BIODIVERSITAS
Volume 24, Number 3, March 2023
Pages: 1417-1430

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240311

Evaluation of the growth and tolerance of maize lines under aluminum stress

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Manuscript received: 8 September 2022. Revision accepted: 4 February 2023.

Abstract. *Herawati, Riadi M, Musa Y, Efendi R, Azrai M. 2023. Evaluation of the growth and tolerance of maize lines under aluminum stress. Biodiversitas 24: 1417-1430.* Breeding and utilization of Al-tolerant maize are among the technological applications for preventing a decrease in the growth rate and grain yield of maize in suboptimal fields. This study aimed to determine the growth and production of maize lines under Al stress, obtain maize lines that are tolerant to Al stress, and determine Al absorption in the tested maize lines. This study comprised two steps. In the first step, maize lines were evaluated in acid soils with a pH of 5.12 and an Al saturation of around 22.13%, while the normal condition was carried out on soil with a pH of 6.60 and 0% Al saturation. The study was arranged in a randomized block with three replications. The maize lines used were 24 genotypes consisting of 20 maize lines and four elite lines as a check (Mal 03, CLYN 231, G102612, B11209). In the second step, maize lines were evaluated in the nutrient solution. This study was arranged in a split-plot design with three replications. The main plot was the concentration of Al at three levels, i.e., 0, 7, and 14 ppm. The subplot comprised seven maize lines representing sensitive, medium tolerant, and tolerant categories. They were Pop. A3-1, Pop. A7-1, CB.Pop 10-1-3-1-2-2, CB.Pop 15-4-2-1-1-1, CB.Pop 23-1-2-1-4-2, Mal 03, and G102612. These studies were conducted at the Indonesian Cereal Research Institute, Maros, South Sulawesi, Indonesia, from August 2021 to January 2022. This study showed that Al stress could reduce plant growth by around 10.84% to 50.51% and grain yield by around 62.23% compared to normal conditions. The traits that were significantly correlated with grain yield under Al stress (r>50%), which could be used in maize selection under Al stress, were the number of live plants, ear diameter, number of kernels per row, shelling percentage, SSI, crown Al absorption, and hematoxylin staining. There were two maize lines tolerant to Al stress, namely CB.Pop 11-2-3-4-2-1 and CB.Pop 15-4-2-1-1-1, with potential yields under Al stress conditions of 2.39 t ha^{-1} and 1.59 t ha^{-1}, respectively. Al absorption averages of roots and crowns of Al tolerant maize lines were 159.33 ppm and 36.33 ppm, respectively.

Keywords: Aluminum stress, growth rate, maize line, tolerance index

INTRODUCTION

Maize (*Zea mays* L.) is a food commodity with a high economic value that can stimulate the growth of the national economy. Maize contains a high level of carbohydrates, namely around 63.03-69.36%. It also contains around 1-3% glucose, sucrose, and fructose, meaning it can be used as a food substitute for rice (Bruyn et al. 2002; Khan et al. 2014; Ely et al. 2016). Aside from being a foodstuff, maize is used in animal feed and industrial raw materials (Khan 2016; Gomez et al. 2017). In 2019, the total demand for maize was 16.64 million tons, which increased by around 1.29% to 16.85 million tons in 2020. In 2021, the demand for maize was expected to increase by about 2.24% from 2020 to 17.23 million tons (PUSDATIN Kementerian Pertanian 2020). Therefore, the demand for maize continues to increase annually, encouraging maize development activities in every region of Indonesia.

The conversion of agricultural land into areas for housing and industry encourages the use of marginal lands, such as acid soil, for maize development. A total of 146.46 million ha (BBSDLP 2017) in Indonesia are classified as acid land. Acid soil poses a major problem to crop production in tropical areas due to its low fertility stemming from poor physical, chemical, and biological properties (Ikiriko et al. 2016). Ultisol and oxisol are soils with an acidic pH. These two soils are thus characterized by an acidic pH (4.6-5.5) and low soil fertility, high Al saturation (22.63-75.64%), high clay content, low organic soil content (OC<2%), low cation exchange, and relatively low availability of macro and micronutrients (Yulnafatmawita and Adrinal 2014; Taisa et al. 2019; Lee et al. 2021). The low soil pH in ultisol and oxisol soils can reduce the efficiency of nitrogen use and nitrogen accumulation in maize (Shibata et al. 2017; Pan et al. 2020). The result is lower productivity for maize grown in acid soils with high Al saturation. Acidic soils are characterized by excesses of H^+, Mn^{2+}, and Al^{3+} with deficiencies of Ca^{2+}, Mg^{2+}, and PO_4^{3-}. Additionally, in acidic soils, hydroxyl-rich aluminum compounds solubilize in the soil solution to an extent and Al replaces polyvalent cations such as Ca^{2+} and Mg^{2+}, while Al will bind P and Mo. The proportion of cation exchange in acid conditions increases due to Al saturation (Krstic et al. 2012; Li and Johnson 2016; Tandzi et al. 2018).

Aluminum (Al) is a chemical element that causes soil acidity. Aluminum is a rhizotomy ion that can inhibit plant growth and productivity in acidic mineral soils (Cunha et al. 2018). At 8%, aluminum is one of the most abundant elements in the earth's crust, behind oxygen and silicon, and reacts with water and air to form oxides and hydroxides (Kvande 2015; Bojórquez-Quintal et al. 2017). According to Silva (2012) and Maron et al. (2013), $Al(H_2O)_6^{3+}$ or Al^{3+} is a form of Al that is very toxic to plants. Excess Al in the soil can inhibit the growth and production of maize plants. High Al saturation (> 25-40%) can decrease the growth of maize (Krstic et al. 2012), sorghum (Too et al. 2014; de Menezes et al. 2018), and rice (Samad et al. 2020). High Al in soil acid is positively correlated with root length and root weight (Joris et al. 2013). Al toxication damages the plasmalemma of root cells and thus inhibits the absorption of nutrients and water (Rahman et al. 2018; Pidjath et al. 2021). High soil acidity in the tropics can result in a 38-80% reduction in maize yields (Tandzi et al. 2015; Tekeu et al. 2015). The potential yield of maize in acid soils with 45-70% Al saturation ranges from 1.55 to 3.76 t ha^{-1} (Evans et al. 2013; Hayati et al. 2014).

Developing soil acidity-tolerant maize with high Al saturation offers one way of increasing growth and productivity in areas with high acidity levels. Acidic soils contain several organic acids from the decomposition of organic matter. Aluminum is released into the ground through the hydrolysis of Al hydroxides, silicates, and Al bound by organic matter in acid soils (Pavlů et al. 2021). Maize that is tolerant to high Al levels can detoxify and prevent the absorption of Al from the soil, increase the pH of the rhizosphere, which reduces the availability of Al, and lower the capacity of the cell walls to bind Al^{3+}, as well as exudes the organic acids and organic phosphorus (Pattanayak and Pfukrei 2013; Xu et al. 2017).

Each genotype of maize developed on land with high Al saturation will have different agronomic traits and yield potential. Plant phenotype and production result from interactions between plants and their environment (Adnan et al. 2020; Anley et al. 2013). In addition, gene interactions with the environment can produce differences in the tolerance index of maize plants to aluminum stress. According to Coelho et al. (2019), Al inheritance in maize is determined genetically and through root growth. The tolerance of maize to Al stress is controlled by the citrate transporter encoded by ZmMATE1 and ZmMATE2 (Sun et al. 2020; Vasconcellos et al. 2021). Root length is an important trait in measuring maize tolerance to Al stress, which is influenced by the action of additive and non-additive genes (Ndeke and Tembo 2019). Al concentrations for testing the tolerance level of maize genotypes through a nutrient culture based on relative root growth ranged from 6-16 ppm (Giannakoula et al. 2010; Zishiri et al. 2022).

