GENETIC ANALYSIS OF TOLERANCE TO ALUMINUM TOXICITY IN MAIZE (*Zea mays*)

BY

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A dissertation submitted to the university of Zambia school of agricultural sciences in partial fulfillment of the requirements for the award of the degree of master of science in plant breeding and seed systems

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DECLARATION

I, **Victoria Ndeke,** hereby declare that that contents submitted in this dissertation is my own work and has never been submitted for any degree at this University or any other institution of learning.

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APPROVAL

This dissertation of **Victoria Ndeke** is approved by the University of Zambia as fulfilling the requirements for the award of the degree of Master of Science in Plant Breeding and Seed Systems

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ABSTRACT

Aluminum (Al) toxicity causes high yield losses and is directly linked to acidic soils. Application of lime can ameliorate this problem, but it is costly and not feasible for small scale farmers as they need to purchase both seed and lime. Developing maize varieties that are tolerant to Al toxicity is cheaper and feasible for small scale farmers. The purpose of this research was to i) identify maize genotypes with tolerance to Aluminum toxicity, ii) evaluate the general and specific combining abilities for the inbred lines and crosses respectively, iii) investigate the type of gene action conditioning tolerance to aluminum toxicity in tropical maize, and iv) determine if shoot length can be used as an indirect selection criterion for Al tolerance. Fourteen maize inbred lines (CZL 083, L151, L552, CZL0814, CML312, L12, L3233, CML511, L917, L2, CML538, L5522, CML 457, and CZL04007) were evaluated in hydroponic conditions containing different concentrations of Al (0, 5, 10, 15 and 20mg/ L) in a 14 x 5 factorial completely randomized design with 3 replications at the University of Zambia, plant physiology laboratory. Five parameters were measured: root length, shoot length, number of root hairs, root and shoot biomass on eleventh day after seed placement. Highly significant differences (P=0.001) were noted for both root and shoot lengths. The results showed that inbred line CML 538 was highly tolerant while inbred line L151 was the most susceptible with mean lengths of 15.44 cm and 6.11 cm respectively. To address objective ii, eleven inbred lines were selected and mated in an 8 male (4 moderately tolerant and 4 susceptible) x 3 female (resistant) North Carolina Design II. Results revealed that GCA effects due to both males and females were highly significantly (P= 0.001) different from zero for root biomass. The shoot length GCA effects (8.75 and 12.81) 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. The SCA effects for the shoot length and root biomass were significant (P= 0.02). Both additive and non-additive gene action were identified to be important for the root length as indicated by a Baker's ratio of 0.49. The association of root length to shoot length was significant (correlation [r] = 0.72).

Keywords: Aluminum, tolerance, General Combining ability, Specific Combining ability, *Zea mays*, Inbred lines, crosses.

DEDICATION

This work is dedicated to my wonderful husband Samson B Mbewe, my lovely children (Cornelius and Tamando), and my brother Martin for their love and support.

To my late my grandmother and mother.

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ACRONYMS AND ABBREVIATTIONS

Al	Aluminum
Ca	Calcium
Ca^{2+}	Calcium ions
CO_3^{-2}	Carbonate ions
CNV	Copy number variation
CRD	Completely randomized design
Fe	Iron
GCA	General combining ability
H	Hydrogen
IITA	International Institute of Tropical Agriculture
JAICAF	Japan Association for International Collaboration of Agriculture and Forestry
K	Potassium
K^+	Potassium ions
MATE	Multidrug and toxic compound extrusion
MDA	Malonyldialdehyde
Mg	Magnesium
Mg^{2+}	Magnesium ions
Mn	Manganese
Na^+	Sodium ions
P	Phosphorous
RB	Root biomass
RH	Root hairs
RL	Root length
ROS	Reactive Oxygen Species
SCA	Specific combining ability
SB	Shoot biomass
SL	Shoot length
USDA	United State Department of Agriculture
UNZA	University of Zambia
WHO	World Health Organization
ZARI	Zambia Agricultural Research Institute

CHAPTER 1: INTRODUCTION

1.1 Importance of maize

Maize is an important cereal crop in the world as it is a staple food in most countries. It is the primary food grain in Mexico, Central America, the Andean Region of South America, Eastern and Southern Africa and is important as a grain in china (Sleper and Poehlman, 2013). In addition, it is a major source of income to farmers among whom many are resource poor in developing countries (Tagne, *et al*., 2008).

It is rich in B-complex vitamins such as B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) and B6 that makes it commendable for hair, skin, digestion, heart and brain (Kumar and Jhariya, 2013). Its grain is also a rich source of starch and minerals while some varieties also contain lysine and tryptophan which are essential amino acids for human beings. The starch extracted from maize grain is used in making confectionary and noodles (Oladejo and Adetunji, 2012).

Maize is processed and consumed differently in the 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 for cooking and industrial application. Maize is widely used as source of dietary energy in sub Saharan Africa. The fresh grains are eaten roasted or boiled on the cob. The grains can be dried and cooked in combination with some edible leguminous crops like cowpeas or beans (IITA, 1982). It is additionally milled and used in preparing, nshima, porridge, and traditional drinks (referred to as *tobwa*, and *munkhoyo* or *chibwatu* in Zambia. It is also used as a main ingredient in the brewing industry and also as a feed for farm animals. According to Ranum *et al*., (2014), Zambia is ranked number three among countries with the highest maize consumption by World Health Organization (WHO) with per capital consumption of 243grams per day.

In Zambia, maize production has been reported to be increasing (Mwambazi, 2017). It recorded a 25 % increment from 2,873,052 metric tonnes in the 2015/2016 season to 3,606,549 metric tonnes in the 2016/17 season. The contributing factors to the high production are increased area planted, favorable agro meteorological conditions. The area under maize increased by 20.5 percent to 1,644,74 hectares in the 2015/2016 season from 1,364977 hectares in the 2014/2015 season (Mwambazi, 2017). The planting area under maize production is greater than other crops in Zambia and smallholders account for 79% of maize production in the country.

1.2 Maize production constraints

Challenges faced by farmers in Zambia include both biotic and abiotic factors. Among the abiotic factors include drought, salinity, floods, low nitrogen and Aluminum toxicity and these are among the primary causes of yield loss. Aluminum toxicity has been found to cause yield losses of up to 80% (Lungu, 2009). Al concentration in the soil rarely exceeds 4 parts per million (ppm), and its interaction with various organic and inorganic components of soil is complex. Generally, Al toxicity may be observed at any pH below 5.5 (Singh, 2009). The initial stage of aluminum toxicity is considered to result from Al-induced membrane instability. Seedlings are more sensitive to Al than are older plants. In many species, Al toxicity also induces deficiency for phosphorous, calcium or iron. Low pH is unfavorable for maize growth especially because in many soils it is combined with high soluble aluminum content, which is toxic and yield depressing. The soils in high rainfall areas which are usually acidic (pH of less than 5) are less suitable for maize. 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 yield reduction of up to 69% in maize (Tandzi *et al.*, 2015). Frequently maize can only be grown in the first year after clearing and burning, when the ash temporarily increases the surface soil pH. Continuous cultivation of such acid soils would require liming (IITA, 1982).

