

## **Investigating the Type of Gene Action Conditioning Tolerance to Aluminum (Al) Toxicity in Tropical Maize**

### **ABSTRACT**

Maize is a third important cereal crop in the world after wheat and rice. In Zambia, it is an important staple crop. Its production is however hampered by both biotic and abiotic factors. Among the abiotic factors, Aluminum (Al) toxicity causes high yield losses and is directly linked to acidic soils. Application of lime can ameliorate this problem, but it is expensive for small scale farmers. Developing maize varieties that are tolerant to Al toxicity is cheaper and feasible for small scale farmers. The purpose of this research was to investigate the type of gene action conditioning tolerance to aluminum toxicity in tropical maize. Eleven inbred lines were mated in an 8 male (4 moderately tolerant and 4 susceptible) x 3 female (resistant) North Carolina Design II. Results revealed that general combining ability (GCA) effects due to both males and females were highly significantly ( $P=0.001$ ) for root biomass. The shoot length GCA effects due to both male and female respectively were significant ( $P=0.01$ ). Similarly, the GCA effects due to females and males for root length were significant,  $P=0.01$  and  $P=0.05$  respectively. CML 511 had the most desirable significant GCA effect value (1.40) for root length among the male lines while CML 538 had the most desirable significant GCA effect value (0.92) among the female lines. The baker's ratio for root length was found to be 0.49 implying that both additive and non-additive gene action were important in conditioning aluminum toxicity tolerance in tropical maize.

**Key words:** Aluminum toxicity, Combining ability, Inbred lines, Gene action

### **1. INTRODUCTION**

Maize is an important cereal crop in the world. (1). It is processed and consumed differently in different parts of the world, the most popular products being maize flour and meal. Apart from human and animal consumption, it is widely used in corn oil production, corn starch industry and the fresh grains are eaten roasted or boiled on the cob [2]. In Africa, maize

production occupies approximately 24% of farmland which is more than any other staple crop. South Africa is currently the largest maize producing country in Africa [5,6] followed by Nigeria, Ethiopia, Egypt, and Tanzania. Most of the maize production in Africa is rain fed.

In Zambia, maize production has been reported to be increasing [7]. However maize productivity is hampered by both biotic and abiotic factors. Among the abiotic factors Aluminum toxicity has been found to cause yield losses of up to 80% [8] and toxic Al species are made available at pH below 5.5 [9]. The initial stage of aluminum toxicity is considered to result from Al-induced membrane instability and seedlings are more sensitive to Al than older plants. In many species, Al toxicity also induces deficiency for phosphorous, calcium or iron. This is because important nutrients like phosphorus (P) become less available to plants while other elements like Al become readily available and may become toxic to plants causing a reduction in crop productivity [8, 10].

Developing maize varieties that are tolerant to Aluminum toxicity is a cheaper and feasible way to small scale farmers. The genetic variability among maize genotypes exists for tolerance to Al toxicity. Al tolerance is genetically inherited [12], thus selection for Al tolerant genotypes is practically achievable in maize.

The direct selection of superior Al tolerant genotypes under field conditions is hindered due to temporal and spatial variations in Al toxic soils and reliable ranking of tolerance in field screening is difficult. The evaluation of field performance under Al stress conditions is also rendered difficult due to field heterogeneity in Al toxic soils which hinders the reliability of the response of genotypes. Moreover, screening at field level is very expensive and time consuming when a large number of genotypes are under evaluation [12]. Understanding the nature of gene action enables the breeder to understand the type of breeding strategy to employ in a breeding program. [13]. The objective of the study was therefore to investigate the type of gene action conditioning tolerance to aluminum toxicity in tropical maize.

## **2. MATERIALS AND METHODS**

## **2.1 Germplasm used and generation of F1 crosses**

Eleven maize inbred lines (CZL 083, L151, L5527, CZL0814, L12, CML511, L917, CML538, L5522, CML 457, and CZL04007) previously evaluated for Al tolerance at University of Zambia (latitude-15.39°S, longitude-28.33°E) were used in this study. These were initially obtained at the Zambia Agriculture Research Institute (ZARI). The F1 crosses were generated at the same institute [Zambia Agriculture Research Institute (ZARI), Mt Makulu]

