (Fig. 8). However, regression of grain yields under control on percent RNRG also showed positive trend with lower R^2 values (R^2 = 0.11 and 0.30) for Sega and Chepkoilel sites respectively (data not shown). This showed the extent of amelioration effects of additional P on

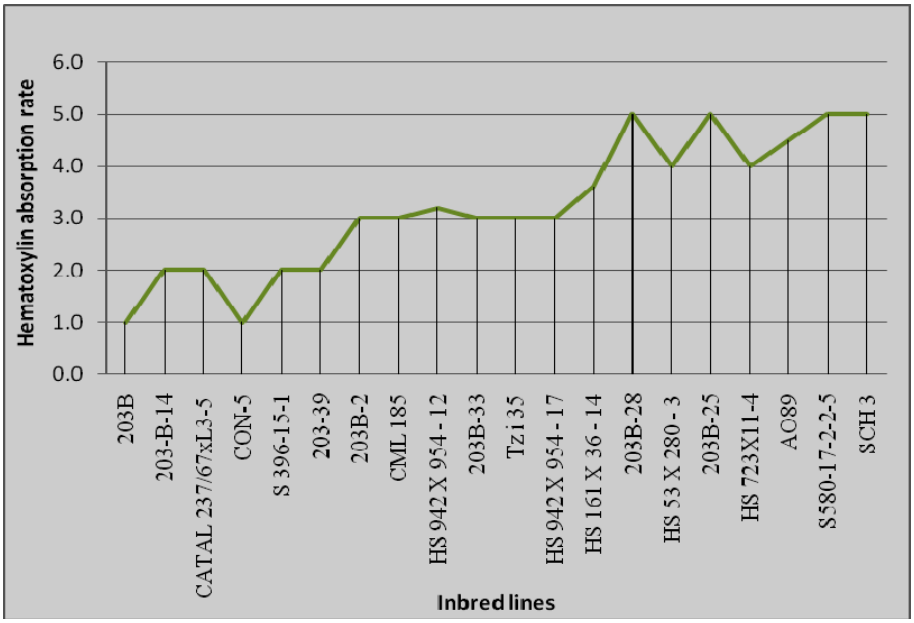


Fig. 4a Mean hematoxylin staining (Hs) values of selected 20 maize inbred lines.

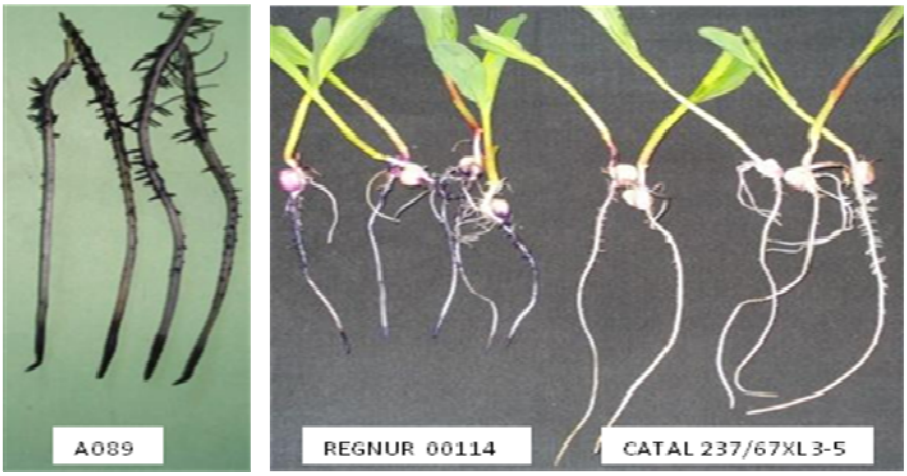


Fig. 4b Maize seedling root apices stained with hematoxylin stain after a 72 h exposure to 222 µM Al in nutrient solution: CATAL 237/67XL3-5—tolerant; REG NUR 00114—Sensitive; A089—Very sensitive.