Aluminum (Al) toxicity symptoms in plants are visible in the form of necrotic spots and lesions along the leaf margins (Malekzadeh *et al.*, 2015). Aluminum stress is known to induce many abnormalities like damage to membranes, generation of reactive oxygen species (ROS), protein denaturation and accumulation of toxic compounds at various organizational levels of the cells. These ROS interrupt normal metabolism in plants by lipid peroxidation of membrane, denaturation of proteins and nucleic acids.

Arora *et al.*, (2017), pointed out that Al stress is primarily visible in the roots. Aluminum toxicity reduces both root and shoot growth. It also causes root discoloration and inhibits lateral root formation (Singh, 2009). Reduction in root growth reduces the absorption of nutrients and water, and consequently, crop yield

(Fageria *et al*., 1988). The root meristem is the sensitive site. Root tips have been found to be the primary site of Al injury, and the distal part of the transition zone has been identified as the target site in maize (Krstic *et al*., 2012). Aluminum is also reported to interfere with cell division in the root and lateral roots and altering root membrane structures and functions. Root respiration is also reduced. An abnormal distribution of ribosomes on the endoplasmic reticulum of root cells results and Interfere with protein synthesis (Arora *et al*., 2017). Aluminum is known to induce a decrease in mitotic activity in many plants, and the aluminum-induced reduction in the number of proliferating cells is accompanied by the shortening of the region of cell division in maize (Krstic *et al*., 2012).

Toxicity can be reduced through lime application by raising soil pH, however this amendment does not remedy subsoil acidity, and liming may not always be practical or cost-effective (Lidon and Barreiro, 2002). Selection of plants tolerant to Al toxicity can be a complementary method to liming to overcome Al toxicity (Fageria *et al*., 1988).

1.3 Statement of the Problem

The high rate of leaching especially in northern Zambia has left the soils acidic and depleted of nutrients hence, making them inherently infertile and generally strongly acidic with high levels of Al content (Banda, 2009). Currently liming is used to control soil acidity, but this is costly for small scale farmers. Over-liming can also have detrimental effects on crop productivity just like soil acidity (Lungu, 2009). It may cause an increase in soil pH resulting in phosphates being unavailable for plant uptake. Over-liming can also cause an increase in pore space in the soil resulting in loss of water retention properties by the soil.

1.4 Justification of the study

Developing maize varieties that are tolerant to Aluminum toxicity is a cheaper and feasible way to small scale farmers, because once farmers purchase tolerant seed, there is no need of purchasing lime. The genetic variability among genotypes within the same species exists for tolerance to Al toxicity. Aluminum (Al) tolerance is genetically controlled (Singh *et al*., 2011); 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 the field screening is difficult. The evaluation of field performance under Al stress conditions is also rendered difficult due to field soil 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 (Singh *et al*., 2011).

Evaluating in hydroponic enhances efficiency of estimating genetic parameters. Plants directly absorb the Al in the solution compared to the field where uptake may be inhibited by soil particles. In addition, there is better control over plant growth. A soil system is difficult to keep in control due to the complex chemical and biological nature of the soil. Plant nutrients are frequently not available to plants due to poor soil structure or unfavorable soil pH value. Other plants growing in soil are also frequent competitors for the essential nutrients in the soil solution. Root length is often evaluated in hydroponic cultures and used as the basis of selection for Al tolerance due to its direct sensitive reaction when exposed to Al solution (Prioli *et al*., 2002, Singh, 2009). Genotypes that exhibit longer roots when exposed to Al concentrated solution are more tolerant (S´onia, 2012, Abate *et al*., 2013, Chanda *et al*., 2015 Tembo, 2018).

In initiating a breeding program, prior knowledge of the combining abilities is essential for a breeder. It provides information on performance of a genotype in cross combinations (Fasahat *et al*., 2016). Understanding the nature of gene action enables the breeder to understand the type of breeding strategy to employ in a breeding program. Additive and non-additive gene action entails recurrent breeding methods and hybridization as optional breeding strategies respectively (Maqbool *et al*., 2018).

Evaluating for Al tolerance may entail primary utilization of roots, which is a distractive approach. It remains to be established weather the use of shoot which is non distractive can be employed as an indirect selection criterion in maize**.**

1.5 Objectives

1.5.1 Overall objective

To carry out genetic analysis for tolerance to Al in tropical maize.

Specific Objective

1. To identify maize genotypes with tolerance to Aluminum toxicity
2. To evaluate the general and specific combining abilities for the inbred lines and crosses respectively.
3. To investigate the type of gene action conditioning tolerance to aluminum toxicity in tropical maize.
4. To determine if shoot length can be used as an indirect selection criterion for Al tolerance.

1.6 Research hypothesis

1. Tropical maize inbred lines that are tolerant to Aluminum toxicity exist
2. Crosses with good specific combining ability and inbred lines with good general combining ability exists.
3. The nature of gene action conditioning Aluminum toxicity is additive
4. Shoot length can be used as an indirect selection criterion for Al toxicity tolerance

1.7 Organization of the dissertation

This dissertation has six chapters. Chapter two comprises of an examination of the literature that was done to help understand the state of the current knowledge and limitations in the knowledge that motivated this study. In chapter three, an explanation of how the materials and methods that were used in the study was done. This explanation consists of names of the inbred lines used in the experiment. This chapter also explained the study design and the methodologies that were used in the different experiments that were part of the whole study. The detailed methods and formulas for each of the experiments in the study were presented. Chapter four presents the results while Chapter five comprise the discussion. Chapter six presents the conclusions and recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1 Origin of maize

Maize originated from Mexico and spread northwards to Canada and southwards to Argentina (Brown *et al*., 1985). It is a member of the Poaceae family (together with wheat, rice, oats, sorghum, barley and sugarcane), the world's most successful family of agricultural crops (Tian *et al*.,2009). Maize belongs to the genus Zea and it is adapted to a wide range of climates making it extensively distributed over the earth compared to any other cereal crop (Lidon and Barreiro, 2002). It grows from 48^0 N to about 40^0S latitude all over the world, and it also grows from below sea level to about 4000 meters. The closet ancestor of maize is believed to be teosinte from which the present day maize evolved (Singh and Kota, 2007).

The ideal soil for maize is a deep, medium-textured, well-drained, fertile soil with a high water holding capacity, but it also grows on a wide variety of soils giving high yields if the crop is well managed. This plant species prefers soils having a pH ranging between 5.5 and 8.0, the optimum range being 5.5 to 7.0 (Lidon and Barreiro, 2002).

2.2 Morphology of maize

Maize belongs to the family of grasses (*Poaceae*). The typical maize plant is a tall (1-4m) annual grass (monocot) which forms a seasonal root system bearing a single erect stem which is made of nodes and internodes. (Anonymous, 2008) (Figure 1). Hochholdinger, (2009), reported that the node is the origin of all lateral outgrowth such as roots, branches, leaves and ears.

The leaves are arranged alternatively along the length of stem (Anonymous, 2011, Wallace and Bressman,1949,). The upper leaves in maize serve the purpose of intercepting light and are therefore major contributors of photosynthates to the grain. The internode that bears the ear is longitudinally grooved in order to allow proper positioning of the cob (Anonymous, 2011). All maize genotypes follow the same general pattern of development, although specific time and interval between stages and total number of leaves developed may vary between different hybrids, seasons, time of planting and location. The leaf is made of three parts namely: the leaf sheath which comes from the node and surrounds the stalk, hiding all of it except a short upper portion; the blade which is composed of the midrib, veins and intracellular tissues, and the ligule which is a collar that prevents water, dirt, and insects from running down the sheath and the stalk. It is located at the hinge the leaf sheath and the blade.

