



# Effect in Hydroponics of Nitrogen and Aluminium Toxicity on Tropical Maize

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## Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

## Article Information

DOI: 10.9734/ARJA/2018/42979

### Editor(s):

(1) Dr. Tancredo Souza, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal.

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Complete Peer review History: <http://www.sciedomain.org/review-history/25594>

Original Research Article

Received 9<sup>th</sup> May 2018  
Accepted 17<sup>th</sup> July 2018  
Published 19<sup>th</sup> July 2018

## ABSTRACT

Maize (*Zea mays* L.) is the most important cereal crop for most countries in the sub-Saharan Africa. Nitrogen (N) deficiency and aluminium (Al) toxicity are among the abiotic stresses leading to low maize productivity. Lack of N and Al toxicity in soil entails, in most cases, application of nitrogenous fertilizer and lime to ameliorate N deficiency and Al toxicity in the soil respectively. However, excessive application of nitrogenous fertilizers and lime may lead to environmental pollution and unavailability of nutrients due to increased pH respectively. To maximize yields and profits, the use of maize genotypes with high N-use efficient which are tolerant to Al toxicity presents a sustainable approach in this scenario. The objectives of this study were therefore i) to evaluate the effect of varying concentrations of N and Al in hydroponics, on genotypic responses of tropical maize and ii) the prospects of selecting genotypes with dual 'N use efficient' and 'Al tolerant trait'. An experiment was carried out in a 9 x 3 x 2 factorial scheme under hydroponic conditions with nine maize genotypes (CML 538, CZL 113, CML 134, CML 537, CML 312, CML 489, CZL 112, CZL 0814 and CML 444) submitted to three nitrogen doses (0, 21.3 and 42.6 mg L<sup>-1</sup>) and two aluminum doses (0 and 20 mg L<sup>-1</sup>), completely randomized design with two replicates. Results showed a significant difference among the genotypes with CZL 0814 genotype identified as the best performing genotype in hydroponics across N and Al concentration levels. However, consideration of variance components showed that N main effect contribution on genotypic performance was lower

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compared to Al and genotypic main effects. These results imply that availability of N for plant uptake could have been affected by the availability of Al in solution. Evaluation for dual trait, N use efficiency (NUE) and Al tolerant requires assessing genotypes for N stress factor and further subject the candidate genotypes to Al stress or vice versa.

*Keywords: Hydroponics; maize; root length; shoot length; variance component.*

## 1. INTRODUCTION

Maize (*Zea mays* L.) is ranked as the third mostly grown crop after wheat and rice in the world. It is also a versatile crop; growing across a range of agro-ecological zones [1]. It is the most important cereal crops and a staple food (for an estimated 50% of the population) for most countries in the sub-Saharan Africa [2]. It is mainly used for human consumption and animal feed and it is an important source of carbohydrates, protein, iron, vitamin B, and minerals. Maize is consumed as a starchy base in a wide variety of porridges, grits, and beer. Green maize (fresh on the cob) is eaten parched, baked, roasted or boiled; playing an important role in filling the hunger gap after the dry season. Every part of the maize plant has economic value: the grain, leaves, stalk, tassel, and cob can all be used to produce a variety of food and non-food products. The calories contribution from consumed maize is about 50% in Southern Africa when compared to other sources [3]. Per capital consumption of maize grain in Zambia was estimated at 140 kg per year [4].

In Zambia, maize is the most important (staple) agricultural crop and about 78% of the total area under cereal production is allocated to the crop [4]. It is grown in most areas, with the exception for wet, dry or infertile places where sorghum and millet are primarily grown [5]. It is regarded as a priority crop of economic importance in Zambia and the government subsidies input availability to ensure improved yields and ultimately food security. The production of maize is, however, hampered by both biotic and abiotic factors.

Among the abiotic factors, nutrient deficient, acidity and drought are the major factors leading to serious yield losses [6,7]. N deficiency and Al toxicity in soils affect crop productivity in maize leading to reduction in biomass production and ultimately yield losses of up to more than 90% [8, 9]. Primarily, Al toxicity impedes plant growth by morphological inhibition and reduction of root growth. It limits the ability of roots to scavenge