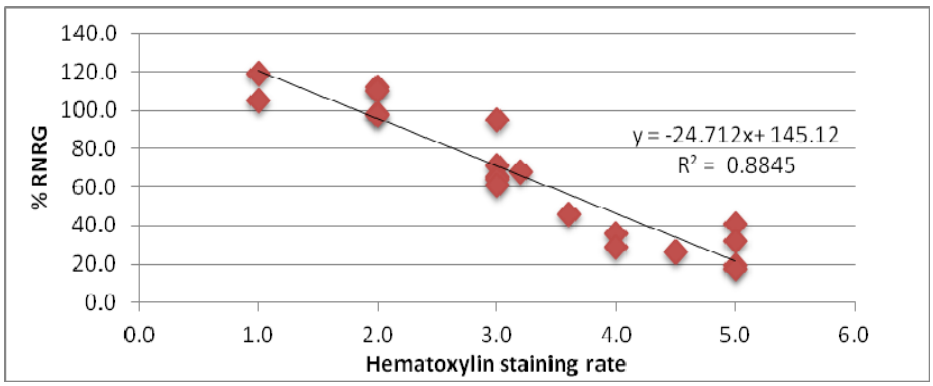


Fig. 5 Relationship between RNRG and hematoxylin staining of selected inbred lines after exposure to Al containing 222 µM concentration for 3 days.

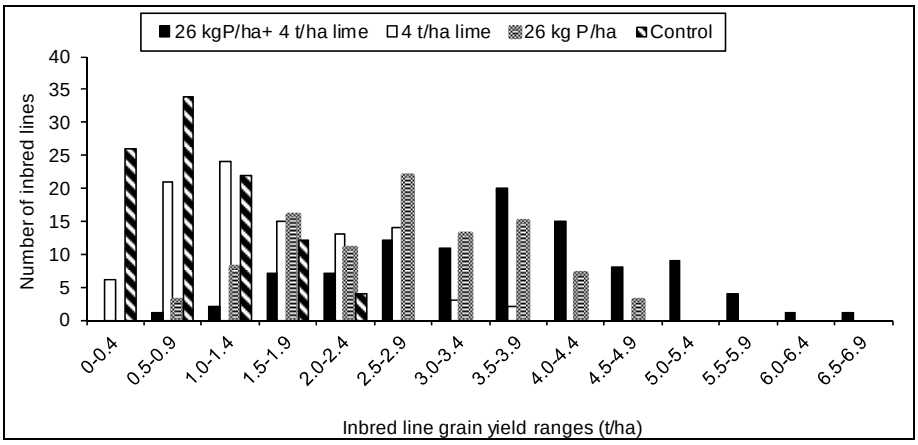
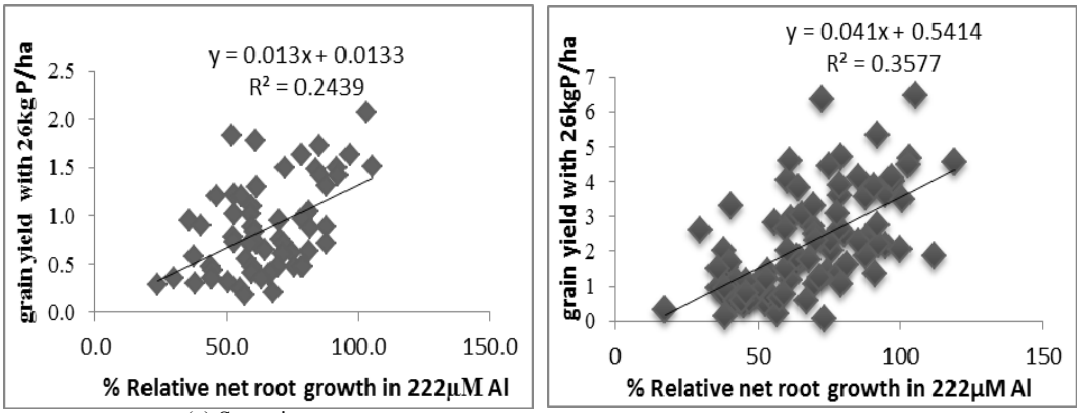


Fig. 6 Trends in grain yield of maize inbred lines screened in Aluminium toxic soils at Sega.