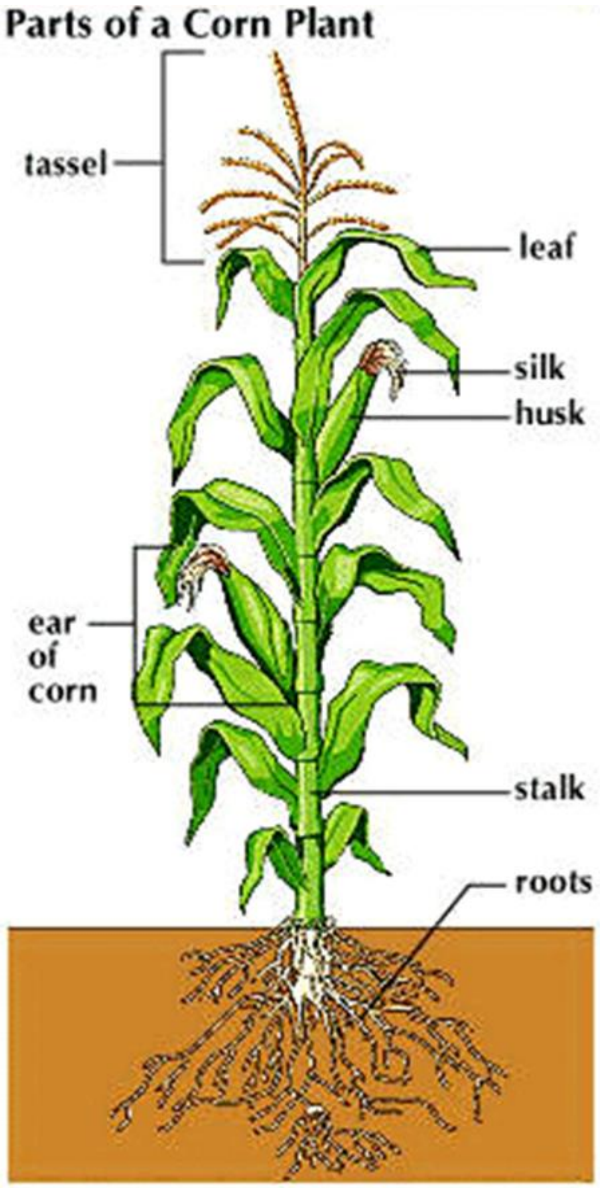

Figure 1: A full grown maize plant with different parts. (Source: Kids, 2019*)*

The root system of maize is made up of roots that are formed during embryogenesis and those formed during postembryonic development. The embryonic root system is made up of a primary root that is formed at the basal pole of the embryo and a variable number of seminal roots (Hochholdinger, 2009). Post-embryonic (shoot borne and lateral roots) root development approximately starts a week after the primary and seminal roots have emerged, as branching of the embryonic roots leads to production of lateral roots that can continue to branch. Together with root hairs, the lateral roots play an important role in the absorption of nutrients and water by increasing the root's surface area (Hauck *et al*., 2015). The embryonic primary and seminal roots make up the major portion of the seedling root stock in the first weeks after germination, while the postembryonic shoot-borne roots form the major backbone of the maize root system later in development. The Shoot borne roots formed below the soil surface are called crown roots and are responsible for lodging resistance while those formed above the ground are called brace roots (Hochholdinger, 2009). Lateral roots have a great influence on root architecture and play a major role in water and nutrient uptake. The stages of growth of maize are broadly divided into the vegetative and reproductive stages (Anonymous, 2011).

Maize is a monoecious plant implying that the sexes are partitioned into separate pistillate (ear), the female flower and staminate (tassel), the male flower (Anonymous, 2011). Maize is predominantly cross pollinated due to this type of flower arrangement. The estimation is that 5 percent or less of the maize grown under field conditions is selfed. Flowers and ovules on the ear develop from the base and proceeds upwards. The receptive silks (moist and sticky) emerge over the husks in a period of three to five days and are capable of growing to 30.5cm long, extending beyond the husk (Anonymous, 2008). The silks have short hairs, trichomes, which form an angle to the stylar canals and help in harboring pollen grains.

Maize is generally protandrous (male flowers reach maturity before female flowers) (Anonymous, 2008, Anonymous, 2011). The male flowers are located in spikelets on the tassel of the plant. It has been approximated that 1000 individual spikelets form on every single tassel and each one bears two florets. Each floret in turn contains three anthers. Pollen is dispersed through pores that open at the tips of the anthers. It is estimated that each tassel produces 2 to 25 million pollen grains. The length of pollen viability depends on the temperature (Nielsen, 2016).

Maize is a C4 plant and is therefore more efficient at utilizing carbon dioxide than C3 plants (Anonymous, 2008). Compared with other plants, maize allows a continued response to increasing radiation up to full sunlight coupled with low levels of photorespiration.

2.3 Classification of Maize

There are different types of maize grown throughout the world with color as a major difference (Ranum *et al*., 2014). Maize kernels have different colors ranging from white to yellow to red to black. Yellow maize is mostly popular in the United States while white maize is preferred in Africa, Central America and the Southern United States. Yellow maize is not popular in Africa due to its association with food aid programs (Ranum *et al*., 2014). It is believed that only the poor people in society consume it. Another reason is that of tradition where people are used to eating a white product in these countries. Maize can also be classified based on size and composition of the endosperm. Thus, in this regard, the kernel type can be described as dent, flint, waxy, flour, sweet, pop, Indian and pod corn (Ranum *et al*., 2014).

2.4 Maize Production

The total area devoted to maize production in the world is more than 129 million hectares, which corresponds to the 470 million tonnes of maize grain (Lidon and Barreiro, 2002). Latest data indicates that about 872 million tonnes of maize is produced worldwide on 177 million hectares (Mafousson *et al*., 2017). Africa accounts for only 7.9% of the world's total production. This could be attributed to continuous cropping over decades without any proper measures in place to regenerate the soil's productivity.

The importance of the maize crop in Southern and Eastern Africa cannot be overemphasized as it accounts for 32% of the consumed calories and the total area of cereal production is up to 29% in these two regions (Seyoum *et al*., 2017). It is grown by the majority of the small scale farmers and therefore plays a major role in the food security for Eastern and Southern Africa. Several biotic and abiotic stresses impend maize productivity.

The United States is the world's largest producer of maize producing approximately1,076.18 million metric tons dominating world maize trade (Anonymous, 2018, Ranum *et al.*, 2014). It is followed by China, Brazil, Argentina, India, Indonesia Mexico, Ukraine, South Africa and Romania (Table 1) The United States and China account for over 60% of the world's production (Sleper and Poehlman, 2013).

In Africa, maize production occupies approximately 24% of farmland which is more than any other staple crop (Anynomous, 2017). Seventy-five (75) million tons of maize were produced by 51 countries in Africa with South Africa producing 14 million tons. South Africa is currently the largest maize producing country in Africa (Daly *et al.*, 2016, Anynomous, 2017) and almost half of its production consists of white maize meant for human consumption. In 2017, South Africa produced 16,820,000 tons (FAOSTAT, 2017). It is followed by Nigeria, Ethiopia, Egypt, and Tanzania. Most of the maize production in Africa is rain fed.