Maize varieties that are tolerant to Al stress are needed to overcome the problem of relatively low maize productivity in acid soils with high Al saturation. Therefore, it is necessary to evaluate the growth and tolerance of maize lines under Al stress as the genetic material for forming Al-tolerant maize varieties. This study aims to determine the growth and production of maize lines under Al stress, obtain maize lines that are tolerant to Al stress, and determine Al absorption in the tested maize lines.

MATERIALS AND METHODS

Study area

This study comprised two steps. In the first step, maize lines were evaluated in the field at the Experimental Farm of the Indonesian Cereal Research Institute, Maros, South Sulawesi, Indonesia. The Al stress was carried out on acid soils with a pH of 5.12 and an Al saturation of around 22.13%, while the normal condition was carried out on soil with a pH of 6.60 and 0% Al saturation (Table 1). Both soils are included in the alluvial soil type. In the second step, maize lines were evaluated in the nutrient solution at the screen house of the Indonesian Cereal Research Institute. These studies were conducted from August 2021 to January 2022. The study area was at an altitude of 5 m above sea level with coordinates 4º58'37"S - 119º34'33"E, and the type of rainfall was C3 according to the Oldeman climatic classification. Rainfall at the time of the study in the field was in the range of 84-542 mm (BMKG 2022).

Procedures

In the first step, the study was arranged in a randomized block design with three replications. The maize lines used were 24 genotypes consisting of 20 maize lines and four elite lines as a check (Table 2). The maize lines from the Indonesian Cereal Research Institute (ICERI), Maros, South Sulawesi, Indonesia. Each genotype was planted in two rows 4 m long with a spacing of 70 cm x 20 cm. The fertilizers used were urea at 150 kg ha^{-1} and NPK at 15:15:15 up to 350 kg ha^{-1} at the age of 10 days after planting (DAP). The second fertilization was carried out at 35 DAP with urea fertilizer up to 200 kg ha^{-1}.

Table 1. Characteristics of the soil field prior to conducting the research

Variable	Normal soil	Acid soil
pH:H_2O (1:2.5)	6.60	5.12
C-Organic (%)	0.89	1.82
N-Total (%)	0.13	0.14
P Bray1 (ppm)	130.00	35.00
P_2O_5-HCl25% (mg/100g)	40.00	16.00
K_2O HCl25%	98.00	47.00
K-dd	0.55	0.44
Ca-dd	23.90	14.24
Mg-dd	1.50	3.22
Na-dd	0.04	0.20
Al Saturation (%)	0.00	22.13
H^+	0.00	0.24
KTK (me/100g)	24.09	24.52
Base saturation (%)	100.00	74.00

Source: Laboratory of soil, plant, fertilizer, and water, BPTP South Sulawesi, Indonesia, 2021

In the second step, the study was arranged in a split-plot design with three replications. The main plot was the concentration of Al at three levels, i.e., 0, 7, and 14 ppm. The subplot comprised seven maize lines representing sensitive, medium tolerant, and tolerant categories based on the SSI and decreased grain yield. They were Pop. A3-1, Pop. A7-1, CB.Pop 10-1-3-1-2-2, CB.Pop 15-4-2-1-1-1, CB.Pop 23-1-2-1-4-2, Mal 03, and G102612.

A total of 20 seven-day-old sprouts for each tested maize were grown for 14 days in tubs containing 5 L of nutrient solution per tube. A basal nutrient solution was used as described by Magnavaca (1982), listed in Table 3. $KAl(SO_4)_2$ as the source of Al. Al concentration of $KAl(SO_4)_2$ was about 10.45%, and it can induce aluminum toxicity in maize, rice, and sorghum by suppressing photosystem II activity, root growth and inhibiting plant physiological activity (Magnavaca, 1982; Boni et al. 2009; Giannakoula et al. 2010; de Freitas et al. 2019). The volume and pH of the nutrient solution were measured every two days. The volume was carried at its initial level by adding stock solution while the pH was taken on a scale of 4.5±0.2 using NaOH and HCl. During plant growth, the media were supplied with oxygen using an aerator. The roots grown in the nutrient solution were washed with distilled water for 15 minutes to remove Al attached to the mucilage. Then the roots were dipped in a solution of hematoxylin (2 g l^{-1} hematoxylin and 0.2 g KI) for 20 minutes and washed again with distilled water for 15 minutes. Next, visual observation of the roots of the plant was carried out. Scoring was based on the method of Evans et al. (2013), with the following scores: very tolerant (1): no black spots on the roots; tolerant (2): fine black spots on the roots; medium tolerance (3): black spots are moderately visible on the roots; sensitive (4): black spots are clearly visible on the roots; and very sensitive (5): black spots are very clearly visible on the roots.

Observation variables included plant height, ear height, number of live plants, leaf length, leaf width, number of leaves, leaf area index (LAI), days to anthesis, days to the silking, anthesis-silking interval (ASI), fresh ear weight, ear diameter, ear length, number of rows per ear, number of kernels per row, seed moisture content, shelling percentage, 100-seed weight, grain yield, stress susceptibility index (SSI), and root Al absorption, crown Al absorption, and hematoxylin staining.

Table 2. List of maize lines used in the research

Genotype	Origin	Type
Pop A1-1	ICERI	Test line (S1 maize line)
Pop A2-1	ICERI	Test line (S1 maize line)
Pop A3-1	ICERI	Test line (S1 maize line)
Pop A4-1	ICERI	Test line (S1 maize line)
Pop A5-1	ICERI	Test line (S1 maize line)
Pop A6-1	ICERI	Test line (S1 maize line)
Pop A7-1	ICERI	Test line (S1 maize line)
CB.Pop 03-7-1-1-1-1	ICERI	Test line (S5 maize line)
CB.Pop 10-1-3-1-2-2	ICERI	Test line (S5 maize line)
CB.Pop 11-1-1-1-1-1	ICERI	Test line (S5 maize line)
CB.Pop 11-2-3-4-2-1	ICERI	Test line (S5 maize line)
CB.Pop 11-3-1-4-2-2	ICERI	Test line (S5 maize line)
CB.Pop 15-1-1-1-1-2	ICERI	Test line (S5 maize line)
CB.Pop 15-1-2-1-2-1	ICERI	Test line (S5 maize line)
CB.Pop 15-4-2-1-1-1	ICERI	Test line (S5 maize line)
CB.Pop 23-1-2-1-4-2	ICERI	Test line (S5 maize line)
CB.Pop 23-2-1-3-2-1	ICERI	Test line (S5 maize line)
CB.Pop 27-5-3-1-2-1	ICERI	Test line (S5 maize line)
CB.Pop 28-5-2-2-2-2	ICERI	Test line (S5 maize line)
CB. op 28-7-3-2-2-2	ICERI	Test line (S5 maize line)
Mal-03	ICERI	Check line (inbred maize)
CLYN-231	ICERI	Check line (inbred maize)
G102612	ICERI	Check line (inbred maize)
B11209	ICERI	Check line (inbred maize)

Table 3. Composition of nutrient solution used for evaluation of maize line in aluminum stress