Control +Lime + P +L+P

Fig. 7 Effects of various treatments on maize growth at Sega site during the long rains of 2010.



(a) Sega site (b) Chepkoilel site

Fig. 8 Relationship between grain yield with additional P in the field (26 kg P ha^{-1}) and RNRG of maize inbred lines grown with P in nutrient solution under Al stress (222 µM Al).

tolerance to Al toxicity under field conditions. It also showed that solution culture screening could predict the response of maize cultivars when tested under Al toxic soils culture by up to 35%, although this would depend on available P and percent Al saturation in the soil. These findings imply that plant breeders should employ an integrated approach of using both solution culture and field screening conditions when selecting cultivars for tolerance to Al toxicity. The low correlation between solution culture screening and field screening could be due to higher interaction of Al and P in nutrient solution since Al imposed in nutrient solution, was higher than that found naturally under field conditions.

These findings compareed well with those of Liao et al. [36] who reported that P-efficient genotypes were more Al tolerant than P-inefficient genotypes. These authors suggested that P could help ameliorate

Al toxicity through Al complexation and possible precipitation of Al in the rhizosphere, in addition to the Al-P interactions in the root apoplast.

The coefficient of determination (R^2 = 24%) observed in Sega was much lower than the one observed at Chepkoilel (R^2 = 35%) probably because of the lower available soil P levels at Sega (2.2 mg P kg^{-1} of soil) compared to Chepkoilel (4.4 mg P kg^{-1} of soil).

3.4 Phenotypic Variation for Tolerance to Aluminium Toxicity among Single Crosses

The phenotypic expression of NSRL and RNRG showed transgressive inheritance. The F1s showed positive, negative and no heterosis (Table 2). Most of the F1s (58%) were more tolerant to Al toxicity than either of their parents (Table 3). This can be attributed to heterosis for RNRG in which the hybrid F_1 exhibited a RNRG that is superior to the means of the two parents (mid-parent heterosis), or the better of the two parents (better/high-parent heterosis) [39]. The genetic basis of heterosis includes dominance, over dominance or epistatic gene effects [40].

The remaining 42% of the single crosses were not heterotic for RNRG. This observation could have been due to negative transgressive inheritance where the offspring performed worse than both parents. HD614, a Kenyan commercial variety bred for high altitude areas, was found to be among the moderately tolerant accessions; however, 32% of the single crosses developed were more tolerant than this variety. The great genetic potential for Al tolerance expressed in the F_1 single crosses could be exploited further to develop varieties (Double crosses, 3-way crosses and synthetics) with tolerance to Al toxicity. These may be more attractive to farmers growing maize in the acid soil regions of Kenya. Fig. 9 shows root growth response of selected single cross maize hybrids in Al stress.

Table 2 Mid-parent and high-parent heterosis of selected F1 single cross maize hybrids tested for tolerance to Al toxicity in nutrient solution.