Table 1: Maize production in 2017 by country, (FAOSTAT 2017)

Country	**Maize Production in 2017 (million tons/year)**
United States of America	370960390
China	259071000
Brazil	97721860
Argentina	49475895
India	28720000
Indonesia	27952000
Mexico	27762481
Ukraine	24668750
South Africa	16820000
Romania	14326100

Among the abiotic factors, Al toxicity is one of the major constraints affecting the productivity of maize in the world. It has been reported to constrain crop production on 67% of the total acid soil area in the world (Abate *et al.*, 2013). Aluminum toxicity has been reported to cause yield losses of up to 69% (Tandzi *et al*., 2018) in maize. The yield reduction varies with the level of acidity in the soil and the genetic potential of maize genotypes.

2.5 Aluminum and acidic soils

The third most abundant element in the Earth's crust after oxygen and silicon is aluminum (Al) (Malekzadeh *et al*., 2015). Aluminum (Al) toxicity is directly related to acidic soils and substantially limits maize yield. Soil acidity can develop on soils where high rates of nitrogen, phosphorus and Sulphur fertilizers are applied (Yerokun, 2009). It is one of the major constraints of crop productivity on acid soils, which occur on up to 40% of the arable lands of the world (Krstic *et al*., 2012, Malekzadeh *et al*, 2015).

Most of the tropical soils are acidic due to continuous exposure to weathering. As the rain water moves downwards, it causes leaching of soluble nutrients such as calcium (Ca), magnesium (Mg), and potassium (K) from the top soil layers and these nutrients are gradually replaced by Al, manganese (Mn), and hydrogen (H), the elements which are highly associated with soil acidity (Pattanayak and Pfukrei, 2013).

When pH drops below 5.5 (acidity situation), aluminosilicate clays and aluminum hydroxide minerals begin to dissolve, releasing aluminum-hydroxyl cations and Al $(H2O)_6^{3+}$ and (Al^{3+}), that then exchange with other cations. On that condition, Al^{3+} also forms the mononuclear species $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, and $Al(OH)_4$ (S´onia, 2012). Al^{3+} is the most predominant form of Al in soils that are acidic and that it readily accumulates in soils due to the electrochemical potential across the plasma membrane (Mickelbart *et al*., 2015). In highly acidic soils of pH below 5, Al^{3+} is solubilized thereby inhibiting root growth and function and leaving plants more vulnerable to drought and mineral nutrient deficiencies (Lyza *et al*., 2013). In sub Saharan Africa, acid soils occupy 29% of the total land area (Muindi *et al*., 2015).

In Zambia, soil acidity is a common problem in the high rainfall areas. This is mainly due to leaching and plant uptake of soil nutrients (Chabala *et al*., 2014). Tandzi *et al*., (2018), noted that high rainfall has an effect on soil acidification when it washes away bases such as Ca^{2+}, Mg^{2+}, K^+, Na^+, and carbonate ions (CO_3^{-2}) from the soils. It is known that the agro-ecological region III of Zambia is dominated by acidic rocks which are also poor in plant nutrients, the leaching of basic cations produces soils that are generally very strongly acidic (Banda, 2009). This in turn solubilize Al from various soil compounds and complexes. It is this solubilized Al that remains in the soil solution and is toxic to many plants.

2.6 Effect of Aluminum on plant growth

The soluble form of Al reacts with soluble phosphorus (available to plants) and converts it to soluble Aluminum phosphate which is not available to plants (Pattanayak and Pfukrei, 2013). Under Al stress conditions, plants become stubby and brittle with root tips and lateral roots turning brown. This causes the root system as a whole to get affected with many stubby lateral roots and without fine branching. As a result, plants become susceptible to different stresses with drought as the most prominent. Abate *et al.*, (2013), also reported thickening of roots and formation of cracks in the root apex due to the uneven and radical expansion of the cortex cells.

Accumulation of high concentrations of Al in soil solution results in increased uptake by most plants and this may lead to poisoning of the plants with adverse effects on growth and development of plants (Yerokun, 2009). Aluminum toxicity is also known to reduce stomatal opening, carbon dioxide assimilation, chlorophyll concentration and to cause chlorosis and leaf necrosis which results in the suppression of photosynthetic capacity of shoots (Abate *et al.*, 2013). When in excess, Al^{3+} can disturb basic cellular functions resulting in yield reduction (Mickelbart *et al*., 2015).

Generally, seedlings are more susceptible to Al than older plants (Pattanayak and Pfukrei, 2013). Malekzadeh *et al*., (2015), reported that when maize seedlings are exposed to Al toxicity, there is a decrease in root and shoot length. Lidon and Barreiro, (1998), found out that Al concentration higher than 9mg/l triggered increase in toxicity. The inhibition of root growth is a major consequence of Al toxicity and is dependent on the activity of free Al^{3+} ions in the solution (S'onia, 2012, Pattanayak and Pfukrei, 2013). This has resulted in root growth inhibition being widely used as a measure to assess Al toxicity. Photosynthetic activity of seedlings is also adversely affected due to a decline in chlorophyll content (Malekzadeh *et al*., 2015). The decrease in pigments content may be attributed either to Al induced inhibition on biosynthesis or reactive oxygen species (ROS) mediated degradation of pigments (Malekzadeh *et al*., 2014, as cited by Malekzadeh *et al*., 2015). Aluminum (Al) has negative effects on the uptake of some macro (Calcium and Magnesium) and micronutrients (Zinc and Manganese) (Mariano and Keltjens, 2005). It can also induce deficiency for Phosphorus (P), and iron (Fe) resulting in development of deficiency symptoms for these minerals (Singh, 2009).

The amount of malonyldialdehyde (MDA) increases significantly in Al stressed maize seedlings (Malekzadeh *et al*., 2015). It is suspected that Al toxicity increases the accumulation of lipid peroxidation product, MDA, which is regarded as an indicator of the loss of structural integrity in membranes subjected to heavy metal stress.

The common methods used to overcome the impacts of Al toxicity include applications of lime, manure, compost, and use of tolerant crop species or varieties. However, in the context of acid soils of tropical Africa, utilization of lime, manure and other organic fertilizer sources have their own technical and or socio economic constraints (Abate *et al*., 2013). Lime procurement can be costly due to distance from the source to the respective end user.

2.6.1 Mechanism of aluminum toxicity tolerance

Plants have evolved a number of mechanisms to help in combating the toxic effect of increased Al levels in the soil (Singh *et al*., 2017). Aluminum tolerance is usually in form of avoidance, which may be achieved by one or more of the following strategies; Aluminum (Al) is excluded from entering the root in some cases. This may be a result of root cell membrane properties or an interaction of Al with mucilage, cell wall e.t.c. This may also be due to the fact that plants release organic acids (citrate, oxalate, and malate) which chelate Al^{3+}, forming stable, non-toxic complexes (Pattanayak and Pfukrei, 2013, Hoekenga *et al.*, 2013). The immediate release of these substances as soon as plants are exposed to Al toxicity has been correlated with differential Al tolerance in a large number of monocots and dicot species (Maron *et al*., 2008, as citied by Pattanayak and Pfukrei, 2013). These organic acids are secreted from the radical apex to the rhizosphere thereby modifying the pH and chelating the toxic Al ions. This may lead to (Al) being compartmentalized in the root resulting in it being excluded from the shoot. They also secrete phosphates (Pi) and phenolic compounds from their roots. At neutral pH, organic acids are found in the cytosol and are transported out through the root when Al stress begins. Localized transporters in the plasma membrane that efflux organic acids into the rhizosphere to reduce pH are the primary Al^{3+} tolerance determinants (Mickelbart *et al*., 2015). Genes called the multidrug and toxic compound extrusion (MATE) transporters that facilitate citrate out of the root tips were identified as Al^{3+} determinants in several grains including maize. Mickelbart *et al*., (2015), described the MATE family as a major genetic determinant of Al^{3+} tolerance in many crop plants (Figure 2).

