MAKERERE UNIVERSITY

GENETIC ANALYSIS OF SORGHUM TOLERANCE TO ALUMINIUM TOXICITY AMONG UGANDAN BREEDING LINES

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DECLARATION

This dissertation report titled genetic analysis of sorghum tolerance to aluminium toxicity among Ugandan breeding lines has never been produced by any person before and I therefore, confidently declare that it is a primary production of my efforts (my original work) which has never been presented/submitted for any award of degree, or even higher award in any private/public University or institution before. However, contributions of others involved, have been clearly indicated with due reference to the literature cited, and acknowledgements of collaborative research and discussions.

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This thesis has been submitted for examination with our approval as supervisors:

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Professor Patrick Okori Date

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Professor Samuel Gudu Date

DEDICATION

I wish to dedicate this thesis report to my lovely mother – Imat Mary Joyce Agoa Oluge (widow) who got involved in my academics long time ago and became my great pillar; my brothers- Mr. Geoffrey Ojuka Oluge, Mr. Alfred Oluge Oluge (late), Mr. Bernard Amuge Oluge of Lira Referral Hospital, Mr. Peter Paul Agong Oluge, Dr. Oluge Christopher Ochom of Busitema University, Arapi Agricultural College campus and to my sisters - Mrs.Hellen Omara and, Mrs. Costa Ebong (late) and Ms Eunice Atoo Oluge of Otuke district local government and their families and sister Akino Harriet for giving me ample time during the course of writing this report. May the Almighty God Richly bless and continuously grant you guidance in all your endeavours.

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List of abbreviations and acronyms

MUARIK: Makerere University Agricultural Research Institute Kabanyolo

ICRISAT: International Crops Research Institute for the Semi-Arid Tropics

NARO: National Agricultural Research Organization

NCII: North Carolina II mating scheme/design

RUFORUM: Regional Universities Forum for Capacity Building in Agriculture

IRL: Initial root length

FRL: Final Rooot Length

NRL: Net Root Length

MSDM: Mean shoot dry matter

MRDM: Mean root dry matter

SDM: Shoot dry matter

GCA: General combining ability

SCA: Specific combining ability

V.C: Variance component
GCA(S): General combining ability of female
GCA(T): General combining ability of male
BS-CGD: Broad-sense coefficients of genetic determination
NS-CGD: narrow sense coefficients of genetic determination
DM: Dry matter

ABSTRACT

Aluminium (Al) toxicity is a major abiotic constraint that limits grain sorghum (*Sorghum bicolor* L. Moench) production on acid soils. It inhibits water and mineral uptake and ultimately, reduces plant vigour and yield. A preliminary study was done to determine the level of variation for tolerance to Al^{3+} toxicity and to identify new sources of Al^{3+} tolerance in the Uganda`s sorghum germplasm. Four day old germinated seedlings of 83 sorghum genotypes (including two tolerant checks O_2 and C_1 and one susceptible check L_5) whose initial root length were measured, were subjected to 0 µM and 148 µM of Al^{3+} supplied as Al_2 $(SO4)_3.16H_2O$ in Hoagland's nutrient solution as described by Magnavaca*et al* (1978). The seedlings were raised in a growth chamber for five days, after which final root lengths were recorded.

Net root growth in the aluminium augmented solution was used to classify the germplasm into phenotypic classes of tolerant, moderately tolerant or susceptible. The above classification was used as a criterion for choosing progenitors for developing segregating populations for studies of inheritance. There were significant differences between the genotypes, Al^{3+} concentration and a significant interaction The genotypes O_2, C_1 and UNGB 2672 exhibited the highest net root length and were classified as tolerant while L_5, UNGB 2759, UNGB 2856 and UNGB 2762 exhibited the lowest net root length and were therefore, classified as susceptible. The above were then used to develop segregating population for inheritance studies.

On the other hand, when the remaining genotypes were analyzed, a high significant difference among the genotypes ($P \leq 0.001$) and a high significant ($P \leq 0.001$) interaction between the treatment levels and genotypes and a significant difference ($P \leq 0.05$) in Al^{3+} level. The genotypes/crosses were also significantly different ($P \leq 0.001$) from each other in regards to haematoxylin staining. Significant but negative correlation between haematoxylin staining and net root length of test genotypes ($r^2 = -0.94$) was discovered. Significant differences were observed for Al^{3+} level, genotypes and interaction all at ($P \leq 0.001$) when the F_2 and parental lines were analyzed. The reduction of net root length at 148 µM to the control computed showed that genotypes with larger values were tolerant to Al^{3+} toxicity; while those with very low ratios were as well very sensitive to Al^{3+}.

On the root biomass study, analysis of variance revealed that there were high significant differences between genotypes (P≤0.001) and Al^{3+} concentration (P≤0.001) and a high significant interaction between Al^{3+} and genotypes (P≤0.01). However, there was a non-significant interaction (P≤0.05) between Al^{3+} and crosses showing that there was increase in Al^{3+} tolerance in the crosses.