Stock solution				Total composition		
Name	Chemical	Concentration (g/L)	Stock (mL/L)	Nutrient	Concentration (mg/L)	Concentration (µM)
Macronutrient	$Ca(NO_3)_2.4H_2O$	270.00	3.08	Ca	141.10	3527.00
	NH_4NO_3	33.80		NO_3-N	152.00	10857.00
				NH_4-N	18.20	1300.00
	KCl	18.60	2.31	Cl	21.05	595.00
	K_2SO_4	44.00		S	18.79	587.00
	KNO_3	24.60		K	91.80	2310.00
	$Mg(N0_3).6H_2O$	142.40	1.54	Mg	20.80	855.00
	KH_2PO_4	17.60	0.35	P	1.40	4.50
	$Fe(NO_3)_3.9H_2O$	20.30	1.54	Fe	4.30	77.00
	HEDTA	13.40		HEDTA	20.60	75.00
Micronutrient	$MnCl_2.4H_2O$	2.34	0.77	Mn	0.50	9.10
	H_3BO_3	2.04		B	0.27	25.00
	$ZnSO_4.7H_2O$	0.88		Zn	0.15	2.29
	$CuSO_4.5H_2O$	0.22		Cu	0.04	0.63
	$Na_2MoO_4.2H_2O$	0.26		Mo	0.08	0.83
				Na	0.04	1.74

Data analysis

First, analyze the data using analysis of variance. If a treatment has a significant effect, the LSD test is carried out at 5%. We then performed Pearson correlation analysis between agronomic traits and yield components with grain yield, which was conducted using STAR 2.0.1. application (IRRI 2014).

Tolerance Index to Al Stress

Tolerance analysis was performed to determine the tolerance of the maize tested under Al stress using the equation proposed by Fischer and Maurer (1978), namely the stress susceptibility index (SSI) based on seed yield:

$$\text{Stress Susceptibility Index (SSI)} = \frac{\left(1-\frac{Ysi}{Ypi}\right)}{\left(1-\frac{Ys}{Yp}\right)}$$

The criteria for determining the tolerance level to Al stress were SSI<0.5 for the tolerant genotype, 0.5≤SSI<1.0 for the medium tolerant genotype, dan SSI≥1.0 for the sensitive genotype.

Percentage yield reduction (PYR):

$$\text{YLP} = \frac{Ypi-Ysi}{Ypi} \times 100$$

Where:
Ysi: Grain yield under Al stress
Ypi: Grain yield at normal condition
Ys : Average grain yield of all genotypes under Al stress
Yp : Average grain yield of all genotypes under normal condition

RESULTS AND DISCUSSION

Growth and yield of maize in the fields under aluminum stress

Analysis of variance for agronomic traits and grain yield in the fields

The results of the variance analysis of agronomic traits and grain yield are represented in Table 4. The effect of environment (normal and aluminum stress condition), genotype, and genotype by environment interaction was different for all traits except for fresh ear weight, ear diameter, ear length, number of kernels per row, and grain yield. The genotype by environment interaction was highly significant (p<0.01) for fresh ear weight, ear diameter, ear length, number of rows per ear, number of kernels per row, seed moisture content, 100 seed weight, and grain yield. The proportion of them is exhibited in Figure 1.

The analysis of variance reveals that the sum of squares based on the effect proportion of environment, genotype, and genotype by environment interaction for the maize line traits showed that the effect of the environment was greater (>35%) than that of genotype (37%) and genotype by environment interaction (<15%) on plant height, ear height, leaf length, LAI, stalk diameter, days to flowering, fresh ear weight, and grain yield. At the same time, the character of grain yield components such as ear diameter, ear length, number of rows per ear, number of kernels per row, seed moisture content, shelling percentage, and 100-seed weight was influenced more by genotype (>42%) than environment (<20%) and genotype by environment interaction (<40%) (Figure 1). It showed that environmental conditions affected the grain yield of the maize lines. The environment played a major role in the phenotype appearance and grain yield of maize, while the genotype by environment interaction was very important in identifying superior maize lines based on their traits (Shojaei et al. 2020).

Tolerance of maize lines in aluminum stress

The SSI can be used to determine the tolerance level of maize lines tested in an Al-stress environment. The values were calculated by comparing the grain yields under aluminum stress and normal conditions, as well as based on yield reduction. SSI can be used to select inbred maize or in maize germplasm collection under stress conditions (Efendi and Azrai 2015; Sánchez-Reinoso et al. 2020).

Based on the SSI, the tested maize lines were grouped into three tolerance levels: sensitive, medium tolerant, and tolerant. The maize lines tolerant to aluminum stress were CB.Pop 11-2-3-4-2-1 and CB.Pop 15-4-2-1-1-1 with yield reduction percentages of 15.81% and 9.20%, respectively. The medium tolerant lines were Pop A1-1, Pop A3-1, Pop A6-1, CB.Pop 10-1-3-1-2-2, CB.Pop 11-3-1-4-2-2, and CB Pop 28-7-3-2-2-2. While the sensitive maize lines were Pop A2-1, Pop A4-1, Pop A5-1, Pop A7-1, CB.Pop 03-7-1-1-1-1, CB.Pop 11-1-1-1-1-1, CB.Pop 15-1-1-1-1-2, CB.Pop 15-1-2-1-2-1, CB.Pop 23-1-2-1-4 -2, CB.Pop 23-2-1-3-2-1, CB.Pop 27-5-3-1-2-1, CB.Pop 28-5-2-2-2-2, and CB.Pop 28-7-3-2-2-2 with percentage decreases in yield ranging from 64.22% to 100% (Table 5).

Table 4. The effect of genotype and environment on agronomic traits and grain yield

Variable	Environment (E)	Genotype (G)	Interaction (G x E)
Plant height	0.00^{**}	0.00^{**}	0.65^{ns}
Ear height	0.01^{**}	0.00^{**}	0.05^{ns}
Leaf length	0.01^{**}	0.00^{**}	0.57^{ns}
Leaf width	0.02^{*}	0.04^{*}	0.35^{ns}
Leaf area index	0.00^{**}	0.00^{**}	0.08^{ns}
Stalk diameter	0.00^{**}	0.16^{ns}	0.10^{ns}
Leaf chlorophyll	0.01^{**}	0.01^{**}	0.17^{ns}
Days to anthesis	0.00^{**}	0.00^{**}	0.64^{ns}
Days to silking	0.00^{**}	0.00^{**}	0.83^{ns}
ASI	0.00^{**}	0.79^{ns}	0.03^{*}
Fresh ear weight	0.00^{**}	0.00^{**}	0.00^{**}
Ear diameter	0.00^{**}	0.04^{*}	0.00^{**}
Ear length	0.00^{**}	0.05^{*}	0.00^{**}
Number of rows per ear	0.00^{**}	0.10^{ns}	0.00^{**}
Number of kernels per row	0.01^{**}	0.00^{**}	0.00^{**}
Seed moisture content	0.12^{ns}	0.31^{ns}	0.00^{**}
Shelling percentage	0.54^{**}	0.06^{ns}	0.04^{**}
100 seeds weight	0.00^{**}	0.16^{ns}	0.00^{**}
Grain yield	0.00^{**}	0.01^{*}	0.00^{**}

Note: **significant effect on 1%, *significant effect on 5%, ^{ns}non significant