In some Al- tolerant plants, growing tissues, accumulate high levels of Al. In such cases, some form of intracellular compartmentation must be involved since true Al tolerance is not known.

In many cases, Al- tolerance may involve nutritional aspects such as tolerance to high NH_4^+ concentration in strongly acidic soils, ability to use NO_3^- in the presence of high NH_4^+ concentration (wheat) and resistance to phosphorus deficiency (wheat, maize, tomato), due to a greater P uptake or tolerance to low P.

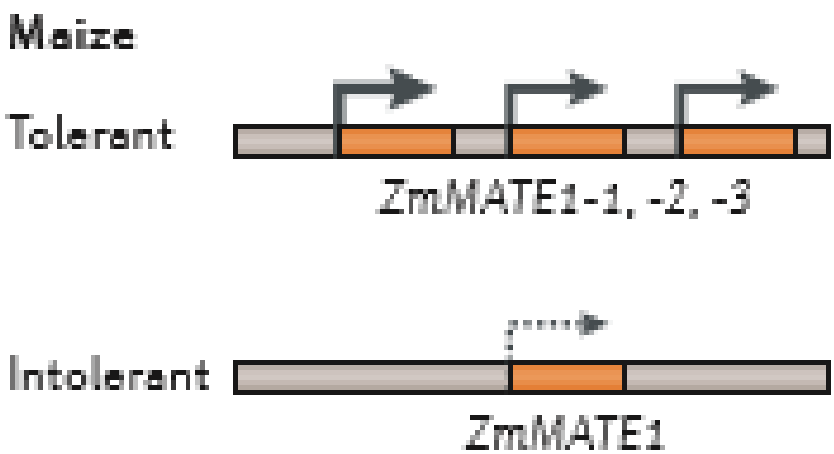

Figure 2: Genetic basis for high Al^{3+} tolerance mechanisms. The multidrug toxic compound extrusion (MATE) family is a key genetic determinant of Al^{3+} tolerance in many crop plants including maize. Tolerance to high Al^{3+} in maize is associated with copy number variation (CNV) of MATEs (Mickelbart *et al*., 2015).

2.7 Evaluation of genotypes

Root growth inhibition has been widely used to assess Al toxicity tolerance since it has been reported as a major consequence in the last century for innumerous species (S´onia, 2012, Abate *et al*., 2013, Chanda *et al*., 2015). It is reported that root growth inhibition could occur within a short time period (30 minutes) in Al-sensitive maize (Llugany *et al*., 1995, as cited by S´onia, 2012). Early symptoms of Al toxicity appear first in the roots because roots are in direct contact with toxic Al^{3+} ions (Gudu *et al.*, 2001). Susceptible genotypes have a tendency to accumulate higher amounts of Al in their root tissues (Polle *et al.*, 1978; Carver *et al.*,1988 as cited by Gudu *et al.*, 2001).

Evaluating maize genotypes for Al toxicity tolerance can be done in the field but this is expensive and time consuming (Gudu *et al.*, 2001). The most common screening method for Al tolerance is nutrient solution. A comparison of root growth of seedlings in a pair of hydroponic solutions with or without Al is a common way by which the screening is done (Abate *et al.*, 2013). This method gives several advantages which include; simplified control over nutrient availability and pH, as well as light condition and non-destructive measurement of tolerance. Previous studies have successfully used hydroponics to identify tolerant maize genotypes (Tembo, 2018; Chanda et al., 2017). No previous attempt has been done to understand the type of gene action conditioning resistance to Al tolerance and combining abilities of utilized inbred lines in mating designs.

2.7.1 Combining ability

The identification of best performing lines and lines that can be used as parents in future crosses is key for the success of any breeding program. Crossing a line to several others provides the mean performance of the line in all its crosses (Fasahat *et al.*, 2016). Sprague and Tatum, (1942) defined general combining ability (GCA) as the average performance of a line in hybrid combinations and specific combining ability (SCA) as those instances in which certain hybrid combinations are either better or poorer than would be expected on the average performance of the parent inbred lines included. It was noted by (Fasahat *et al.*, 2016) that GCA is an effective tool used in the selection of parents based on the performance of their progenies, usually the F1s, but it has also been used in F2s and later generations. The concepts of GCA and SCA are useful for characterizing inbred lines in crosses (Hallauer *et al.*, 2010). Tandzi *et al.*, (2018), stressed that heterosis and good combining ability are a prerequisite for developing good and economically viable maize hybrids. The knowledge on the nature of combining ability effects and their resulting variances play a significant role in deciding the selection procedure for exploiting heterosis (Anyanga *et al.*, 2016).

Magnavaca *et al*., (1987), conducted combining ability field studies for Al tolerance in maize and found that GCA variations were more important than SCA variations. Sprague and Tatum, (1942), interpreted GCA as an indication of genes having largely additive effects and SCA as indication of genes having dominance and epistatic effects.

2.7.2 Type of gene action

The genetic analysis of Al tolerance has been an active area of research (Hoekenga, *et al*.,2003) and a lot of progress has been made in understanding its physiological basis. Aluminum tolerance in maize inbreds using field studies has been reported to be controlled by a single gene with multiple alleles; Al tolerance seems to be the pleiotropic effect of the heat resistance gene *Lte*2 (Singh, 2009).

There exists allelic variation and copy number variation (CNV) for Al tolerance in crop germplasm which have been exploited to enable enhancement of yield stability (Mickelbart *et al*., 2015). Allelic variation and CNV are related to increase in gene expression of multidrug and toxic compound extrusion (MATES) and are known to cause Al^{3+} tolerance conferred by specific haplotypes of the Alt_{SB} (encoding sbMATE) and the ZmMATE1 loci of sorghum and maize respectively. The transgenic expression of MATEs are to confer Al^{3+} tolerance but the translation to yield stability is not yet known.

On the other hand, Abate *et al.*, (2013), reported that Al tolerance in maize is controlled by quantitative genes. The differences in findings of the type of gene action conditioning resistance trait to Al tolerance could be due to different materials under study. Both the nature and magnitude of gene action are important factors in a breeding program.

CHAPTER 3: MATERIALS AND METHODS

3.1 Germplasm used and location of experiment

The experiment was conducted at the University of Zambia (UNZA), School of Agricultural Sciences laboratory (latitude-15.39^{0}S, longititude-28.33^{0}E). Fourteen maize inbred lines (CZL 083, L151, L5527, CZL0814, CML312, L12, L3233, L2, CML511, L917, CML538, L5522, CML 457, and CZL04007) were used in this study. These were provided by the Zambia Agricultural Research Institute (ZARI), maize breeding program. These inbred lines were chosen because they are the most commonly used in the breeding program and possess certain traits such as drought tolerance.