Generally, there was a decrease in mean root biomass with increase in aluminium concentration meaning that the genotypes were affected differently by the different concentrations of $Al^{3+,}$ hence differential significant interaction. Nevertheless, some crosses had better root mean dry biomass than the parents involved in the cross.

For root dry mater, significant GCA(S) (**P≤0.001)** was observed at all Al^{3+} levels, while (GCA(T)) were significant at only 0 and 148 Al^{3+} concentration (**P≤0.05)** but non-significant at 222 µM Al^{3+} (**P≤0.05)**. The specific combining ability of the crosses were significant at P≤0.05 across all levels of Al^{3+}.There was a very high Baker`s ratio, BS-CGD and NS-CGD across treatment indicating the importance of additive gene effects for dry root weight.

Significant GCA effects were observed for the all the female parents for root dry matter with L5 consistent significant positive GCA effects (P≤0.001) among the females parents across treatments. All the male parents had non-significant general combining ability (GCA) effects (P≤0.05) across concentration except C1 which was significant at P≤0.05 at only 0 Al^{3+} concentration but positive GCA effects across the Al^{3+} concentration.

In regards to SCA effects for dry root weight, all crosses had non-significant SCA effects (p**≤0.05)** across the Al^{3+} concentration. High positive and negative SCA estimates were observed for L5xC1 and UNGB2856xO2 and L5xO2 and UNGB2856xC1respectively across treatment although the remaining crosses had very small values of the SCA effects across treatment level.

For shoot dry weight, there were high significant differences between genotypes, Al^{3+} concentration and a high significant interaction between Al^{3+} and genotypes all at (P≤0.001) with a decrease in shoot biomass as Al^{3+} concentration was increased from 0-222 µM Al^{3+}.

However, there was a non-significant interaction between Al^{3+} and crosses ($P \leq 0.05$) when only the crosses were analyzed. This possibly showed that there was increase in Al^{3+} tolerance in the crosses as shown by increase in mean shoot dry matter.

In regards to combining ability of the F_2 generation, there were high significant differences ($P \leq 0.001$) for both GCA and SCA.

Significant GCA effects of shoot dry matter (**$P \leq 0.001$**) were observed for the all the female and male parents for shoot dry matter across the Al^{3+} concentration. However, the female parents UNGB2856 and UNGB2762 and male parents O2 and UNGB2672 had negative GCA effects across the Al^{3+} concentration. This means that C1, L5 and UNGB2759 were the best general combiners across the concentration.

Variation in the SCA effects for shoot dry weight among the crosses was observed. L5xC1 and UNGB2856xO2 had high significant positive ($p \leq 0.001$) SCA effects, L5xO2 and UNGB2856xC1 had significant negative SCA effects ($p \leq 0.001$) across Al^{3+} concentration. However, UNGB2759xUNGB2672, UNGB2762xC1 and UNGB2762xUNGB2672 had a non-significant negative ($p \leq 0.05$) SCA effects across Al^{3+} concentration.

CHAPTER ONE

INTRODUCTION

1.1 Biology and economic importance

Sorghum (*Sorghum bicolor* (L. Moench) is a tropical grass grown primarily in semi-arid parts of the world, particularly in Africa, India, and Asia, where it is an important staple food crop (Rohrbach *et al*., 2002). It is indigenous to Africa and Asia and is believed to have been domesticated in Sub-Saharan Africa, particularly in the Nile basin, from where it spread to other parts of the world (Kimber, 2000). Cultivated sorghums are divided into 7 basic races (agronomic types): Kafir (S. Africa) - short plants, Milo-Caudatums (E. Africa), Feterita-Guineas (Sudan), Durra (East Africa, Middle East and India), Sballu (India), Koaliang (China) and Hegari (Sudan). These races can be distinguished by their growth habit, panicle characteristics, seed shape and color and adaptation. Additionally, hybrid races can be identified from crosses among the basic races. Very high levels of genetic diversity exist among and within races. sorghum is an important food and feed crop, and is becoming an industrial crop used in biofuels and brewing manufacture while the stalks are used to manufacture paper (Munyinda *et al*, 2008). It is an appropriate staple crop in semi-arid agro-ecologies of Eastern Africa because of relative tolerance to heat and drought and lesser demand for high soil fertility compared to maize. Sorghum can thus contribute immensely to the livelihoods of the vulnerable communities living in semi-arid environments (World Bank, 2005).

1.2 Crop requirements, adaptive traits and management practices

Sorghum is a C4 crop species with wide adaptation, from temperate to tropical climates. It has a dense, deep root system and can tolerate high temperatures, low rainfall, and low soil fertility. Sorghum is a short-day species, but many cultivars are day-length insensitive and it has a competitive advantage over maize on marginal lands in dry, hot areas. However, it is best adapted to slightly cooler, dry climates with optimal growth occurring at 30 °C. About 450 to 1000 mm of rainfall per year is required to grow a sorghum good crop. It is adapted to a wide range of soil types, from waterlogged to sandy soils, and low to high fertility. Yield potential is similar to maize and wheat under many conditions, but somewhat less than maize and wheat in better environments (3-4 t/ha). When moisture is limiting, yield of 0.3 to 1 t/ha is typical

(FAOSTAT, 2009). The mechanisms for tolerance to drought in sorghum include: dense, deep penetrating root system, ability to reduce transpiration through leaf rolling and stomatal closure under stress conditions, waxy leaves prevent water loss and ability to reduce metabolic processes to near dormancy under extreme drought.