A crossing block was laid using North Carolina Design II in which three tolerant lines were designated as females (CZL 083, CZL 0814 and CML 538) and the other eight as males (CML 457, L12, L5527, L917, CZL 04007, CML 511, L151, and L552) among which four were moderately tolerant and the other four were susceptible. Hand pollination was done as follows; the ear shoots of plants designated as female were covered with shoot bags before the emergence of silks in order to avoid unwanted pollination. On the other hand, the tassels of plants designated as males were covered for a day with tassel bags. The following morning the plants were carefully bended and the tassels covered with bags were shaken gently so that the pollen could fall in the bags. Thereafter, the bag with pollen was carried to pollinate the desired female plants. Hand pollination was done by quickly removing the shoot bag from the ear and applying pollen immediately. The pollinated ear remained covered with tassel bag and labeled for identification. A total of 24 F1 progenies were generated.

## **2.2 Evaluation of F1 progenies in hydroponics**

Evaluation of F1 progenies were done at the University of Zambia laboratory (latitude-15.39°S, longitude-28.33°E). A 24 (genotypes) x 2 (Al levels) factorial completely randomized design (CRD) with 3 replications was used to evaluate the genotypes. A total of 144 experimental units (plots) were used and the treatments were randomly assigned to each plot (test tube). Each test tube being a diameter of 2.3 cm and height of 14.5 cm. The levels of Al used were 0mg/ L and 20mg/ L, and these were purposely chosen being concentrations in solutions with the most favorable and most limiting for plant growth respectively as determined by Tembo [14].

### 2.3 Nutrient solution used in hydroponics

The modified protocol described by Kerridge et al. [15], was used to prepare the nutrient solutions (Table 1). The pH was adjusted to 4.2 using HCl and NaOH buffer solutions before being transferred to the test tubes. Petri dishes, test tubes, seed and polyethylene stoppers were sterilized using 35% commercial bleach of the JIK brand that contains 0.39% sodium hypochlorite (NaClO).

**Table 1: Nutrient solution used in hydroponics study at University of Zambia, school of Agriculture**

Nutrient	Con (mg/ L)	Chemical Formula	Compound Name
N	42.61	$\text{NH}_4\text{NO}_3$	Ammonium Nitrate
K	23.5	$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	Potassium hydrogen phosphate trihydrate
Zn	0.16	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	Zinc sulphate heptahydrate
Mg	14.6	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	Magnesium sulphate heptahydrate
Cu	0.06	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Copper sulphate pentahydrate
Fe	1.60	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	Iron sulphate heptahydrate
Ca	48.10	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	Calcium chloride dehydrate
Mo	0.03	$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	Sodium molybdate dehydrate
Mn	0.03	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	Manganese sulphate monohydrate
B	0.32	$\text{H}_3\text{BO}_3$	Boric acid
Al	Varied*	$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	Aluminum potassium sulphate dodecahydrate

N- Nitrogen, K-Potassium, Zn- Zinc, Mg- Magnesium, Cu- Copper, Fe- Iron, Ca- Calcium, Mo- Molybdenum, Mn- Manganese, B- Boron, Al- Aluminum, \*- Factorial scheme under hydroponic combinations with either 0 or 20 mg/ L aluminium dose

### 2.5 Placement of Maize Seedlings

The seeds were germinated on petri dishes lined with filter paper wetted with water and placed in the germination chamber for 5 days at 25°C. Seedlings of uniform root length (approximately 2 cm) were selected and transferred to test tubes containing nutrient solutions with either of the two concentrations of Al. These seedlings were supported over the nutrient solution by polyethylene stoppers and test tubes were covered with black polyethylene bags throughout the experiment, to prevent algae from growing in the solution. The nutrient

solution was aerated twice a day using an aquarium air pump and the volume was maintained by adding to the test tubes more solution after aeration.

## 2.6 Data analysis

The genotypes were evaluated on the 11th day. The shoot and root lengths were measured immediately after harvesting using a 30 cm ruler. The number of root hairs were also counted. The roots and shoots were separated and placed in the oven for 24hrs at 75°C after which the root and shoot biomass were weighed using a balance.

The root length was used in determining the type of gene action conditioning Al toxicity as used in related studies [16, 17]. Analysis of variance was performed using a fixed model and means of root length, shoot lengths and numbers were separated using the Fisher protected Least Significant Difference (LSD) method, at a significant level of  $\alpha = 0.05$ . All the data analysis was carried out using GenStat statistical package [18]. Means were separated using the Fisher's protected LSD.