for nutrients and restricts the depth of penetration, resulting in a poorly developed root system, leading to nutrient deficiencies and eventually reduced grain yields. In soil medium an increase in Al reduces cation exchange capacity and increases leaching of N-nitrate. Genotypic differences to Al tolerance in maize genotypes is due to genetic variations in i) exclusion of Al from the root tips, and ii) absorbance, but tolerance of Al in root cells [10]. Lack of N nutrient and Al toxicity in soil, entails in most cases, application of nitrogenous fertilizer and lime to ameliorate N deficiency and Al toxicity in the soil respectively. However, on the other hand excessive application of N and lime leads to environmental contamination which may lead to unavailability of other nutrients due to increased pH respectively [11,12]. Furthermore, application of inorganic fertilizers (N fertilizers and lime) is costly and not feasible to resource constrained small scale farmers. To maximize yields and profits, the use of maize genotypes with high N-use efficient and tolerance to Al toxicity is the sustainable and affordable approach for resource poor farmers. Assessing of maize genotypes to mineral ions or cations is more effective hydroponically as compared to soil medium due to easy availability of minerals to root surfaces [13,14]. The objectives of this study were therefore to i) evaluate the effect of varying concentrations of N and Al in hydroponics, on genotypic responses of tropical maize and ii) the prospects of selecting genotypes with dual 'N use efficient' and 'Al tolerant trait'.

## 2. MATERIALS AND METHODS

### 2.1 Study Location and Germplasm

The maize inbred lines utilised in the experiment were obtained from the Golden Valley Agricultural Research Trust (GART) maize improvement center in Chisamba district of Zambia. The hydroponic study was conducted in laboratory in Lusaka at the University of Zambia (28° 19' E; 15° 23' S), Department of Plant Science, at the School of Agricultural Sciences.

## 2.2 Experimental Design, Treatments and Nutrient Solution Management

An experiment was carried out in a 9 x 3 x 2 factorial scheme under hydroponic conditions with nine maize genotypes (CML 538, CZL 113, CML 134, CML 537, CML 312, CML 489, CZL 112, CZL 0814 and CML 444) submitted to three nitrogen doses (0, 21.3 and 42.6 mg L<sup>-1</sup>) and two aluminum doses (0 and 20 mg L<sup>-1</sup>), completely randomized design with two replicates, in a total of 108 experimental units. Each unit constituted 50 ml solution in a test tube.

The nutrient solutions were prepared using a modified protocol by Kerridge and Kronstad [15] (Table 1). The 42.6 mg L<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub> was added as an optimal requirement for N, while the sub-optimal and control were 21.3 and 0 mg L<sup>-1</sup> respectively. The 20 mg L<sup>-1</sup> Al was used as a concentration upper limit used in discriminating genotypic responses to Al concentration as performed by Chanda et al. [16].

The pH was initially adjusted to 4.2 to make available the toxic species of Al<sup>3+</sup> and Al (OH)<sup>2+</sup> which are toxic to plants and adjustments were done using HCl and NaOH buffer solutions before being transferred to 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) (Reckitt Benkiser East Africa Limited, Nairobi, Kenya).

## 2.3 Placement of Maize Seedlings

The germination of maize genotypes was done on separate petri dishes lined with filter paper

soaked in distilled water and placed in the germination chamber for 5 days at 25°C. Seedlings of uniform root length (approximately 15 mm) were selected and transferred to test tubes containing nutrient solutions with different combinations of Al and N concentrations. 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 (Sonic 9905).

## 2.4 Data Collection and Analysis

The evaluation of genotypes was done on 11<sup>th</sup> day following the procedure of Kerridge and Kronstad [15]. The shoot and root lengths were measured immediately after harvesting using a 30 cm ruler. The number of root hairs were also counted.

Analysis of variance was performed using a fixed model and means of roots lengths, shoot length and numbers were separated using the fisher protected Least Significant Difference (LSD) method, at a significant level of  $\alpha = 0.05$ . In addition a multiple fisher protected Least Significant Difference at a significant level of  $\alpha = 0.05$  was also performed to compare more than two means where appropriate. Correlation analysis for genotypic means on measured roots and shoots was also performed. All the data analysis was carried out using GenStat statistical package [17]. Variance components were computed assuming a fixed model, as demonstrated by Searle et al. [18].