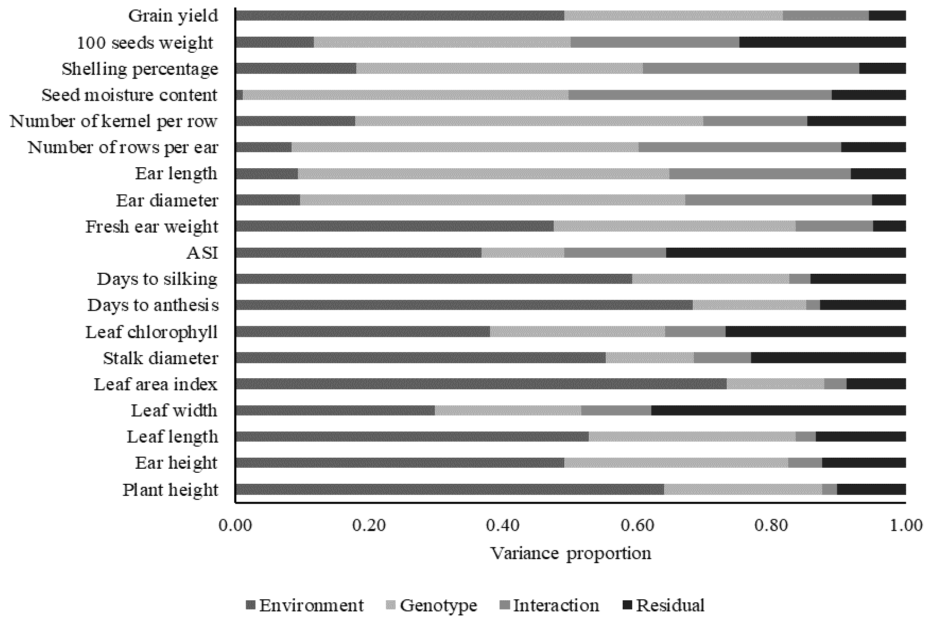


Figure 1. Proportions of the value of the sum of squares from the analysis of variance of the influence of environment, genotype, and genotype by environment interaction and the error on certain traits of 24 maize lines

Based on the regression analysis results in Figure 2, maize lines with high grain yields under Al stress tended to be tolerant to Al stress with relatively small SSI values (R^2=0.6343). However, in general, the level of plant tolerance to stress based on SSI was more related to the ability of plants to suppress yield reduction under stress conditions (Table 6).

Agronomic performance and grain yield of maize lines under aluminum stress

The agronomic performance and grain yield of the 24 maize lines in normal and aluminum stress conditions are shown in Tables 6 and 7. Aluminum stress could reduce plant height (34.41%), LAI (50.51%), stalk diameter (36.09%), leaf chlorophyll (14.72%), ear diameter (10.84%), shelling percentage (17.26%), and grain yield (62.23%) and increase the value of ASI (149.06%) compared normal condition.

The tolerant lines CB.Pop 11-2-3-4-2-1 and CB.Pop 15-4-2-1-1-1 had average plant heights of 96.50 cm and 103.38 cm, respectively, which were not significantly different from the four check lines. The heights of the medium tolerant line ranged from 97.29 to 141.50 cm. The plant heights of the sensitive line ranged from 84.26 to 135.71 cm. Here, the highest line was Pop. A7-1 (135.71 cm), which was not significantly different from the heights of the check lines G102612 (94.46 cm) and B11209 (77.08 cm). The ability of Al to suppress the growth of maize lines was tested by binding various essential nutrients and water in the soil and inhibiting root growth. According to Tyagi et al. (2020), aluminum suppresses cell division, reduces root respiration and oxidative phosphorylation, and inhibits the translocation of certain nutrients and water. Reduced cell division results from inhibiting the transporter function by auxin through exposure to aluminum (Zhang et al. 2020). The influence of Al on plant growth begins with inhibiting root elongation as a manifestation of the interaction between the root tips and aluminum. Roots respond rapidly to Al toxicity in less than one hour. Al affects the performance of apoplasts and root symplasts (Batista et al. 2013).

Table 5. The average grain yield of the 24 maize lines in normal conditions and aluminum stress, the percentage grain yield reduction, and the stress susceptibility index

Line	Grain yield (t/ha^{-1})		Percentage decrease in yield (%)	SSI
	Normal	Acid		
Pop A1-1	4.36	2.37	45.55	0.73MT
Pop A2-1	3.77	0.95	74.74	1.20P
Pop A3-1	5.29	3.49	33.96	0.55MT
Pop A4-1	4.99	1.79	64.22	1.03P
Pop A5-1	6.00	1.47	75.49	1.21P
Pop A6-1	4.25	2.68	36.93	0.59MT
Pop A7-1	5.81	0.48	91.68	1.47P
CB.Pop 03-7-1-1-1-1	3.04	1.06	65.10	1.05P
CB.Pop 10-1-3-1-2-2	3.74	2.12	43.30	0.70MT
CB.Pop 11-1-1-1-1-1	3.35	0.97	71.14	1.14P
CB.Pop 11-2-3-4-2-1	2.84	2.39	15.81	0.25T
CB.Pop 11-3-1-4-2-2	3.57	1.57	56.16	0.90MT
CB.Pop 15-1-1-1-1-2	1.90	0.00	100.00	1.61P
CB.Pop 15-1-2-1-2-1	1.76	0.45	74.13	1.19P
CB.Pop 15-4-2-1-1-1	1.75	1.59	9.20	0.15T
CB.Pop 23-1-2-1-4-2	4.25	0.86	79.87	1.28P
CB.Pop 23-2-1-3-2-1	2.17	0.65	69.92	1.12P
CB.Pop 27-5-3-1-2-1	3.73	0.92	75.43	1.21P
CB.Pop 28-5-2-2-2-2	1.49	0.30	79.58	1.28P
CB.Pop 28-7-3-2-2-2	3.70	1.58	57.19	0.92MT
Mal 03	3.25	1.27	61.01	0.98MT
CLYN-231	3.17	1.16	63.31	1.02P
G102612	2.52	0.70	72.39	1.16P
B11209	2.77	0.71	74.18	1.19P
Average	3.48	1.31	62.10	1.00

Note: P: sensitive SSI>1.0; MT: medium tolerant 0.5≤SSI<1.0; dan T: tolerant SSI<0.5

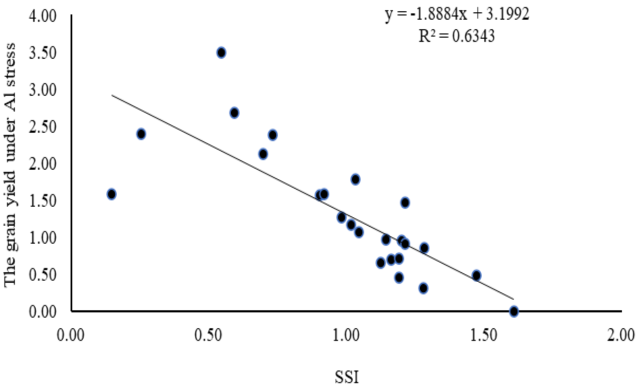


Figure 2. The correlation between the grain yield under Al stress and the stress susceptibility index (SSI)

LAI is the ratio between the leaf area and soil surface area covered. It is widely used to describe the canopy structure of plants. LAI determines the pattern of light availability in the canopy, controls the process of leaf development, and balances energy and water use by leaves, as well as photosynthesis (Liu et al. 2015). The pattern of the plant canopy determines the penetration of sunlight that can be used in the photosynthesis process (photosynthetically active radiation).