The GCA and SCA were estimated as done by Singh and Chaudhary [19]. In addition, narrow and broad sense heritability were also estimated. Narrow sense heritability ( $h^2$ ) which is a measure of the proportion of additive variance in the overall variance was estimated as follows;

$$h^2_{n.s} = \sigma^2_{gca_m} + \sigma^2_{gca_f} / \sigma^2_{gca_m} + \sigma^2_{gca_f} + \sigma^2_{sca} + \sigma^2_e$$

Broad sense heritability, which  $h^2_{b.s}$  is the proportion of both additive and dominance variances in the overall variance was estimated as follows;

$$h^2_{b.s} = \sigma^2_{gca_m} + \sigma^2_{gca_f} + \sigma^2_{sca} / \sigma^2_{gca_m} + \sigma^2_{gca_f} + \sigma^2_{sca} + \sigma^2_e$$

Where:  $\sigma^2_{gca_m}$  is the variance component due to GCA male,  $\sigma^2_{gca_f}$  is the variance component due to GCA female,  $\sigma^2_{sca}$  is the variance component due to SCA, and  $\sigma^2_e$  is the error variance

The ratio of combining ability variance components (Baker's ratio) was estimated as by [20];

$$\text{Baker's ratio} = \sigma^2_{gca_m} + \sigma^2_{gca_f} / \sigma^2_{gca_m} + \sigma^2_{gca_f} + \sigma^2_{sca}$$

The variance components for GCA and SCA were calculated as described by Baker [19].

$$\sigma^2_{sca} = MS_{mf} - MS_{mfc}/rc, \sigma^2_{gca_m} = MS_m - MS_{mf} - rf\sigma^2_{mc}/rcf, \sigma^2_{gca_f} = MS_f - MS_{mf} - rm\sigma^2_{fc}/rcm$$

Where MS- mean square value; m- number of male lines, c- levels of Al concentration, f- number of female lines

### 3. RESULTS

#### 3.1 Nature of inheritance for Al tolerance

Results (Table 2) showed that GCA effects due to both males and females were highly significant for root biomass ( $P= 0.001$ ), shoot length. Similarly, the GCA effects due to females and males were significant at  $P= 0.01$  and  $P= 0.05$  for the root length respectively. The SCA effect were only significant for shoot length and root biomass ( $P= 0.02$ ). All the interaction effects except for SCA x concentration for root biomass were significant.

**Table 2: Mean squares for 8 x 3 North Carolina design F1 crosses evaluated at the University of Zambia, School of Agricultural Sciences Laboratory**

Source of variation	d.f	Mean Squares			
		RL	SL	RH	RB
GCA <sub>m</sub>	7	25.32*	8.75**	757.52*	37.98***
GCA <sub>f</sub>	2	41.01**	12.81**	207.37	58.46***
		3059.47**			1005.42
CONC	1	*	124.23***	18871.89***	***
SCA	14	9.04	5.64*	173.52	14.86*
GCA <sub>m</sub> x CONC	7	41.67***	4.18***	574.70***	25.16***
GCA <sub>f</sub> x CONC	2	16.59***	3.21***	172.88***	24.66***
SCA x CONC	14	6.27***	1.85***	182.69***	4.765
Error	96	1.63	0.45	5.99	2.439

\*\*\*, \*\* and \* data significant at  $P= 0.001$ ,  $0.01$  and  $0.05$  respectively, d.f - degrees of freedom, RL - root length, SL - shoot length, RH - number of root hairs, RB - root biomass, Conc- concentration, GCA<sub>m</sub>- general combining ability effects due to male, GCA<sub>f</sub>-general combining ability effects due to females, SCA- specific combining ability.

#### 3.1.1 Evaluation of GCA effects

Further analysis showed that the male line L917 had a negative significant GCA effects of -2.35, -1.29, -4.28 and -1.30 for root length, shoot length, number of root hairs and root biomass respectively (Table 3). The other male line CML 457 had negative significant GCA values for the number of root hairs (-4.55) and root biomass (-1.30) while the male line CZL 04007 only had a negative significant GCA value for the number of root hairs (-8.18). On the other hand, a male line CML 511 had a positive significant GCA effect (8.58) for the number of root hairs. Among female lines, CML 538 exhibited a positive significant GCA effects of 0.92, 0.59, 2.13 and 0.96 for root length, shoot length, number of root hairs and root biomass

respectively. The female line CZL 083 had negative significant GCA effects for shoot length root biomass. Furthermore, female line L5527 had a positive significant GCA effect for root biomass (Table 3).