Sorghum is a fast-maturing crop with high photosynthetic efficiency and a high rate of dry matter accumulation. Most varieties will mature within 90 to 140 days. The crop can be 'ratooned' like sugarcane. If the heads and stems are cut back, the plant can re-sprout from the roots. This is a useful characteristic for both subsistence farmers and sorghum breeders.

1.3 Statement of the problem

In the four decades before 2000, the agricultural sector of East and Central Africa experienced a decline in labour productivity (Omamo *et. al.,* 2006). Currently, while agriculture is growing at a rate of about 2% per annum in Uganda, the population growth rate is over 3% (2002 national census) during the first decade of the 2000s. This trend is exacerbated by endemic production constraints (biotic and abiotic factors), including climate change effects of drought, and increased pests and diseases. Yet, the current and future demands for food call for increased agricultural productivity and even doubling the production of cereals (Pingali and Pandey, 2001). In this regard, sorghum which plays a central role in the livelihoods strategy of farming communities of semi-arid and drought prone areas of Uganda, needs to be improved upon by developing resilient and vibrant varieties that can respond to both biotic and abiotic constraints, and, above all, provide improved aluminium tolerance and thus, introduce a management system for income, food, and nutrition security for the increasing population.

Major factors accounting for low sorghum productivity include the cultivation of inherently low-yielding varieties, poor soil fertility, drought, striga, and other pests and diseases (Nichol and Oliveira, 1995; ECARSAM, 2005). Moreover most sorghum production in many parts of Eastern Africa also occurs in soils with high aluminium toxicity (Samac and Tesfaye, 2003; Obura, 2008). The dissolution of just a small fraction of the aluminium compounds in the soils release phytotoxic forms [Al(OH)2+ and Al(OH)3+] of Al^{3+} into the soil solution impairing root development and in turn limiting water and soil nutrient uptake inadvertently reducing productivity of affected plants (Kochian *et al.,* 2004; Zhang *et al*., 2007). The problem is exacerbated by the use of ammonium fertilizers and acid rain (Beebe *et al.,* 2008). Aluminium

toxicity thus poses a threat to sorghum production especially for resource-constrained communities exacerbating food insecurity in marginal agro-ecologies where the crop is often cultivated. Aluminium toxicity has been reported in western Kenya, a major sorghum producer, which shares a lot of geological and soil properties as eastern Uganda, another important producer of sorghum (Bray *et al.*, 2000; Kanyanjua *et al*., 2002). Yet there is paucity of information on the availability of novel sorghum genotypes suitable for these agro-ecologies especially in Uganda. As part of the efforts to develop aluminium tolerant cultivars, this study is one among others aimed breeding high yielding farmer preferred tolerant cultivars for marginal acid soils of Uganda.

1.4 Study objective

To develop sorghum genotypes tolerant to aluminium toxicity among Ugandan farmer preferred varieties and elite breeding lines.

1.4.1 Specific objectives

(i) Establish level of aluminium tolerance of selected sorghum accessions.

(ii) Establish the inheritance and mode of gene action controlling tolerance to aluminium toxicity in selected sorghum lines.

(iii) To determine combining ability, genetic variability, heterosis, and heritability for shoot and root dry matter (DM) yield of sorghum seedlings grown in nutrient solutions containing varying Al^{3+} concentrations.

1.5 Research hypotheses

(i) There is genetic variation for tolerance to aluminium toxicity in Ugandan sorghum land races that could be exploited in breeding programmes.

(ii) Tolerance to aluminium toxicity is governed by additive and or non-additive genes.

(iii)Tolerance to aluminium toxicity in sorghum is closely associated with shoot and root dry matter (DM) yield of sorghum seedlings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Aluminium toxicity often occurs in acidic soils and is one of major abiotic stresses that limit sorghum and other crop productivity worldwide (Samac and Tesfaye, 2003; Magalhaes *et al.*, 2004; Kochian *et al.*, 2004). In the world, over 40% of the potential arable lands are acidic due to excess H^+ and Al^{3+} ions in the soil (Delhaize *et al.*, 2004). In East Africa, most sorghum production occurs on soils with pH≤5.5, while in some parts, Al saturation is high (4-55%). This dramatically affects the availability of phosphorus a major macro-nutrient needed by crop plants (Kanyanjua *et al*., 2002). In Tanzania for example, acid soils cover more than 15% of the agricultural land (MARI, 2006), and while in Kenya they affect over 7.5% of arable land (Kanyanjua *et al*., 2002). Presence in the soil of aluminium compounds may increase soil acidity and / or subject crop plants to aluminium (Al^{3+}) toxicity through abundance of hydrogen (H+) and aluminium (Al^{3+}) ions. Excess acidity (H+ ions) in acid soils affects plant root membrane permeability and therefore, interferes with cation systems needed for nutrient absorption (Ligeyo and Gudu, 2005). This restricts the plant growth, especially in the roots, which in turn limits water and mineral uptake leading to poor plant growth and low grain yield (Kochian *et al.*, 2004).