The Al-tolerant maize lines CB.Pop 11-2-3-4-2-1 and CB.Pop 15-4-2-1-1-1 had LAI values of 1.87 and 1.60, respectively, while the leaf chlorophyll contents at 40 DAP were 41.05 and 43.35 units, respectively. Meanwhile, the sensitive line had LAI values ranging from 1.50 to 3.24, where the highest LAI was found in the CB.Pop 03-7-1-1-1-1 (3.24) with a leaf chlorophyll content of 38.87 units and the lowest LAI was found in the CB.Pop 15-1-1-1-1-2 (1.50) with a leaf chlorophyll content of 31.28 units. In research conducted under Al stress conditions, Batista et al. (2013) demonstrated that the epidermal tissue of the leaves was covered by a thin cuticle and characterized by the slow development of the epidermal and cortical cells. In vascular tissue, the metaxylem and protoxylem have a small diameter and do not have a secondary wall. High Al concentrations inhibited the growth of leaf mesophyll tissue due to a decrease in the number and size of cells in the tissue. This decreased the chlorophyll content of *Spinacia oleracea* L. leaves by around 44.44% in 200 µM $AlCl_3$ (Karimaei and Poozesh 2016). Aluminum toxicity reduces photosynthetic activity, chlorosis, and necrosis in leaves, as shown in Figure 3 (Qu et al. 2020). Other studies have reported Al toxicity symptoms similar to Ca deficiency, including the upsetting of petioles and the curling of young leaves (Ashraf et al. 2019).

Table 6. Performance of the 24 maize lines in normal condition

Genotype	PH (cm)	LAI	SD (cm)	LC (unit)	ASI	ED (cm)	R	Y (t ha^{-1})
PopA1-1	180.71d	5.50bd	2.27	46.07	1.67	4.04cd	0.78a	4.36abcd
PopA2-1	184.04d	5.20bd	2.08	45.79	1.33	4.37abcd	0.76a	3.77cd
PopA3-1	188.42cd	5.03bd	2.20	49.69d	2.00	4.45abcd	0.72a	5.29abcd
PopA4-1	193.33acd	5.37bd	2.07	46.90	1.00	4.65abcd	0.73a	4.99abcd
PopA5-1	170.38d	5.43bd	3.30abcd	49.69d	2.00	4.58abcd	0.72a	6.00abcd
PopA6-1	190.04cd	5.96bd	2.30	53.45bd	0.67a	4.43abcd	0.72a	4.25abcd
PopA7-1	200.08abcd	5.94bd	2.26	51.18d	0.67a	4.67abcd	0.74a	5.81abcd
CB.Pop 03-7-1-1-1-1	182.08d	4.68	2.50b	45.48	1.67	3.80	0.74a	3.04
CB.Pop 10-1-3-1-2-2	161.79	4.54	2.03	42.75	2.00	3.97c	0.71a	3.74cd
CB.Pop 11-1-1-1-1-1	180.29d	5.21bd	1.97	47.98	1.00	3.87c	0.75a	3.35
CB.Pop 11-2-3-4-2-1	138.38	3.67	2.18	48.52	2.33	3.99cd	0.70	2.84
CB.Pop 11-3-1-4-2-2	179.29d	5.62bd	2.19	43.78	1.00	4.08cd	0.71a	3.57c
CB.Pop 15-1-1-1-1-2	144.13	4.51	2.35	42.87	1.33	3.76	0.62	1.90
CB.Pop 15-1-2-1-2-1	145.79	4.04	2.35	43.10	2.00	3.58	0.65	1.76
CB.Pop 15-4-2-1-1-1	144.88	4.08	2.31	49.85d	1.00	3.79	0.64	1.75
CB.Pop 23-1-2-1-4-2	151.29	4.52	1.97	47.40	1.67	3.94c	0.74a	4.25abcd
CB.Pop 23-2-1-3-2-1	154.33	4.52	2.33	44.09	2.33	4.03cd	0.61	2.17
CB.Pop 27-5-3-1-2-1	189.17cd	5.49bd	2.39	51.89d	2.00	3.69	0.79a	3.73c
CB.Pop 28-5-2-2-2-2	186.33cd	4.90	2.65bd	47.50	1.33	3.22	0.62	1.49
CB.Pop 28-7-3-2-2-2	180.25d	4.84	3.12abd	47.73	1.67	3.78	0.77a	3.70c
Mal03(a)	171.33	5.46	2.38	51.65	2.00	3.90	0.62	3.25
CLYN-231(b)	177.71	4.11	1.78	44.82	1.33	3.90	0.83	3.17
G102612(c)	166.04	5.92	2.42	48.16	1.33	3.58	0.72	2.52
B11209(d)	143.71	4.12	1.85	42.42	0.67	3.70	0.76	2.77
Average	170.99	4.94	2.30	47.20	1.50	3.99	0.71	3.48
CV (%)	7.10	10.60	18.90	9.20	50.40	4.20	7.40	17.00
LSD 5%	19.81	0.86	0.71	7.17	1.24	0.28	0.09	0.97

Note: Numbers followed by the same letter as the comparison line differed significantly from those at $LSD_{\alpha=5\%}$. PH: plant height, LAI: leaf area index, SD: stalk diameter, LC: leaf chlorophyll, ASI: anthesis-silking interval, ED: ear diameter, SP: shelling percentage, and Y: grain yield

Table 7. Performance of 24 maize line under aluminum stress

Genotype	PH (cm)	LAI	SD (cm)	LC (uni)	ASI	ED (cm)	SP	Y (t ha^{-1})
PopA1-1	118.42d	3.28bcd	1.59d	39.54	5.67	3.64	0.74abd	2.37abcd
PopA2-1	115.29d	2.40	1.46d	37.73	3.67	3.96d	0.48	0.95
PopA3-1	141.50cd	3.57bcd	1.65d	43.14	4.00	4.26abcd	0.69abd	3.49abcd
PopA4-1	122.25d	2.30	1.37	37.83	3.00c	4.20abcd	0.69abd	1.79abcd
PopA5-1	103.04	2.51	1.51d	42.89	5.33	4.00cd	0.70abd	1.47cd
PopA6-1	131.63cd	3.12bd	1.83abcd	41.58	4.33	4.31abcd	0.70abd	2.68abcd
PopA7-1	135.71cd	3.18bd	1.35	41.48	2.33bc	4.28abcd	0.26	0.48
CB.Pop 03-7-1-1-1-1	131.88cd	3.24bcd	1.53d	38.87	4.33	3.32	0.69abd	1.06
CB.Pop 10-1-3-1-2-2	111.96d	2.54	1.35	41.67	3.67	3.42	0.71abd	2.12abcd
CB.Pop 11-1-1-1-1-1	133.88cd	2.70	1.43d	37.89	4.00	3.76d	0.44	0.97
CB.Pop 11-2-3-4-2-1	96.50	1.87	1.48d	41.05	1.67bcd	4.00cd	0.71abd	2.39abcd
CB.Pop 11-3-1-4-2-2	134.54cd	2.77	1.43d	41.59	3.67	3.82d	0.75abd	1.57cd
CB.Pop 15-1-1-1-1-2	84.26	1.50	1.44d	31.28	5.33	0.00	0.00	0.00
CB.Pop 15-1-2-1-2-1	85.42	2.04	1.59d	36.51	2.67c	3.15	0.55	0.45
CB.Pop 15-4-2-1-1-1	103.38	1.60	1.63d	43.35	3.33	3.83d	0.63abd	1.59cd
CB.Pop 23-1-2-1-4-2	88.71	1.97	1.41	41.08	4.00	3.09	0.64abd	0.86
CB.Pop 23-2-1-3-2-1	87.79	1.97	1.52d	35.26	3.33	3.75d	0.66abd	0.65
CB.Pop 27-5-3-1-2-1	119.25d	2.23	1.50d	41.20	3.00c	3.17	0.61l	0.92
CB.Pop 28-5-2-2-2-2	124.79cd	2.73	1.61d	46.95	3.00c	3.07	0.53	0.30
CB.Pop 28-7-3-2-2-2	97.29	1.78	1.39	35.68	2.33bc	3.89d	0.67abd	1.58cd
Mal03(a)	117.21	3.27	1.45	47.10	1.00	3.78	0.55	1.27
Clyn-231(b)	135.25	1.86	1.35	44.01	5.33	3.79	0.55	1.16
G102612(c)	94.46	2.30	1.34	39.16	6.00	3.61	0.71	0.70
B11209 (d)	77.08	2.00	1.10	39.20	4.67	3.29	0.53	0.71
Average	112.14	2.45	1.47	40.25	3.74	3.56	0.59	1.31
CV (%)	15.30	22.90	13.10	6.90	47.90	6.20	7.00	19.80
LSD 5%	28.18	0.92	0.32	4.59	2.94	0.36	0.07	0.43