3.2 Experiment 1: Evaluation of Germplasm in Hydroponics

A 14 (genotypes) x 5 (Al levels) factorial arranged in a completely randomized design (CRD) with 3 replications was used to evaluate the genotypes**.** A total of 210 experimental units (plots) were used and the treatments were randomly assigned to each plot (test tube). Each test tube being a diameter of 2.3cm and height of 14.5 cm.

3.2.1 Nutrient solution used in hydroponics

The modified protocol described by Kerridge and Kronstad (1968), was used to prepare the nutrient solutions (Table 2). Five levels of Al (0, 5, 10, 15 and 20mg/L) were used in this study with 0mg/L being used as a control. 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).

3.2.2 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^{o}C. Seedlings of uniform root length (approximately 2 cm) were selected and transferred to test tubes containing nutrient solutions with different concentrations of Al. These seedlings were supported

Table 2: Nutrient solution used in hydroponics in the study

Nutrient	Conc (mg/ L)	Chemical Formula	Compound Name
N	30.45	NH_4NO^3	Ammonium Nitrate
K	17.45	$K_2HPO_4.3H_2O$	Potassium hydrogen phosphate trihydrate
Zn	0.18	$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate
Mg	37.02	$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
Cu	0.06	$CuSO_4.5H_2O$	Copper suphate pentahydrate
Fe	1.99	$FeSO_4.7H_2O$	Iron sulphate heptahydrate
Ca	44.10	$CaCl_2.2H_2O$	Calcium chloride dehydrate
Mo	0.02	$NaMoO_4.2H_2O$	Sodium molybdate dehydrate
Mn	0.02	$MnSO_4.H_2O$	Manganese sulphate monohydrate
B	0.46	H_3BO	Boric acid
Al	¥	$AlK(SO_4)_2.12H_2O$	Aluminum potassium sulphate dodecahydrate

N- Nitrogen, K-Potassium, Zn- Zinc, Mg- Magnesium, Cu- Copper, Fe- Iron, Ca- Calcium, Mo- Molybdnum, Mn- Manganese, B- Boron, Al- Aluminum, ¥ - varied, Conc-Concentration 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.

3.3 Experiment 2: Nature of Inheritance for Al tolerance

3.3.1 Crossing of inbred lines

Of the fourteen inbred lines evaluated in the laboratory for Al toxicity tolerance, three of them were identified as tolerant, four as moderately tolerant and four as susceptible. A crossing block was planted at Mt. Makulu research station using the North Carolina Design II in which the 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). Hand pollination was done as follows; the ear shoots of plants designated as female were covered with shoot bags before the emergency of silks in order to protect the silks from being contaminated by any pollen before the desired pollen was applied. Plants were observed every day and new ears were covered as they emerged. Some plants had ear shoots with sharp tips, these were cut with a knife before being covered to prevent the shoot bag from being damaged. The tassels of plants designated as males were covered for a day with brown bags called tassel bags. The bags were then pulled down passed the first flag leaf and folded firmly at the base around the sheath and stem of the tassel after which they were stapled in place. To collect the pollen, 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 the recently obtained pollen was carried to the desired female plants in the morning around (08-09hrs). Hand pollination was done by quickly removing the shoot bag from the ear and applying pollen immediately. The pollinated ear was then quickly covered with a tassel bag. The tassel bag used was labelled with the name of the male and female. A total of 24 F1 progenies were generated.

3.3.2 Evaluation of Crosses

Twenty-four crosses were evaluated (24 genotypes x 2 Al levels) hydroponically using a factorial arranged in a completely randomized design with three replications. A total of 144 experimental units were used and the treatments were randomly assigned to each plot. The nutrient solution was prepared as done in section 3.2. 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 respectively as determined by Tembo (2018).

3.4 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^{O}C after which the root and shoot biomass were weighed using a balance.

The root length was used to determine the most tolerant genotype because it has been widely used as a selection criterion for Al toxicity tolerance (Chanda *et al*., 2015, Tembo *et al*., 2016).

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 (VSN International, 2015).

The GCA and SCA were estimated as done by Singh and Chaudhary, (1985) and is as presented:

GCA = mean of parent – test mean or overall mean

SCA = observed mean of the cross - (GCA_m+GCA_f) +test mean

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;

$$\mathbf{h^2_{n.s}} = \sigma^2_{gca}m+\sigma^2_{gca}f \,/\, \sigma^2_{gca}m+\sigma^2_{gca}f+\sigma^2_{sca}+ \sigma^2_e$$

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;

$$\mathbf{h^2_{b.s}} = \sigma^2_{gca}m+\sigma^2_{gca}f+\sigma^2_{sca/}\ \sigma^2_{gca}m+\sigma^2_{gca}f+\sigma^2_{sca}+ \sigma^2_e$$

where $\sigma^2_{gca}m$ is the variance component due to male GCA,

$\sigma^2_{gca}f$ is the variance component due to female GCA,

σ^2_{sca} is the variance component due to SCA, and

σ^2_{e} is the error variance

The ratio of combining ability variance components (Baker's ratio) was estimated as by Baker, (1978);

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 Singh and Chaudhary, (1985).

$\boldsymbol{\sigma^2}$**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} - $rm\sigma^2_{fc}/rcm$

A correlation analysis (VSN International, 2015) was done using summary statistics in genestat to find out the degree of association of root length to other measured parameters. A two sided test of correlation was used to identify significant levels of correlation.

CHAPTER 4: RESULTS

4.1 Screening for germplasm in hydroponics

Significant differences were obtained with concentration and genotypic main effects for all measured parameters (P = 0.001) (Table 3). The interaction (Concentration x Genotype) effect was equally significant.

4.1.1 Genotypic mean performance of measured parameters across Al concentration

Further analysis showed that parental inbred line CML 538 had the longest mean root length of 15.44cm followed by CZL 083 with 10.05cm across Al concentrations (Table 4). L151 had the shortest mean root length of 6.11cm. Considering shoot length for the genotypes, inbred line CZL 0814 recorded the longest mean shoot length, 15.77cm followed by L5527 with 13.47cm across Al concentration. The shortest mean shoot length was observed with CZL 04007 7.28cm. The average number of root hairs for the parental inbred lines ranged from 18.87 to 54.2 across Al concentrations. The highest mean number 54.22 was observed with L3233 followed by L5522 with 49.57. The lowest mean number of root hairs was 18.87 recorded by CML 312. The root biomass ranged from 177.90mg-481.90mg. The highest value was 481.90mg recorded by CML 511 followed by CML 538 with 435.50mg. The least weighing genotype was CZL 04007 with 177.90mg. Considering shoot biomass, L 5522 had the highest weight of 462.2mg followed by CML 583 which weighed 429.10mg. The lowest shoot biomass weight was 216.20mg recorded by CZL 04007.

4.1.2 Mean performance of measured parameters at different Al concentration across genotypes

The trend showed that root length and other measured parameters reduced as the concentration of Al increased (Table 5, Figure 3). However, with mean shoot length across genotypes, no significant differences were obtained between 15 mg/ L and 20 mg/ L.