2.2 Aluminium toxicity symptoms and effects in sorghum

Extensive genetic variability with respect to aluminium tolerance exists in plants at both inter- and intra-specific levels (Ishikawa and Wagatsuma, 1998). For example, while maize shows considerable genetic tolerance to soil acidity, the majority of commercial genotypes are so sensitive to aluminium toxicity that breeding for better adapted maize cultivars seems to be the best strategy for improving farming in regions with soil acidity.

Sorghum is much more closely related to many tropical cereals that have complex genomes such as maize. In fact sorghum and maize diverged from a common ancestor ~12 million years ago (mya) (Gaut, *et al.,* 1997; Swigoňová *et al.,* 2004). Thus sorghum a close relative of maize but with a less complex genome than the latter and could therefore share a lot in common including adaptation to aluminium toxicity (Al^{3+} toxicity) (Paterson *et al.,* 2004; Magalhaes *et al*., 2004;

Magalhaes *et al*., 2007). Al^{3+} is transported in the xylem as a complex with citrate and within the plant it affects a number of metabolic processes that further curtail plant growth (Ma and Hiradate, 2000). The major symptom of Al^{3+} toxicity is a rapid inhibition of root growth (Zhang *et al.,* 2007). Aluminium inhibits root cell expansion and elongation and if over the long term, cell division as well. Aluminium-induced callose is deposited in plasmodesmata, which in turn blocks cell-to-cell movement of molecules, and interferes with intercellular communication, inhibit cytoskeletal dynamics and interact with both microtubules and actin filaments (Sivaguru *et al.,* 2000; 2003). This inhibition of the growth of the roots further causes reduces plant vigor and yield (Kochian *et al.,* 2005). Roots injured by high aluminium become stubby and thick, dark colored, brittle, poorly branched and rubberized with a reduced root length and volume (Nguyen *et al*., 2003).

Toxicity symptoms of Al^{3+} are similar to nutrient deficiencies, though these general symptoms appear to be the consequence of the inhibition of the root development caused by targeted action of aluminium at the tips. In addition to rapid accumulation in cell walls and apoplasm of roots, aluminium rapidly accumulates in the plasma membrane and the symplasm of sensitive plants, affecting many processes involved in root growth (Rout *et al.,* 2001; Ciamporova, 2002) where in certain cases the root rapid inhibition of radical growth seen in the primary and lateral root apexes as thick masses and turn brownish-gray. These symptoms become evident after a few minutes or hours of the plants being exposed to micro molar concentrations of Al^{3+} in hydroponic solutions (Rengel and Zhang, 2003). Radical inhibition coincides with a decline in cell division and elongation of the root cells, which then induces significant lignification of the cell wall by crossing with pectins (Rout *et al*., 2001; Jones *et al*., 2006). This alteration prevents water absorption that is essential for the transport of nutrients through the apoplast, eventually causing a decrease in yield and grain quality (Raman *et al*., 2002; Zheng and Yang, 2005). The shoot is also inhibited due to limiting supply of water and nutrients. Prolonged exposure of plants to Al^{3+} induces rapid change in other biochemical and physiological processes after cessation of cell elongation (Rengel and Zhang, 2003). This is why the symptoms at foliar level resemble phosphorus deficiency, preventing plant growth, turning mature leaves dark green, stems purple and killing leaf apexes (Wang *et al*., 2006).Therefore, both Al^{3+} tolerant and Al^{3+} sensitive plants

accumulate Al^{3+} ions when grown in acid soils rich in Al^{3+} (Gaume *et al.,* 2001; Jensen *et al.,* 2002; Watanabe and Osaki, 2002).

Later stages of Al^{3+} toxicity are associated with lipid peroxidation and oxidative stress (Yamamoto *et al.,* 2001). Due to the many cellular responses to Al^{3+} treatment, a number of changes in gene expression have been associated with Al^{3+} treatment including genes associated with oxidative stress and pathogen invasion (Rodriguez Milla *et al.,* 2002). Because pathogen invasion leads to production of active oxygen species and general oxidative stress, it is perhaps not surprising that disease defence response genes are expressed in response to Al^{3+} treatment. Al^{3+} is closely linked to other DNA-associated molecules, such as phosphorylated proteins (histones) (Kochian, 2005). Al^{3+} toxicity may also affect the mechanism that controls the organization of cytoskeletal microtubules as well as the polymerization of tubulin by delaying disassembly during mitosis (Franzios *et al*., 2003). This would affect the direction of the microtubules, which is closely related to cell expansion (Zheng and Yang, 2005).

Moreover, high levels of aluminium impair the growth of roots of the plants, and when they are accompanied by periods of water deficiency, even in the rainy season, productivity is significantly reduced, thus incapacitating the cultivation of sorghum in areas with acid soils (Lima *et al.,* 1995). Many authors have reported that aluminium fixes phosphorous into less available forms to plants, reduces root respiration interferes with cell division and with the enzymes responsible for the deposition of polysaccharides in the cell wall, increases cell wall rigidity, interferes in the absorption, transport and use of some chemical elements (calcium, magnesium, phosphorous and potassium) and water and has the capacity of precipitating the nucleic acids (Ryan *et al.*, 1995).