F1 Single crosses	NRL 0 μM Al		NRL 222 μM Al		RNRG	
	MPh (%)	HPh (%)	MPh (%)	HPh (%)	MPh (%)	HPh (%)
KML 036 × MUL 863	13.85	-15	134	92.7	110.8	90.1
S596-41-2-2 × REG 007-361	-3.8	10.6	57.5	29.9	63.2	37.9
KML 036 × S396-15-1	-40	-43.2	-2.1	-20.6	55.9	19.4
MUL 863 × MUL 1007	72	32.8	115.4	67.7	50	35.4
MUL 125 × POOLB 26-1	-47.6	-64.5	36	-56.6	39.6	24.6
MUL 817 × MUL 863	134	87	100	83.4	34	26.7
MUL 817 × MUL 216	51	15.8	101.27	80.5	33.3	14.2
MUL 817 × MULX125	4.5	-27.2	8.8	-27.3	23.3	13.8
MUL 822 × S558-2-2-3-7	19.5	-13.4	-1.7	-4.4	23	14.2
CML 181 × MUL 817	116.4	108	146.9	134.1	18.75	5.5
MUL 216 × CML 202	44.9	-18.4	35	14.2	14	-8
KML 026 × MUL817	219	183.2	237	209.5	2.8	-15.2
MUL 125 × MUL 863	23.4	-18.3	7.5	-31	-0.8	-12.3
REG N007-361 × MUL 817	110.8	100	102.2	93.5	-7	-8.6
MUL 116 × MUL 104	-12.6	-14.2	-4.3	-13.9	7.6	5.6
CML 181 × REG N007-361	79.6	65.5	90.7	73.5	-10.7	-19.4
POOL B26-1 × MUL 817	65.3	35.8	27.2	12.9	-22	-25
POOL A6-1 × CML 202	108.3	95.2	67.2	54.5	-22.5	-22.5
MUL 817 × S558-2-2-3-7	88.6	69.9	0	-9.3	-41	-47

RNRG: Relative net root growth, NSRL 222 μM Al Net seminal root length in Al at 222 μM concentration; NRL—0μM A Net seminal root length at no Al; MP%—Percent mid-parent heterosis; HP%—Percent high parent heterosis.

Table 3 Means for net root lengths, relative net root growth, root reduction and Al tolerance status of selected maize single crosses and their parents.

Single crosses and Parents	Net root Length 0 μM	Net root Length 222 μM	Relative Net root Growth	Percent Root Reduction	Al Status
KML 036 × MUL 863	37.8a-h	34.7j-o	0.97g	3.2a	T
KML 036 × S396-15-1	25.0a-c	23b-m	0.92fg	7.6 ab	T
KML O26	20.2a	16.7a-h	0.85e-g	14.6a-c	T
MUL 863 × MUL 1007	53.1e-j	34.9k-o	0.84d-g	15.7a-d	T
MUL 125 × POOLB 26-1	23.8a-c	17.8a-i	0.81c-g	19.3a-e	T
MUL 817 × MUL × 125	48.8a-i	30.1g-n	0.74b-g	25.9a-f	T
S558-27-2-1	29.4a-f	17.1a-i	0.68a-g	32.2 a-g	MT
MUL 125	67.1h-j	41.4n-p	0.65a-g	34.5 a-g	MT
MUL 817 × MUL 216	57.2e-i	31.6i-o	0.64a-g	35.8 a-g	MT
MUL 1007	39.9a-h	20.8a-k	0.62a-g	37.8 a-g	MT
CML 202	23.7a-c	12.1a-d	0.62a-g	38.4 a-g	MT
MUL 822	33.4a-g	18a-i	0.6a-g	39.5 a-g	MT
POOL A6-1	27.1a-d	14.3a-f	0.6a-g	39.7 a-g	MT
REG 007-361	23.7a-c	12.7a-e	0.58a-g	41.6 a-g	MT
CML 181 × REG N007-361	47.6a-i	26.9e-n	0.58a-g	41.9 a-g	MT
MUL 817 × REG 007-361	52.6c-i	28.7f-n	0.58a-g	42.3 a-g	MT
MUL 216 × CML 202	40.3a-h	20a-j	0.57a-g	43.1 a-g	MT
MUL 125 × MUL 863	54.8d-i	28.5f-n	0.57a-g	43.1 a-g	MT
MUL 817	26.3a-d	13.9a-f	0.56a-g	43.6 a-g	MT
MUL 116 × MUL 104	44a-h	22.8b-l	0.56a-g	44.4 a-g	MT
MUL 125 × MUL 1007	57.3e-j	28.2f-n	0.55a-g	44.8 a-g	MT
MUL 228 × MUL 216	64.3h-j	33.2j-o	0.53a-f	47.1 b-g	MT
MUL 116	49.6a-i	21.2a-k	0.53a-f	47.1 b-g	MT
REG N007-361 × MUL 817	52.7c-i	26.9e-n	0.53a-f	47.4 b-g	MT
POOL B26-1	37.4a-h	14.4a-f	0.52a-f	47.9 b-g	MT
POOL A6-1 × CML 202	52.9c-i	22.1b-l	0.48a-e	52.2 b-g	S
S558-2-2-1-4	87.1j	37.8m-p	0.43a-d	57.1 c-g	S
POOL B26 - 1 × MUL 817	50.9b-i	20.1a-k	0.42a-d	57.9 d-g	S
KML 036	44.3a-h	18.3a-k	0.42a-c	58.4 e-g	S
S596-41-2-2	25.3a-d	8.5ab	0.41a-c	59.2 e-g	S
MUL 216	49.4a-i	17.5a-i	0.41a-c	59.2 e-g	S
POOL A6-1 × S558-2-2-1-4	49.6a-i	17.9a-i	0.39a-c	61.1 e-g	S
MUL 817 × S558-2-2-3-7	48.1a-i	15.5a-g	0.37ab	63.3fg	S
REG NUR-00114	23.5a-c	7a	0.32a	68.3g	S
Grand mean	42.6	23.3	0.62	38	S
SE	0.8	0.4	0.01	1.1	

Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukeys range test. T—tolerant to Al toxicity; MT—medium tolerant to Al toxicity; S—sensitive to Al toxicity

4. Conclusions

There is a wide variation for tolerance to Al toxicity among the inbreds and the single crosses. Using this variation, this study has developed both Al tolerant inbred lines and single crosses from diverse sources. Nutrient culture screening for Al toxicity can predict field selection under Al toxic soils by between 24%-35% depending on the Al saturation of the particular soil and the levels of available phosphorus. This implies that plant breeders should employ an integrated approach of using both solution culture and

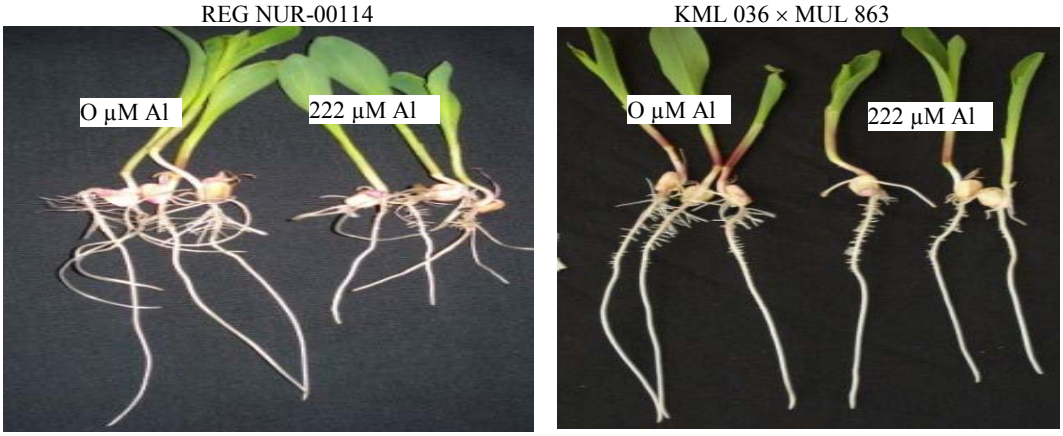


Fig. 9 Root growth response to Al stress by the sensitive Al standard (REG NUR-00114) and the most tolerant Single cross (KML 036 × MUL 863).

field screening conditions when selecting cultivars for tolerance to Al toxicity. Some of the Kenyan inbreds identified in this study were more tolerant than the inbreds derived from CATETO. These include 203B and some of its derivatives which remain the most Al tolerant genotype among Kenyan maize germplasm. Additionally, some of the single cross hybrids identified in this study showed superior tolerance to Al toxicity and could be used directly or as parental material for future hybrids for acid soils. They include: KML 036 × MUL 863, KML 036 × S396-15-1, MUL 863 × MUL 1007, MUL 125 × POOLB 26-1, MUL 817 × MUL × 125.

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