Note: Numbers followed by the same letter as the comparison line differed significantly from those at LSDα=5%. PH: plant height, LAI: leaf area index, SD: stalk diameter, LC: leaf chlorophyll, ASI: anthesis-silking interval, ED: ear diameter, SP: shelling percentage, and Y: grain yield

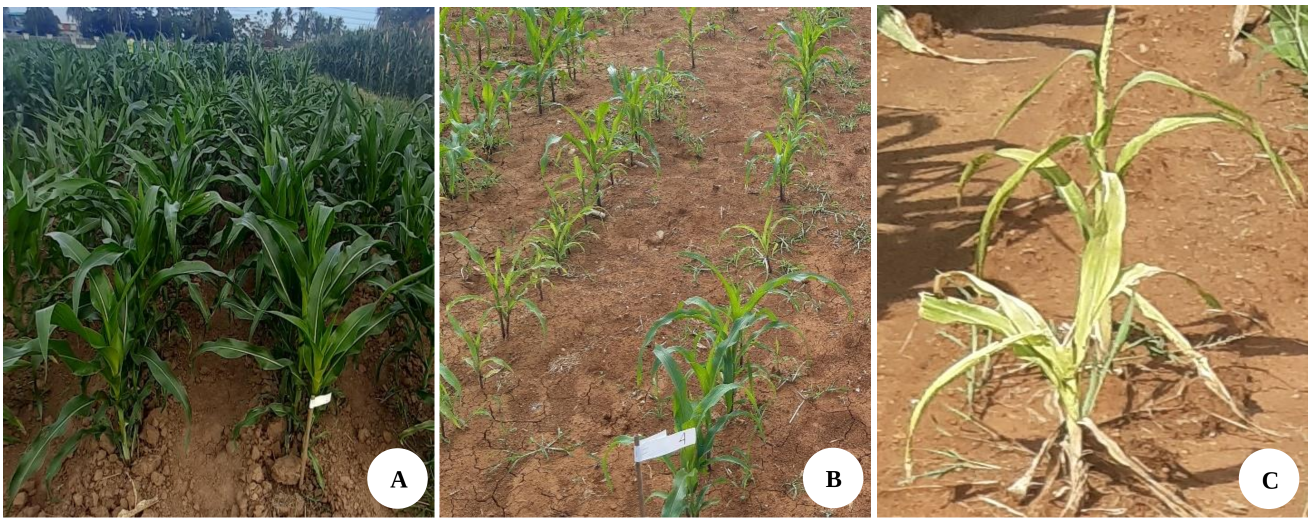


Figure 3. The performance of maize line. A. the performance of maize line at the age 30 DAP under normal conditions. B-C. the performance of maize line at the age 30 DAP under aluminum stress

The stalk diameter of the Al stress-tolerant lines ranged from 1.60 to 1.87 cm. The medium tolerant lines had a stalk diameter ranging from 1.35 and 1.83 cm, where the largest diameter was in the Pop A6-1 line (1.83 cm) and was significantly different from the four check lines. The lines sensitive to Al stress had smaller stem diameters than the tolerant and medium tolerant lines, ranging from 1.35 to 1.61 cm, and where the average sensitive line was significantly different from the check line B11209 (1.10 cm).

The ASI variable shows the difference between male and female flowering ages. The ASI of a tolerant line such as CB.Pop 11-2-3-4-2-1 was 1.67 days shorter than the average ASI value in the lines sensitive to Al stress (2.33-5.33 days). According to Chassaigne-Ricciulli et al. (2021), an ASI of 1-3 days allows synchronization in the pollination and fertilization process and, thus the potential for maximum production. The flowering phase is inhibited when Al mostly bound phosphorus to form insoluble phosphate in Al stress. In addition, water availability due to the bonding of Al and other metals in acidic soils causes dehydration of plant tissue; this affects cell division and tissue expansion, leading to the inhibition of pollen shedding and silk elongation (Opala 2017; Leng et al. 2022).

The number of seeds in the ear determines ear diameter. The tolerant and medium tolerant lines had an ear diameter of around 3.42 to 4.31 cm. While the ear diameter of the sensitive lines ranged from 0 to 4.28 cm. The influence of Al stress and the gene of the maize led to the presence of seedless ears. High levels of Al in soil inhibit the performance of the P and K elements that play a role in the assimilate transport process, pollen formation, and seed filling to produce small, seedless ears (Figure 4). Even the CB.Pop15-1-1-1-1-2 line under Al stress conditions had only two plants with ears; however, they contained no seeds, meaning the yield component and grain yield variables could not be measured (Table 7).

The average yield of tolerant maize CB.Pop 11-2-3-4-2-1 under Al stress conditions was 2.39 t ha^{-1}, which was significantly different from that of the check lines, that is Mal-03 (1.27 t ha^{-1}), CLYN-231 (1.16 t ha^{-1}), G102612 (0.70 t ha^{-1}), and B11209 (0.71 t ha^{-1}). Meanwhile, tolerant line CB.Pop 15-4-2-1-1-1 had a grain yield of 1.59 t ha^{-1}, significantly different from the sensitive check lines (G102612 and B11209). The medium tolerant line had a grain yield range of 1.57-3.49 t ha^{-1}. Here, the highest average grain yield was found in the Pop A3-1 line (3.49 t ha^{-1}), which was significantly different from the grain yields of the check lines Mal-03 (1.27 t ha^{-1}), CLYN-231 (1.16 t ha^{-1}), G102612 (0.70 t ha^{-1}), and B11209 (0.71 t ha^{-1}). However, the POP A3-1 line had a higher grain yield than the CB.Pop 11-2-3-4-2-1 and CB.Pop 15-4-2-1-1-1 also had a greater reduction in grain yield (33.96% or 1.79 t/ha) than the two tolerant lines (15.81% and 9.20%). The tolerant lines were able to suppress yield reduction under Al stress conditions. The Pop A4-1 sensitive line had a grain yield of 1.79 t ha^{-1}, which differed from the four check lines. Meanwhile, the sensitive line CB.Pop 15-1-1-1-1-2 did not produce because it had seedless ears. Vasconcellos et al. (2021) found that Al toxicity caused an 18.7% decrease in the grain yield of maize lines. Aluminum entering root cells can inhibit the uptake and transport of P, Ca, Mg, and K, as well as cause lipid peroxidation or oxidative degradation of fats.