2.3 Management of aluminium toxicity in acidic soils

Some plants have developed mechanisms that enable them overcome the stresses of acidic soil and aluminium toxicity (Kochian *et al.,* 2005). Using lime to neutralize H^+ and Al^{3+} ions is one of many alternatives to alleviate soil acidification, by applying it to the soil surface does not solve the acidity problem in the lower layers (Matsumoto *et al.,* 2001), and liming to great depths is not possible because of technical and economic issues. On the other hand, heavy application of lime may have adverse effects on some crops in a rotation or cause deficiencies of certain

nutrients (Kochian *et al.*, 2005). Thus, the development of cultivars with improved tolerance to acid soil stress and or tolerant to aluminium and exploitation of adaptation mechanism by plants to Al^{3+} toxic acids is of great importance in order to address the problem of low yields in sorghum and agricultural production in these soils (Jones *et al.*, 2006). Accordingly, the development of segregating populations is important in inheritance studies and mapping of genes and/or loci related to aluminium tolerance is as well useful in breeding programs.

2.4 Genetics of aluminium tolerance in plants

Recently genes that encode membrane transporters have been identified and characterized and associated with tolerance to aluminium Al^{3+} toxicity (Sasaki *et al.*, 2004; Furukawa *et al.*, 2007). These genes confer resistance to toxicity for aluminium in different cereals such as *Triticum aestivum*, *Hordeum vulgare*, *Sorghum bicolour* and other species (Table 1). In sorghum a MATE gene (*SbMATE*) was identified as an aluminium-activated citrate transporter (Magalhaes *et al*., 2007). The monogenic inheritance of genes encoding proteins responsible for transporting organic acids in cereals such as *Triticium aestivum* and *Hordeum vulgare* (barley) facilitates the prospects of improving these species for tolerance to Al^{3+} in acidic soil. Knowledge of the molecular physiology of aluminium tolerance and the genetics that control this trait, may allow significant advances in the development of tolerant varieties in sensitive cereals.

Table 1. MATE and ALMT-family genes encoding organic anion transporter proteins for aluminium resistance in cereals and other plant species.

Gene	Plant species	Reference
TaALMT1	Wheat (*Triticum aestivum* L.)	Sasaki *et al.*, 2004
ScALMT1	Rye (*Secale cereale* L.)	Fontecha *et al.*, 2007
HbMATE	Barley (*Hordeum vulgare* L.)	Wang *et al.*, 2007
SbMATE	Sorghum (*Sorghum bicolor* L.)	Magalhaes *et al.*, 2007
AtMATE	Arabidopsis (*Arabidopsis thaliana*)	Liu *et al.*, 2008
AtALMT1	Arabidopsis (*Arabidopsis thaliana*)	Hoekenga *et al.*, 2006
BnALMT1	Rape (*Brassica napus* L.)	Ligaba *et al.*, 2006

2.4.1 Mechanism of aluminium tolerance inheritance in sorghum

It has been indicated that Al^{3+} tolerance in plants is largely influenced by a putatively orthologous series of at least two major loci that are inherited as major Al^{3+} tolerance genes in sorghum (Magalhaes *et al*., 2007). Tolerance to Al^{3+} toxicity in sorghum is controlled by a major

gene *Alt$_{SB}$*, located on chromosome 3 (Magalhaes *et al*., 2004). This locus might result in Al^{3+} tolerance through citrate exudation from roots. The quantitative trait locus (QTL) located on chromosome 1 of rice is orthologous to the *Alt$_{SB}$* sorghum gene, while the QTL found on chromosome 3 of rice is orthologous to the *Alt$_{BH}$* wheat genes (chromosome 4DL) and to barley *Alp* on chromosome 4H (Magalhaes *et al*., 2004).

2.5 Screening methods for aluminium tolerance

Several native and crop species exhibit significant genetic based variability in their responses to Al^{3+} toxicity. This variability is useful to plant breeders for the production of Al^{3+} tolerant crops. Selection and breeding of crops for Al^{3+} tolerance is a useful approach to increased production on acid soils. For selection of genotypes tolerant to Al^{3+}, a precise screening technique to evaluate sensitivity of plants to Al^{3+} is needed. This requires a rapid and reliable system to discriminate between Al^{3+} tolerant and Al^{3+} sensitive genotypes (Springer, 2004). Generally, the Al^{3+} screening technique can be classified into laboratory screening and field screening. Laboratory screening methods include screening of plants with solution-soaked paper and solution culture methods (Narasimhamoorthy *et al.,* 2007), soil-petri-dish method (Stass *et al.,* 2007), and screening in pots in a greenhouse (Tazeem *et al.,* 2009).