**Table 1. Hydroponic solution used in the experiment**

Nutrient	* Conc. (mg L <sup>-1</sup> )	Chemical formula	Compound name
N	* Varied	NH <sub>4</sub> NO <sub>3</sub>	Ammonium nitrate
K	23.5	K <sub>2</sub> HPO <sub>4</sub> .3H <sub>2</sub> O	Potassium hydrogen phosphate trihydrate
Zn	0.16	ZnSO <sub>4</sub> .7H <sub>2</sub> O	Zinc sulphate heptahydrate
Mg	14.6	MgSO <sub>4</sub> .7H <sub>2</sub> O	Magnesium sulphate heptahydrate
Cu	0.06	CuSO <sub>4</sub> .5H <sub>2</sub> O	Copper sulphate pentahydrate
Fe	1.67	FeSO <sub>4</sub> .7H <sub>2</sub> O	Iron sulphate heptahydrate
Ca	48.1	CaCl <sub>2</sub> .2H <sub>2</sub> O	Calcium chloride dehydrate
Mo	0.03	NaMoO <sub>4</sub> .2H <sub>2</sub> O	Sodium molybdate dehydrate
Mn	0.03	MnSO <sub>4</sub> .H <sub>2</sub> O	Manganese sulphate monohydrate
B	0.32	H <sub>3</sub> BO <sub>3</sub>	Boric acid
Al	* Varied	AlK(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O	Aluminium potassium sulphate dodecahydrate

\* Concentration, \* factorial scheme under hydroponic combinations of three nitrogen doses (0, 21.3 and 42.6 mg L<sup>-1</sup>) and two aluminum doses (0 and 20 mg L<sup>-1</sup>)

### 3. RESULTS

They were significant differences ( $P < 0.001$ ) among genotypes with regards to measured parameters (root length, root hairs and shoot length) across N and Al concentrations. The interaction between genotype x N content across Al concentrations for measured root length and root hairs was found to be highly significant ( $P < 0.001$ ). The 3- way interaction (genotype x Al x N) was also found significant across all measured parameters (Table 2). The highest mean root length (11.6 mm), mean number of root hairs (41.3) and shoot length (14.5 mm) were recorded by CZL112, CZL112 and CZL0814 respectively (Table 3).

A trend showed that all the three mean measured parameters across genotypes and N concentrations reduced with an increment in Al content (Table 4). However significant mean differences among N concentration for measured parameters occurred only for root length elongation across genotypes and Al concentrations (Table 5).

Further analysis revealed a weak but significant genotypic correlation ( $P = 0.002$ ,  $r = 0.28$ ) between root and shoot length mean values across Al and N concentration. An analysis on variance components revealed the components due to genotypic and Al as the major contributors to parameter responses among the main effects (Table 6).

**Table 2. Mean squares for measured parameters evaluated in hydroponics at the University of Zambia, School of Agricultural Sciences, Zambia**

Source of Variation	d.f.	Mean square		
		Root length	Root hairs	Shoot length
Genotype	8	46.8***	759.3***	124.7***
Aluminium	1	1090.8***	26245.5***	552.1***
Nitrogen	2	74.0***	420.1	41.2
Genotype x Al	8	18.8*	255	25
Genotype x N	16	28.4***	591.2***	11.6
Al x N	2	135.3***	126.8	13
Genotype x Al x N	16	29.7***	585***	26.6*
Error	54	8.9	169.9	13.2

\*, \*\*\* Data significant at  $P = 0.05$  and  $P = 0.001$  respectively

**Table 3. Genotype means for measured parameters across Al and N concentration evaluated in hydroponics at the University of Zambia, School of Agricultural Sciences, Zambia**

Genotype	Root length (cm)	Root hairs (numbers)	Shoot length (cm)
CZL 112	11.6a	41.3a	14.0a
CML 537	8.4b	21.9bc	13.7a
CML 444	7.2bc	15.5bc	5.3d
CML 312	7.0bc	20.2bc	11.9a
CML 538	7.0bc	13.7c	8.0cd
CZL 0814	5.7c	20.7bc	14.5a
CZL 113	5.7c	25.2b	13.9a
CML 134	5.6c	20.4bc	8.9bc
CZL 04007	5.2c	20.2bc	11.8ab
<b>* LSD (<math>\alpha = 0.05</math>)</b>	<b>2.4</b>	<b>10.7</b>	<b>3.0</b>

\* Fisher Protected Least Significant Difference. Means within columns followed by the same letter(s) are not significantly different at  $P \leq 0.05$  level as computed by Multiple Fisher Protected Least Significant Difference

**Table 4. Means for measured parameters across genotype x N concentration evaluated in hydroponics at the University of Zambia, School of Agricultural sciences, Zambia**

Aluminium ( $\text{mg L}^{-1}$ )	Root length (cm)	Root hairs (numbers)	Shoot length (cm)
0	10.2	37.7	13.6
20	3.9	6.5	9.1
<b>* LSD (<math>\alpha = 0.05</math>)</b>	<b>1.2</b>	<b>5.0</b>	<b>1.4</b>