Correlation of several traits with grain yield under aluminum stress

Based on the results of the correlation analysis, plant height, ear height, leaf length, LAI, stalk diameter, leaf chlorophyll, number of live plants, ear length, ear diameter, number of rows of seeds per ear, number of seeds per row, shelling percentage, and the SSI were significantly correlated with grain yield under Al stress with correlation coefficients ranging from 0.14 to 0.62 (Figure 5). This indicated that if the value of the characters were high, the grain yield of the maize tested under Al stress would also increase. Optimum plant height, LAI, stalk diameter, and leaf chlorophyll can survive and produce under Al stress. The study's results showed that the maize, which was tolerant and medium tolerant to Al stress, had a higher average plant height, LAI, stem diameter, and leaf chlorophyll than the Al stress-sensitive lines (Table 8). According to Bonelli and Andrade (2020), plant height and LAI are related to the capture of photosynthetically active radiation, which plays a role in the distribution of nitrogen elements and the formation of assimilate.

Root morphophysiology of Al-tolerant and sensitive maize lines

In the second step, we evaluated several maize lines representing tolerant, medium tolerant, and sensitive categories through nutrient culture for 14 days in a screen house using Magnavaca nutrient solution media. It is to confirm the maize line tolerance grown in the field and identify the growth and Al absorption of roots and crown of these lines.

Analysis of variance for seedling traits

The genotype by environment interaction was significant for root Al absorption and crown Al absorption ($p<0.01$) as well as for hematoxylin staining ($p<0.05$). It indicates that the performance of the maize lines was not the same at each concentration of Al in the nutrient solution for these traits. The aluminum concentration was also highly significant for all seedling traits.

Table 8. Mean square of aluminum concentrations, genotype, and interaction factor at seedling traits

Source of variance	Aluminum	Genotype	Interaction
Root Al absorption	174963.00^{**}	92673.40^{**}	11111.90^{**}
Crown Al absorption	2520.11^{**}	649.22^{ns}	219.35^{**}
Hematoxylin staining	7.39^{**}	4.03^{**}	0.26^{*}

Note: **significant effect on 1%, *significant effect on 5%, ^{ns}non significant

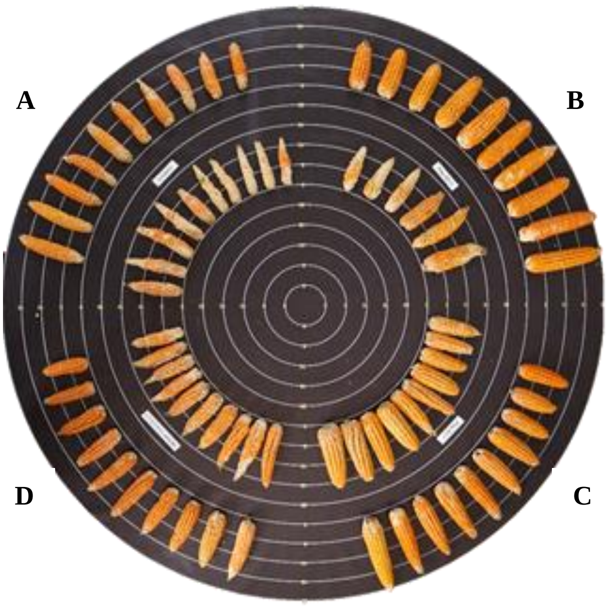


Figure 4. The ear performance of several maize lines under normal conditions (top tier) and Al stress (bottom tier): A. G102612, B. Pop. A7-1, C. Pop. A3-1, D. CB.Pop. 15-4-2-1-1-1

Correlation of seedling traits and grain yield

The correlation between seedling traits and grain yield is represented in Table 9. The root Al absorption was significantly positively correlated with crown Al absorption (0.45) and SSI (0.65). This indicates that if root Al absorption is high, crown Al absorption is also elevated, and the maize line is very sensitive to Al.

After a 14-day exposure to Al in the nutrient solution, tolerant maize line CB.Pop 15-4-2-1-1-1 had an average root Al absorption of around 159.33 ppm, while medium tolerant Al Pop A3-1, CB.Pop 10-1-3-1-2-2 and Mal 03 had an average root Al absorption of 244.33-310.00 ppm. The sensitive lines Pop A7-1, CB.Pop 23-1-2-1-4-2 and G102612 had an average root Al absorption of around 299.67-494.67 ppm (Figure 6.A). Based on this, Al-tolerant maize lines absorb less Al at the roots than sensitive lines. Zerrouk et al. (2020) showed that the roots of Al-sensitive maize lines absorbed 97.50% more Al at a concentration of 2.43 ppm than tolerant lines. At the same time, Du et al. (2020) found that the roots of Al-sensitive maize lines absorbed 85.71% more Al at a concentration of 1.62 ppm than tolerant lines. In maize, Al^{3+} ions that have entered the cytoplasm are rapidly detoxified in the cells by forming complex compounds with organic acids in the form of citric acid (Tekeu et al. 2015). Yuan and Ren-Kou (2014) showed that total reducing substances exudate in the maize root tip was significantly correlated with Al concentration, where the concentration of 30 mM Al total reducing substances exudate in the maize root was around 2800 mmol/kg dry root. However, at a concentration of 50 mM, the reducing substances maize root exudate increased by about 4000 mmol/kg dry root.

Al-tolerant maize line CB.Pop 15-4-2-1-1-1 had a lower crown Al absorption than the moderately tolerant and sensitive lines, around 37 ppm at a concentration of 7 ppm Al and 40 ppm at 14 ppm Al (Figure 6.B). Medium tolerant lines Pop A3-1 and CB.Pop 10-1-3-1-2-2 and check line Mal 03 had crown Al absorptions of 39, 46, and 37 ppm at a concentration of 7 ppm Al, respectively, while at a concentration of 14 ppm, these values increased by 41.03%, 10.87%, and 5.41%, respectively. The Al-sensitive maize lines had higher crown Al absorptions, namely, Pop A7-1, which was around 49 ppm at a concentration of 7 ppm Al and 90 ppm at 14 ppm Al and CB.Pop 23-1-2-1-4-2 with around 54 ppm at a concentration of 7 ppm Al and 66 ppm at 14 ppm Al. According to Maron et al. (2013), in plants tolerant to AI toxicity, most of the Al is retained in the roots and slightly translocated to the top of the plant.

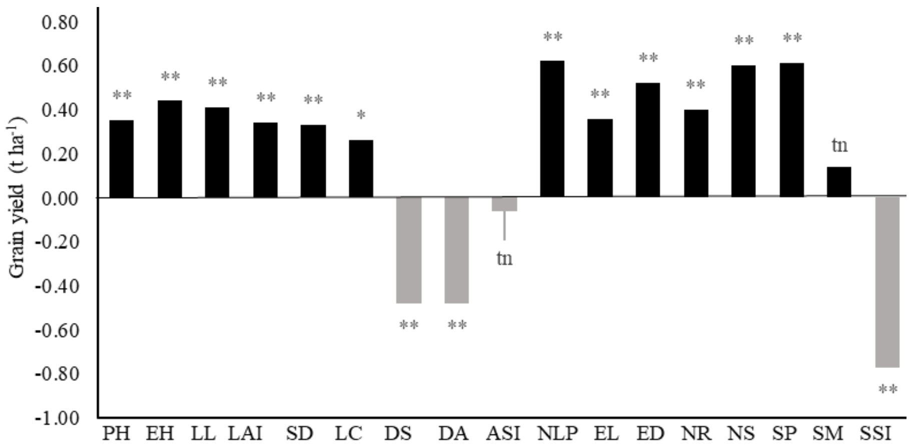


Figure 5. Correlation of agronomic traits, yield components, and SSI with grain yield under aluminum stress. Note: PH: plant height, EH: ear height, LL: leaf length, LAI: leaf area index, SD: steam diameter, LC: leaf chlorophyll, DS: days to silking, DA: days to anthesis, ASI: anthesis silking interval, NLP: the number of live plants, EL: ear length, ED: ear diameter, NR: the number of rows per ear, NS: number of kernel per row, SP: shelling percentage, SM: seed moisture content, dan SSI: stress susceptibility index