For sorghum, screening in the field, and in pots or nutrient solution are commonly used for selection of Al^{3+} tolerant genotypes (Mohammed and Ezeaku, 2006). A rapid screening method is needed to select a large number of new genotypes or new inbred lines in plant breeding, such as solution-soaked paper, solution culture and soil-petri dish methods used to evaluate Al^{3+} tolerant sorghum. All of these rapid screening techniques use the response to Al^{3+} of the rate of seedling germination and root development. However, the method using such growth responses can curtail the accuracy of screening (Yashida and Yashida, 2000). Detection systems not dependant on the rate of seedling or root development, would greatly improve the success of screening procedure (Abdel-Hady, 2006). Screening by using haematoxylin staining of seedling roots requires less time and simpler pH management than the other methods, and is very useful for selection or screening a relatively large population in breeding program. Measurement of Al^{3+} tolerance is based on the staining pattern of the root. The haematoxylin staining method is a very common technique for the evaluation of Al^{3+} tolerance in wheat (Kashif *et al.,* 2004) and barley (Shahinnia *et al.,* 2005). Field screening for Al^{3+} tolerance would be the best approximate

for selecting Al^{3+} tolerant plants. In practice, however, reliable ranking of tolerance in the field screening is difficult because the Al^{3+} concentration in soil may not be uniform and because environmental factors interact with soil Al^{3+} to mask the expression of Al^{3+} tolerance (Naserian *et al.*, 2007). Screening by using the growth response to Al^{3+} added to the soil in pots in a greenhouse (referred to as growth-response method hereafter) may be superior in this respect.

2.6 Sectional conclusion

Aluminium toxicity is one of major abiotic stresses that limit sorghum productivity in acidic soils worldwide (Magalhaes *et al.*, 2004). This causes a rapid inhibition of root growth by inhibiting root cell expansion and elongation and finally, cell division over a long term Zhang *et al.*, 2007). This inhibition of the growth of the roots further causes reduced plant vigor and yield (Kochian *et al.*, 2005). Majority of farmer preferred sorghum genotypes in most East African genotypes are sensitive to Al^{3+} toxicity that breeding for better adapted sorghum cultivars seems to be the best strategy for improving farming in regions with the soil acidity. This study is designed to support screening of a wide collection of Ugandan accessions for reaction to Al^{3+} toxicity a research gap that need to be undertaken to support sorghum breeding work in Uganda and the wider region.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The north-eastern quadrant of Africa has been proposed as one of the centres of domestication of crop which is presumed to have occurred about 5000 year ago (Doggett, 1975). Other authors however suggest that the crop could have been domesticated in the savannah zone south of the Sahara from where different races of the present cultivated races spread to the rest of the continent (Harlan, 1971; Harlan and De wet 1972; Clark, 1976). These seminal reports suggest that indeed eastern Africa is an important home to the crop with the Sudano-Sahelian regions being particularly important. Tolerance to Al^{3+} toxicity was first found in breeding lined derived from Sudano-Sahelian regions of Africa (Magalhaes *et al.,* 2004, 2007). Uganda falls in this region and could thus hold accessions with rare and useful alleles that could be exploited in sorghum improvement. This thesis research thus aimed at characterising local sorghum accessions for tolerance to Al^{3+} toxicity. The studies was conducted using sorghum accessions assembled from various Ugandan agro-ecologies and screened for sensitivity to aluminium toxicity using a solution culture and haematoxylin staining method. Detailed description of the methodologies used is provided below.

3.2 Description of experimental site

The study was conducted at Makerere University Agricultural Research Institute (MUARIK). Makerere University Agricultural Research Institute (MUARIK) is located at 0^o28^oN, 32^o37^oE north of Kampala city, Wakiso district approximately 18 Km from the city centre and is at an altitude of 1,200 m above sea level. The annual rainfall it receives is 1,300 mm over the two (2) rainy seasons of April – July and October – December while the daily minimum and maximum temperatures are about 16.5 and 28°C respectively. The daily relative humidity may fall below 40% during the dry season. The screening experiments (solution cultures) were done in the controlled plant growth environment chamber at MUARIK.

3.3 Plant material used in the study

One hundred and sixty seven sorghum accessions were used in this study. The standard sorghum checks were obtained from International Crops Research Institute for the Semi-Arid Tropics

(ICRISAT) through Moi University. The phosphorus efficient and in-efficient materials were obtained from Moi University Sorghum Research Team led by Professor Samuel Gudu while the local land races were collected from various agro-ecologies in Uganda and neighbouring countries by Makerere University and the Sorghum research team at the National Semi –Arid Agricultural Research Institute (NaSAARI) located in Serere District in Eastern Uganda. General description of the germplasm used is as presented in Table 2. Genotypes O2 and C1 were used as aluminium standard tolerant reference checks, while L5 is an aluminium standard sensitive reference, (Wenzl *et al*. 2006). Two inter-related studies (1 & 2) and a third study were conducted and are described below.

Table 2. Summarized description of parents/accessions used in the study.