\* Fisher Protected Least Significant Difference

**Table 5. Means for measured roots across genotype x Al concentration evaluated in hydroponics at the University of Zambia, School of Agricultural Sciences, Zambia**

Nitrogen (mg L <sup>-1</sup> )	Root length (cm)
0	8.58a
21.3	5.73b
42.6	6.85b
<b>* LSD (α = 0.05)</b>	<b>1.4</b>

<sup>‡</sup> Fisher Protected Least Significant Difference. Means followed by the same letter(s) are not significantly different at P ≤ 0.05 as computed by the multiple Fisher Protected Least Significant Difference

**Table 6. Variance components for measured parameters evaluated in hydroponics at the University of Zambia, School of Agricultural Sciences, Zambia**

Source	Variance components		
	Root length	Root hairs	Shoot length
Genotype (G)	25.3	392.93	74.33
Aluminium (Al)	20	482.88	9.98
Nitrogen (N)	3.6	13.9	1.55
G x Al	13.2	113.47	15.73
G x N	78	1685.2	0
Al x N	14.04	4.789	0
G x Al x N	166.4	3320.8	107.2
Error	8.9	169.9	13.2

#### 4. DISCUSSION

The productivity of maize greatly depends on soil fertility. N deficiency and soil acidity leads to serious yield losses and ultimately affecting maize production. Therefore, this work aimed at evaluating the effect of varying concentrations of N and Al in hydroponics, on genotypic responses of tropical maize inbred lines.

In this research, significant differences were obtained among genotypes with regards to root length, number of root hairs and shoot length across Al and N content. Further evaluation (Table 2) revealed that the genotype CZL112 had the highest mean elongated root (11 cm), including higher mean number (41.3) of root hairs implying that it probably could be the most efficient at utilising N and tolerating Al. Previous work deduced that root elongation is the function of Al toxicity and N use efficient with Al tolerant genotypes and good N-use efficient genotypes exhibiting relatively longer roots when compared

to lesser efficient genotypes [19,20]. Upon further analysis of the shoot length, the genotype CZL0814 exhibited the longest mean shoot length across N and Al concentrations despite elongating relative shorter roots as compared to CZL 112. The implication is that the genotype CZL 0814 could be relative more efficient at utilising N and tolerating Al with regards to shoot length. The positive weak but significant (P= 0.002) correlation (r= 0.28) exhibited by a relationship between root and shoot length indicates that other factors other than root elongation probably have an effect on genotypic Al tolerance and N utilisation. Genotypes can have same capacity to tolerate Al in solution but tolerance of Al in root cells (cytoplasm) may differ among genotypes [8,10]. In this regard CZL 0814 can be considered better candidate than CZL112 at utilising N and tolerating Al. Significant differences between Al concentrations was obtained for all measured parameters across genotypes and N concentration. The mean performance for all parameters being significantly lower in 20 Al as compared to mean performance at 0 Al concentration (Table 4). These results are similar to an earlier study which deduced that mean root length and shoot length decreases with an increment in Al concentration due to impairment of the central cylinder inside the roots [19]. The 3- way interaction (G x Al x N) was significant across all measured parameters implying that they were significant differences among the means of the treatment combinations. This could be attributed to variations in response of different genotypes to different concentration combinations of N and Al.

Significant differences among N concentration was obtained only for root elongation with a mean highest longer root length (8.58 cm) obtained at 0 mg L<sup>-1</sup> N. This is similar to work done by Ranjitha et al. [20] on wheat which deduced that the mean root length increases with lower concentrations of N in solution. However, consideration of variance components (Table 6) among the main effects showed that the variance component for N main effect was more than five times lower than the components for genotype and Al main effects for all the measured traits. The implication is that omission of N factor in the experiment will have little effect on parameter response in the presence of Al ions at low pH. This is in agreement with previous work done on soya bean which deduced that root elongation was more sensitive to Al that N- nutrient availability [21].

## 5. CONCLUSION

The N- nutrient availability to maize is to a large extent affected by the presence of Al in solution. Therefore, an efficient method to select genotypes for better performance at utilising N in acidity soils or nutrient solutions can best be done by evaluating for N user efficiency and thereafter screening the candidate genotypes for Al tolerance or vice versa. It is imperative to ensure that the soils are tested and ameliorated for acidity before N fertilizer is applied to ensure optimised use of N.

## ACKNOWLEDGEMENT

The author acknowledges Mr. Sydney Mpimpa and Mr. Alex Bwalya for their assistance with Laboratory work.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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