**Table 3: Significant GCA effects of parental lines used in the study of all measured parameters**

Parental lines	RL	SL	RH	RB
CML 457 <sup>m</sup>	-0.70	0.01	-4.55**	-1.30**
L12 <sup>m</sup>	-0.32	-0.68	1.18	1.67***
L5527 <sup>m</sup>	0.36	0.67	-2.12	2.69***
L917 <sup>m</sup>	-2.35**	-1.29**	-4.28**	-1.30**
CZL 04007 <sup>m</sup>	0.88	0.07	-8.18***	-0.52
CML 511 <sup>m</sup>	1.40	-0.07	8.58***	-0.75
L151 <sup>m</sup>	-0.29	0.69	-1.02	0.36
L5522 <sup>m</sup>	1.01	0.60	10.38***	-0.80
SE <sub>m</sub>	0.74	0.39	1.41	0.90
CZL 083 <sup>f</sup>	-0.79	-0.23***		-1.20***
CZL 0814 <sup>f</sup>	-0.14	-0.36***		0.24
CML 538 <sup>f</sup>	0.92*	0.59***		0.96***
SE <sub>f</sub>	0.45	0.06		0.55

NS-non significant; \*, \*\*, \*\*\*data significantly different from zero at P= 0.05, P= 0.01 and P= 0.001 respectively; RL- root length, SL-shoot length, RH- number of root hairs, RB- root biomass, SB- shoot biomass, SE- standard error of effect, <sup>m</sup> - represents GCA effects associated with male and female parent. <sup>f</sup> - GCA effects associated with female parent. SE- Standard error of the effect

### 3.1.2 Evaluation of SCA effects

Further analysis on significant parameters for SCA effects (Table 2), revealed that the crosses L 917 x CML 538 and CML 511 x CZL 0814 had a positive significant SCA effect values of 2.59 and 1.86 for root biomass respectively (Table 4). The crosses CML 457 x CZL 083, L5527 x CZL 083, L12 x CML 538 and L12x CZL 083 had a positive significant SCA effect value for shoot length. The crosses L552 x CML 538 and L5522 x CZL 083 had negative and positive significant SCAs for shoot length and root biomass respectively (Table 4).

### 3.3.3 Estimation of genetic parameters

The Bakers ratio for root length, shoot length, number of root hairs, and root biomass was found to be 0.49, 0.21, 1.0, and 0.28 respectively (Table 5). The broad sense heritability for root length, shoot length, number of root hairs, and root biomass was found to be 0.36, 0.64, 0.66, and 0.49 respectively. With narrow sense heritability, the values were computed as

0.18, 0.13, 0.66, and 0.14 for root length, shoot length, number of root hairs, and root biomass respectively.

**Table 4: Evaluation of Genotypic SCA effects for shoot length and root biomass**

<b>Crosses</b>	<b>SL</b>	<b>RB</b>
CML 457 X CZL 083	0.70**	-0.41
L5527 X CZL 0814	0.50*	0.86
L917 X CZL 0814	-0.97***	-0.52
CZL 04007 X CML538	0.34	-0.52
CML 511 X CML 538	-0.63**	0.71
L151 X CML 538	-0.09	-1.07*
L151 X CZL 0814	-0.05	0.14
L5527 X CZL 083	0.48*	-0.85
L5522 X CML 538	-0.69**	-1.24*
L151 X CZL 083	0.14	0.93
L917 X CML 538	1.92***	2.59***
L5527 X CML 538	-0.98***	-0.01
CZL 04007 X CZL 0814	-0.17	0.20
CML 511 X CZL 083	-0.24	-1.96***
L917 X CZL 083	-0.95***	-2.07***
L5522 X CZL 083	-0.54*	2.59***
L12 X CML 538	0.52*	-0.71
L12 X CZL 083	0.58*	1.42**
CML 457 X CZL 0814	-0.31	1.15
CML 457 X CML 538	-0.40	0.26
CML 511 X CZL 0814	0.87***	1.26*
CZL 04007 X CZL 083	-0.17	0.32
L12 X CZL 0814	-1.10***	-0.74
L5522 X CZL 0814	1.23***	-1.35*
<b>SE</b>	<b>0.67</b>	<b>1.56</b>