Table 3: Mean squares for analysis of variance of measured parameters evaluated at the university of Zambia, school of Agricultural Sciences

Source of variation	d.f	RL	SL	RH	RB	SB
Conc (C)	4	357.86 ***	440.13***	14927.42***	1073940.0***	1304368.0***
Gen (G)	13	81.58 ***	77.37 ***	2400.59***	101756.0***	75704.0***
C x G	52	11.65***	27.90***	633.54***	71962.0***	85174.0***
Error	140	0.98	1.64	12.85	17342	9023.0
Total	209					

***Data significant at P= 0.001, d.f - degrees of freedom, ms - mean square, RL -root length, SL - shoot length, #RH - number of root hairs, RB - root biomass, SB -shoot biomass, Conc-Concentration, Gen- Genotype

4.2 Nature of inheritance for Al tolerance

Results (Table 6) 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 (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 interactions effect except for SCA x concentration for root biomass were significant.

4.2.1 Evaluation of general combining ability 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 7), with female line CML 538 having 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 male line CML 457 had negative significant GCA values, -4.55 for the number of root hairs and -1.30 root biomass while CZL 04007 had a negative significant GCA value of -8.18 for the number of root hairs. CML 511 had a positive significant GCA effect 8.58 for the number of root hairs. The female line CZL 083 had negative significant GCA effects for shoot length root biomass. L5527 had a positive significant GCA effect for root biomass (Table 7).

Table 4: Genotypic mean performances for measured parameters across Al concentrations

Genotype	RL	SL	# RH	RB	SB
CML 538	15.44	12.68	49.07	435.50	429.10
CZL 083	10.05	12.63	49.40	344.80	427.90
CZL 04007	9.97	7.28	25.45	177.90	216.20
CZL 0814	9.04	15.77	28.57	209.30	381.10
L3233	8.37	11.26	54.22	223.70	294.30
L2	8.34	11.07	20.54	326.70	454.20
CML 511	7.89	11.71	20.93	481.90	360.70
L917	7.43	8.05	40.03	292.00	328.80
L5522	7.42	10.49	49.57	354.70	465.80
CML 312	7.39	10.68	18.87	276.40	274.70
CML 457	7.37	8.60	30.47	310.00	398.80
L5527	7.05	13.47	42.67	343.80	366.20
L12	6.34	9.61	22.87	314.70	405.30
L151	6.11	9.71	28.07	361.70	362.30
MEAN	8.45	10.93	34.34	318.10	369.00
LSD $_{(\alpha = 0.05)}$	0.72	0.93	2.60	95.33	68.76

LSD- Fishers protected least significant difference test performed at P=0.05, RL-root length, SL - shoot length, #RH - number of root hairs, RB - root biomass, SB -shoot biomass.

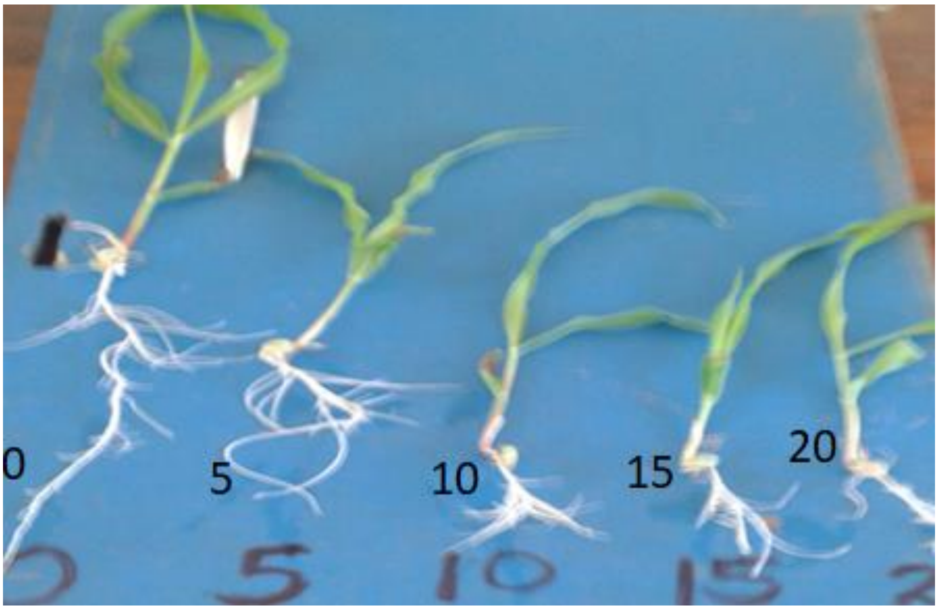

Figure 3: *General trend on mean root length performance with increased levels of Al. Corresponding root length on Numbers from 0 to 20 displayed show general root responses in 0 mg/ l Al to 20 mg/ l AL concentration*

Table 5: Mean values of measured parameters in varying Al concentration across Genotypes

Al (mg/L)	RL (cm)	SL (cm)	#RH	RB (mg)	SB (mg)
0	11.46	14.68	57.77	496.50	548.80
5	10.44	13.02	44.75	396.10	476.70
10	9.63	11.83	38.69	381.40	396.90
15	5.77	7.68	18.02	226.20	333.20
20	4.92	7.45	12.24	90.20	89.30
$LSD_{(\alpha=0.05)}$	0.43	0.55	1.551	56.97	41.09

LSD- Fishers protected least significant difference test performed at P=0.05 RL -root length, SL - shoot length, #RH -number of root hairs, RB - root biomass, SB - shoot biomass, Al – Aluminum

Table 6: Mean squares for 8 x 3 North Carolina design for all measured parameters evaluated at 0mg/l Al and 20mg/l Al.

Source of variation	d.f	Mean Squares			
		RL	**SL**	**RH**	**RB**
GCA_m	7	25.32*	8.75**	757.52*	37.98***
GCA_f	2	41.01**	12.81**	207.37	58.46***
CONC	1	3059.47***	124.23***	18871.89***	1005.42 ***
SCA	14	9.04	5.64*	173.52	14.86*
GCA_m x CONC	7	41.67***	4.18***	574.70***	25.16***
GCA_f 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

***Data significant at P= 0.001, ** data significant at P= 0.01, * data significant at P= 0.05, d.f - degrees of freedom, RL - root length, SL - shoot length, #RH - number of root hairs, RB - root biomass, Conc- concentration, GCA_m- general combining ability effects due to male, GCA_f -general combining ability effects due to females, SCA- specific combining ability

Table 7: GCA effects of parental lines used in the study of all measured parameters

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

NS-non significant, *data significantly different from zero P= 0.05** data significantly different from P= 0.01, ***data significantly different from at P= 0.001, RL- root length, SL- shoot length, RH- number of root hairs, RB- root biomass, SB- shoot biomass, SE- standard error of differences, ^{m,f} represents GCA **effects** associated with male and female **parents.**

4.2.2. Evaluation of specific combining ability effects

Analysis for SCA, revealed that the crosses L 917 x CML 538 and CML 511 x CZL 0814 had a positive significant SCA value of 1.92, 2.59 and 0.87, 1.86 for shoot length and root biomass respectively (Table 8). The crosses CML 457 x CZL 083, L5527 x CZL 083, L12 x CML 538 and L12x CZL 083 had a positive significant SCA 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. The SCA for the shoot length and root biomass were both negative but significant for the cross L12 x CZL 0814 and L917 x CZL 083. The crosses L5527 x CML 538 and CML 511 x CML 538, L917 x CZL 0814 had negative but significant shoot length while CML 511 x CZL 083 had negative significant root biomass.