Variety/Description	Source	Number of lines	Code	Al^{3+} reaction status
1. Tolerant check	ICRISAT	2	0_2, C_1	Tolerant
2. Susceptible check	ICRISAT	1	L_5	Susceptible
P –efficient	Moi University	2	N140d, P_5	Unknown
P –inefficient	Moi University	2	K_{5e}, P_3	Unknown
Accessions	Ugandan collections	160	UNGB series	Unknown

3.4 Study 1: Reaction of Ugandan sorghum accessions and their progeny to Al^{3+} toxicity

3.4.1 Background to the study

The level of tolerance/ or sensitivity of the Ugandan sorghum germplasm to Al^{3+} is unknown and could be determined using both solution culture and haematoxylin staining techniques. Different sorghum varieties have shown to have varying aluminium tolerant levels and thus a large genetic base can provide an opportunity for identifying sources of genetic tolerance to aluminium. The reliance on a single or a few sources of tolerance can result to low crop yield in Al^{3+} toxic soils. Therefore, locally adapted sources of Al^{3+} tolerance would be valuable in the crop improvement. The objective of this study was to evaluate/determine the level of tolerance of the sorghum accessions for aluminium tolerance/ or sensitivity.

3.4.2 Experimental design

The study involved use of related experiments, that is on, growth and analysis of the reaction of sorghum accessions to Al^{3+} in a hydroponic system based on analysis on root growth parameters and haematoxylin Harris staining of roots for damaged by Al^{3+} (Magnavaca *et al.,* 1987; Cancado *et al.,* 1999). In the first experiment, only the nutrient media augmented with $Al^{3+}$was used to characterize all the 160 accessions. The experiment was replicated twice and promising genotypes used subsequently in cropping experiments. In the second experiment, both root growth parameters and haematoxylin Harris straining was used. Detailed description of each experiment is provided below.

3.4.2.1 Assessment of sorghum genotypes to Al^{3+} toxicity based on root growth parameters

Aluminium induced inhibition of root growth (first experiment) and staining (second experiment) were used to quantify aluminium tolerance/ or sensitivity of the sorghum genotypes, using the basal nutrient solution as the growth media and haematoxylin staining solution (Magnavaca *et al.,* 1987; Cancado *et al.,* 1999). This experiment was set up following a split plot design with two replications for the nutrient solution. The experimental design was chosen because of the large number of genotypes and in an attempt to control variation within treatments. Treatments comprised of seedlings were subjected to two levels of Al^{3+} i.e. 0 μM (control) and 148 μM with the source of Al^{3+} being $AlK(SO4)_2.12H_2O$. The main plot was the two levels of aluminium applied (while the genotypes constituted the sub-plots randomized using computer. Each main plot contained ten (10) testable genotypes and two (2) standard checks; one (1) tolerant and the other sensitive to aluminium. Prior to planting, test sorghum seeds were washed with distilled water, surface sterilized in 1% sodium hypochlorite (NaOCl) for 5-8 minutes, and rinsed 8 times in sterile distilled water. The surface sterilized seed was then pre-germinated using moist paper towels contained in petri-dishes in an incubator at 25 – 27 ^{o}C in the dark for four (4) days. Prior to transfer to the hydroponic growth system (Figure 1), the initial root length (IRL) was measured. Subsequently, seedlings were put in plastic cups measuring (2.5 cm x 3.5 cm) held together by a styrofoam plate measuring 31 cm x 36 cm and transferred to trays containing 5-8 litres of nutrient solutions. Four seedlings per genotype per treatment were used in the analysis. The seedlings were continuously aerated using aeration pump (FIMA air compressor) for 5 days. The pH of the nutrient solution varied from 4.0 - 4.2 as standardized

using 0.1M HCl. Temperature and light were maintained at 25 – 27 ºC and 550 µmol photons per square metre per second, respectively. Final root length (FRL) was measured from the root tip to the base on the 5th day after transfer to nutrient solution. The net root length (NRL) was used to group sorghum into tolerant, moderately tolerant and sensitive phenotypic classes. Data were subjected to analysis of variance and means separated by Fisher`s Protected Least Significant Difference at 5% probability level (Steel *et al.,* 1997) using Genstat version 14 (Lawes Experimental Trust: Rothamstead Experimental Station UK, 2007).

Figure 1. Hydroponics screening system for sorghum seedling in cold room

3.4.2.2. Assessments of sorghum genotypes to Al^{3+} based on haematoxylin staining

Haematoxylin screening experiment on cereals is often performed as a rapid assessment method on the performance of the accessions in relation to Al^{3+} tolerance. In so doing, the genotypes will be grouped basically into two extreme categories; one tolerant and the other sensitive to Al^{3+} with the middle class being intermediate. Measurement of Al^{3+} tolerance is based on the staining pattern of the root. It is a very common technique for evaluation of Al^{3+} tolerance in wheat (Kashif *et al.,* 2004) and barley (Shahinnia *et al.,* 2005) as selection and screening of a relatively large population in a breeding program is made possible. In this study, seedlings of 83 sorghum genotypes were subjected to the haematoxylin staining as described by Cancado *et al.* (1999).