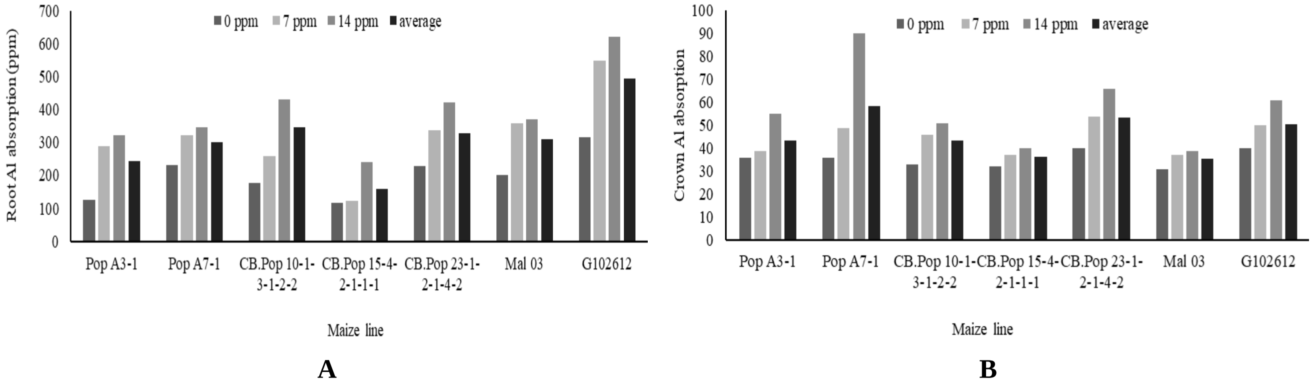


Figure 6. Root Al absorption and crown Al absorption of 7 maize line after evaluated in nutrient solution for 14 days with 0, 7, and 14 ppm of Al

Table 9. Correlation of root Al absorption, crown Al absorption, hematoxylin staining, and SSI value with grain yield

Variable	Root Al absorption	Crown Al absorption	Grain yield under Al	SSI
Root Al absorption	1.00	0.45 *	-0.43 ns	0.65 **
Crown Al absorption	0.45 *	1.00	-0.55 *	0.73 **
Hematoxylin staining	0.75 **	0.73 **	-0.53 *	0.84 **
Grain yield under Al	-0.43 ns	-0.55 *	1.00	-0.66 **
SSI	0.65 **	0.73 **	-0.66 **	1.00

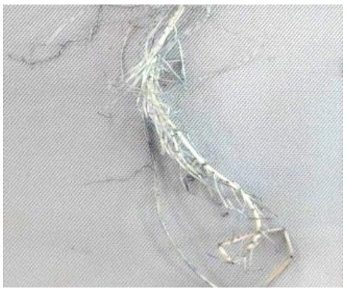
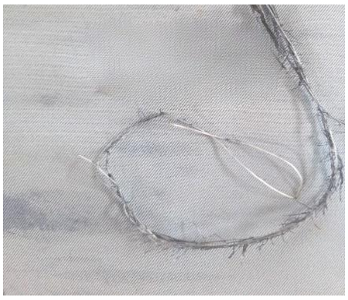
Note: *significant correlated on 5%, **highly significant correlated on 1%, ^{ns}non significant

Estimation of Al tolerance of maize lines by hematoxylin staining

Hematoxylin staining is a qualitative method based on staining patterns on the root tips. Hematoxylin forms a complex bond with Al that is triggered by the oxidation of hematoxylin to hematin in the presence of $NaIO_2$. Hematin will produce a dark blue color when complex bonds are formed with Al. Al accumulates in the symplast to enable Al penetration (Llewellyn 2013). According to (Xu et al. 2017), the hematoxylin staining method has great potential in evaluating maize, especially for early screening.

Visual observation of root staining showed that hematoxylin was relatively effective in distinguishing between the lines that were tolerant and sensitive to Al. In this study, hematoxylin absorption was assessed based on the method of Evans et al. (2013), with scores ranging from 1 (very tolerant) to 5 (very sensitive). Table 9 showed that the hematoxylin staining score significantly correlated with root Al absorption, crown Al absorption, and SSI. Al-tolerant line CB.Pop 15-4-2-1-1-1 had a lower score for the hematoxylin staining (≤2), with fine black spots on the roots at a concentration of 7 ppm Al. However, at a concentration of 14 ppm Al, a fairly clear black spot appeared on the roots, thus indicating that the line was medium tolerant at this concentration (Figure 7). It indicates that the maize line has a critical limit to survive under certain stress conditions. Zishiri et al. (2022) also reported no hematoxylin stains on the roots of Al-tolerant inbred lines.

Medium tolerant line Pop. A3-1 and CB.Pop 10-1-3-1-2-2 had scores of ≤3, with black spots quite clearly visible on the roots. From the observations, the score for the hematoxylin staining of the line was the same as the check line Mal-03, which was a medium tolerant comparison. The sensitive lines Pop. A7-1, CB.Pop 23-1-2-1-4-2, and G102612, meanwhile, had a hemotoxylin absorption score of ≤4, with black spots clearly visible on the roots at 7 ppm Al and 14 ppm Al. Dark black stains were found from the tip of the root to its base. This indicated that the sensitive strains accumulated more Al in the root apoplast than the tolerant maize lines. In Al-sensitive maize, aluminum enters the symplast quickly, and Al can be detected in the root tip vacuole after 4 hours of exposure to Al (Ren et al. 2022).

Genotype	Concentrations 0 ppm	7 ppm	14 ppm
Pop. A3-1			
Pop. A7-1			
CB.Pop 10-1-3-1-2-2			
CB.Pop. 15-4-2-1-1-1			
CB.Pop. 23-1-2-1-4-2			
Mal-03			

G102612

A B C

Figure 7. The roots of some genotypes with hematoxylin staining after being grown in nutrient culture media for 14 days with concentrations of Al: (A) 0 ppm, (B) 7 ppm, and (C) 14 ppm

In conclusion, Al stress could reduce plant growth by around 10.78% to 34.42% and grain yield by around 63.36% compared to normal conditions. The traits that were significantly correlated with grain yield under Al stress (r>50%), which could be used in maize selection under Al stress, were the number of live plants, ear diameter, number of kernels per row, shelling percentage, SSI, crown Al absorption, and hematoxylin staining. There were two maize lines tolerant to Al stress, namely CB.Pop 11-2-3-4-2-1 and CB.Pop 15-4-2-1-1-1, with potential yields under Al stress conditions of 2.39 t ha^{-1} and 1.59 t ha^{-1}, respectively. Al absorption averages of roots and crowns of Al tolerant maize lines were 159.33 ppm and 36.33 ppm, respectively.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Indonesian Cereal Research Institute, Maros, South Sulawesi, Indonesia for their support and contribution to this research.

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