\*, \*\*, \*\*\* -data significantly different from zero at P= 0.05, 0.01 and 0.001 respectively,

RL-Root length, SL- Shoot length, RH- Number of root hairs, RB- root biomass, SE- standard error of the effect



**Table 5: Estimates of variances and baker's ratio**

<b>Variances and ratios</b>	<b>RL</b>	<b>SL</b>	<b>RH</b>	<b>RB</b>
$\sigma^2\text{SCA}$	0.46	0.63	0.00	1.68
$\sigma^2\text{GCA}_f$	0.45	0.12	0.91	0.49
$\sigma^2\text{GCA}_m$	0.00	0.04	10.67	0.15
$h^2\text{b.s}$	0.36	0.64	0.66	0.49
$h^2\text{n.s}$	0.18	0.13	0.66	0.14
Baker's ratio	0.49	0.21	1	0.28

$\sigma^2\text{SCA}$ -variance due to specific combining ability,  $\sigma^2\text{GCA}_f$ - variance due to female general combining ability,  $\sigma^2\text{GCA}_m$ - variance due to male general combining ability,  $h^2\text{b.s}$ - broad sense heritability,  $h^2\text{n.s}$ - narrow sense heritability, RL-Root length, SL- Shoot length, RH- Number of root hairs, RB- root biomass

#### 4. DISCUSSION

Assessments of Al tolerance based on root growth has been used extensively in genetic and molecular studies in crop plants [21]. Studies show that root growth inhibition is a major effect of Al toxicity and the extent of inhibition depends on both the genotype and Al concentration [21]. The root system in maize is crucial for plant establishment as well as water and nutrient uptake [22].

CML 538 had desirable GCA effects for root length, shoot length and number of root hairs. CML 511 and L5522 also had desirable GCAs for the number of root hairs. As elucidated by Tembo et al. [17], CML 538 can be crossed with high yielding single cross hybrids that are not tolerant to Al toxicity to generate high yielding Al tolerant three way cross hybrids. The root length and number of root hairs are critical as they help plants to access water and nutrients from the soil. The desirable GCA effect (0.92) exhibited by CML 538 is a confirmation of its tolerance to Al toxicity. In this study, significant positive GCA and SCA

were desirable while negative combining ability effect were undesirable as they depict unfavourable response to Al toxicity tolerance.

L917 displayed undesirable GCA effects for root length, shoot length and number of root hairs. Thus L917 can be crossed with CML538 (desirable GCA) to create a mapping population to use in identifying associated QTL's to Al tolerance [23]. The desirable SCA effects observed from crosses between the desirable (CML 538) and the undesirable (L 917) combiner parents (L917 X CML 538) for shoot length and root biomass could be due to non-additive effects [24]. The baker's ratio of 1 for the number of root hairs indicate the absolute importance of additive gene action for this parameter while 0.21 and 0.28 shows the predominance of non-additive gene effect for both the shoot length and root biomass respectively [20]. Both additive and non-additive gene action were important for the root length as indicated by a Baker's ratio of 0.49. This study is in agreement with Magnavaca [25] who found that both additive and non-additive gene action played a role in the inheritance of Al tolerance in maize by using backcrossed generation ( $BC_1$  and  $BC_2$ ),  $F_1$ ,  $F_2$  and their parents ( $P_1$  and  $P_2$ ). This study endeavored to further understand the nature of inheritance to Al tolerance by evaluation  $F_1$  hybrids using North Carolina design II mating design.

The finding for root length being determined by both additive and non-additive gene action as indicated by the Baker's ratio of 0.49 for root length, may imply that hybridization and approaches that utilize recurrent selection (population improvement) can be employed as a breeding program. However, the revelation of low narrow sense heritability (0.18) influencing Al trait, entails that hybridization is the best option in breeding for Al toxicity.

## CONCLUSION

The type of gene action conditioning Al toxicity tolerance in tropical maize was investigated to be both additive and non-additive gene action implying that hybridization and approaches that utilize recurrent selection (population improvement) can be employed as a breeding strategy for this trait. However, the revelation of low narrow sense heritability (0.18), prioritizes hybridization as the best option as it will be quicker and cheaper to employ. The finding of low narrow sense heritability influencing Al trait entails involvement of many cycles if population improvement strategy is employed.

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