Table 8: Evaluation of crosses on the significant genotypic SCA effects for shoot length and root biomass

Crosses	SL	RB
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*
SE	**0.67**	**1.56**

* data significantly different from zero at P= 0.05, ** data significantly different from zero at P= 0.01, ***data significantly different from zero at P= 0.001, RL-Root length, SL- Shoot length, RH- Number of root hairs, RB- root biomass, SE- standard error of differences

An estimation of genetic parameters and Bakers ratio was found to be 0.49, 0.21, 1.0, and 0.28 for root length, shoot length, number of root hairs, and root biomass respectively (Table 9). 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.

Table 9: Estimates of variances and baker's ratio

Variances and ratios	RL	SL	RH	RB
σ^2SCA	0.46	0.63	0.00	1.68
σ^2GCA_f	0.45	0.12	0.91	0.49
σ^2GCA_m	0.00	0.04	10.67	0.15
h^2b.s	0.36	0.64	0.66	0.49
h^2n.s	0.18	0.13	0.66	0.14
Baker's ratio	0.49	0.21	1	0.28

σ^2SCA-variance due to specific combining ability, σ^2GCA_f- variance due to female combining ability, σ^2GCA_m- variance due to male combining ability, h^2b.s- broad sense heritability, h^2n.s- narrow sense heritability.

The narrow sense heritability was 0.18, 0.13, 0.66, and 0.14 for root length, shoot length, number of root hairs, and root biomass respectively.

4.3 Correlation analysis among the measured parameters for the F1s

The correlation coefficients between the root length to other measured parameters are summarized in Table 10. Correlation of root length to root biomass, number of root hairs, and shoot length were 0.73, 0.82 and 0.72 respectively and these were highly significant (p= 0.001) while for shoot biomass r =0.20 was positively correlated to root length but non- significant (Table 10).

Further the amount of phenotypic variation explained (r^2) was determined and results are shown in Table 11. The amount of phenotypic variation explained between the root length and the number of root hairs was 67% and that between the root length and shoot length was 52%.

Table 10: Associations among measured parameter for the F1 genotypes

	RB	#RH	RL	SB	SL
RB					
RH	0.58***				
RL	0.73***	0.82***			
SB	0.22	0.15	0.20		
SL	0.55***	0.64***	0.72***	0.27	

***Correlations significant at p=0.001, RL - root length, SL - shoot length, #RH - number of root hairs, RB - root biomass, SB - shoot biomass

Table 11: Phenotypic variation explained (r^2) among the measured parameters

	RB	#RH	RL	SB	SL
RB					
RH	0.34				
RL	0.53	0.67			
SB	0.05	0.02	0.04		
SL	0.30	0.41	0.52	0.07	

RL - root length, SL - shoot length, #RH - number of root hairs, RB - root biomass, SB - shoot biomass

CHAPTER 5: DISCUSSION

Soil acidity, which entails high Al concentration in the soil leads to low crop productivity and in turn production. It was for this reason that popular parental inbred lines were evaluated for tolerance to Al to identify appropriate parental lines for further research.

Assessments of Al tolerance based on root growth has been used extensively in genetic and molecular studies in crop plants (Bidhan and Bhadra, 2014). In this study, CML 538, CZL 083 and CZL 0814 were identified as tolerant. 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 (Bidhan and Bhadra, 2014). This could be due to the fact that the roots are in direct contact with the Al in solution. The root system in maize is crucial for plant establishment as well as water and nutrient uptake (Pace *et al*., 2014).

The trend was the same for all the measured parameters as they showed an enhanced performance in low levels of Al across all the genotypes. It was also observed that toxic levels of Al in nutrient solution significantly affected crop performance as is evident from decreased mean seedling root growth, number of root hairs, shoot length, as well as shoot and root biomass (Table 5). This coincides with Bidhan and Bhadra (2014), who observed a similar trend in rice. The decrease in growth parameters observed at high levels of Al could be due to toxic Al^{3+} ions accumulating in the root system of the genotypes. High levels of Al could also cause other nutrients in the solution to be unavailable for plant uptake. 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*., (2016), 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 good 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 not responding favorably 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 (Tembo *et al*., 2014). The desirable SCA effects observed from crosses between the good (CML 538) and the bad (L 917) combiner parents (L917 X CML 538) for shoot length and root biomass could be due to epistatic effects (Fasahat *et al*., 2016). The cross L917 x CZL 083 had undesirable SCA effects for both the shoot length and root biomass. This could be attributed to the fact that both lines had undesirable GCA effects.

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 (Baker, 1978). Both additive and non-additive gene action are important for the root length as indicated by a Baker's ratio of 0.49. This study is in agreement with Magnavaca *et al*., (1987), who found that both additive and non-additive gene action played a role in the inheritance of Al tolerance in maize. The finding of different gene action influencing root length and shoot length might mean that there are different genes influencing them. Non additive gene action entails the production of hybrid maize while additive gene action entails the launch of an open pollinated maize breeding program or a recurrent breeding approach scheme.

The nature of gene action and the heritability are key in developing an effective breeding program. The low values (0.18 and 0.36) of narrow and broad sense heritability respectively imply that the trait cannot easily be transferred to offsprings. For root length being determined by both additive and non-additive gene action, approaches that utilize recurrent selection and hybridization can be employed in the breeding program. However, due to the low narrow sense heritability, hybridization remains the best option in breeding for Al toxicity. The finding of non-significant specific combining ability for root length, number of root hairs and shoot biomass could probably imply that the genotypes could have been from the same heterotic group or they share a common parent.

The correlation for the root length to the shoot length was 0.72 implying a reasonable high correlation. However, the moderate phenotypic variation explained (r^2) value of 0.52 explained that shoot length cannot be entirely used as a selection criterion for Al toxicity tolerance. The use of shoot length as a selection criterion can therefore only supplement the direct use of root length.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Genotypes that are tolerant to Al toxicity were identified. Parental inbred lines CML 538 and CZL 083 were found as the most tolerant to Al toxicity. These exhibited mean root lengths of 15.44 cm and 10.04 cm respectively. High levels of Al (15mg/ L and 20mg/ L) in nutrient solution significantly decreased seedling root growth, number of. root hairs, shoot length, as well as shoot and root biomass.

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. The cross between L917 and CML 538 had desirable SCA for root length, shoot length and number of root hairs. The crosses L151 x CZL 083, L5527 x CZL 083, and L12 x CML538 had desirable SCA for the number of root hairs.

In this study, it was found that both additive and non-additive gene action control Al toxicity tolerance in maize as indicated by the Baker's ratio of 0.49 for the root length. Furthermore, the moderate Phenotypic variation explained r^2 (0.52) for the association of root to shoot length indicate that shoot length cannot be used for indirect selection but can supplement use of root length as a selection criterion.

6.2 Recommendations

Further research should be done on selected tolerant and non-tolerant genotypes in an endeavor to further study the mechanism of tolerance. Tolerant inbred lines and crosses with good combining abilities should also be evaluated under field conditions.

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