The experiment was set up following a split plot design with two (2) replications in a controlled plant growth chamber. This experimental design was chosen because of the large number of genotypes and in an attempt to control variation within treatments. Test genotypes were screened in a hydroponic Al^{3+} augmented nutrient solution set-up as described in section 3.4.2.1. Each tray contained ten (10) test genotypes and two (2) standard checks; one (1) tolerant and the other sensitive to aluminium. The main plot was either 0.2% haematoxylin solution containing 0.02%

potassium iodide solution, augmented nutrient solution and the distilled water control, while the test genotypes were sub-plots. After 3 days of the experiment, the sorghum seedlings (suspended in Styrofoam trays as described above) were transferred from the 148 µM Al nutrient solution in to distilled water and subjected to gentle shaking (20 rpm) in a mechanical shaker for 15 minutes. The seedlings were then transferred into the haematoxylin solution and shaken gently as above for 20 minutes after which the seedlings were finally placed in another fresh distilled water and again shaken for another 15 minutes before the visual scoring of haematoxylin staining was made on individual seedlings. All test genotypes were visually scored for root staining intensity due to complex formation with Al^{3+} ions on a scale of 1-5 as follows: seedlings with non-stained were classified as very tolerant, (scale1), faintly stained roots as tolerant (scale 2), moderately stained as moderately tolerant (scale 3), well stained roots as sensitive (scale 4), and with deeply stained roots as very sensitive (scale 5) (Figure 2).

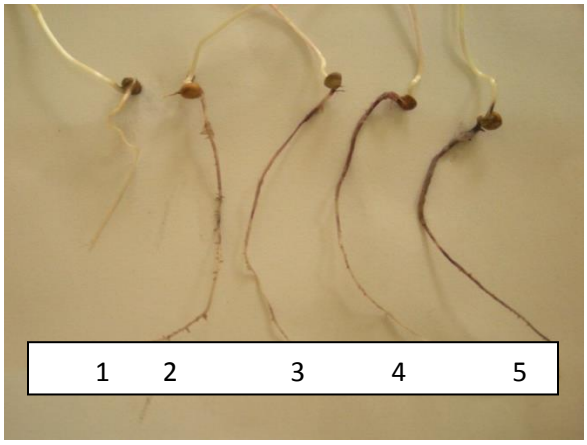

Figure 2. Haematoxylin staining score chart used to assess reaction to Al^{3+} toxicity.

3.5 Study 2: Genetic analysis of tolerance to Al^{3+} toxicity in selected sorghum genotypes

3.5.1 Experiment 1. Population development and inheritance studies

Based on their performance and phenotypic classes for reaction to aluminium toxicity, three tolerant and four sensitive sorghum cultivars including two (2) tolerant checks and one (1) susceptible check were selected for development of a segregating population. The population was developed through crosses in the screen house at MUARIK using a North Carolina II mating design (NCII) (Table 3). Each parent was planted in buckets using soil that had been sterilized

and fertilized appropriately with NPK (17:17:17) Planting was staggered to allow for flowering synchronization and the crossing was done by hand pollination of emasculated flowers. Three (3) to four (4) crossing blocks were established to ensure adequate F_1 seed. The F_1 plants derived from each cross were self-pollinated and seven F_2 populations generated for Al^{3+} tolerance and genetic studies.

Table 3. North Carolina II mating scheme used to generate populations for combining ability

Parent (Female)	Parent (male)		
	O2 (T1)	C1 (T2)	UNGB2672 (T3)
L5 (S1)	X	X	X
UNGB2759 (S2)	X	X	X
UNGB2856 (S3)	X	X	X
UNGB2762 (S4)	X	X	X

X = crosses; T1 = T2 = T3 = Tolerant lines; S1 = S2 = S3 = Sensitive lines

3.5.2 Experiment 2. Reaction of F_2 seedlings to Al^{3+} aluminium based on haematoxylin staining of test plant roots

3.5.2.1 Introduction

As a whole, cereal crops (Poaceae) provide an excellent model for studying Al^{3+} tolerance because of their abundant genetic resources, large, active research communities, and importance to agriculture. In addition, work in one cereal species such as sorghum can rapidly translate into impact throughout the family. The objective of this study was to evaluate the eight F_2 sorghum family seed for aluminium tolerance.

3.5.2.2 Experimental design and data analysis

F_2 progeny and their parents were subjected to Hydroponic analysis of Al^{3+} tolerance in the nutrient medium and haematoxylin Harris analysis as described in sections 3.4.2.1 and 3.4.2.2 respectively. Table 4 shows the test lines used in the study. The nutrient solution experiment was replicated four times at three Al^{3+} levels of 0, 148 and 222 µM while the haematoxylin Harris staining procedure was replicated twice at only Al^{3+} level of 148 µM. The data was handled as described in sections 3.4.2.1.

3.5.3 Experiment 3. Genetic analysis of tolerance to Al^{3+} toxicity based on seedling, shoot and root biomass production

3.5.3.1 Background to the study

Aluminium (Al^{3+}) toxicity is one of the factors limiting crop production on acid soils. However, genotypic differences exist among plant species or cultivars in response to Al^{3+} toxicity. Among and within crop and plant species there is genetic variation in response to Al^{3+} toxicity (Kochian *et al.,* 2004; Yang *et al.,* 2011). This natural genetic variability provides a suitable solution for breeding crops that are tolerant to Al^{3+}. As such improving our knowledge of the genetics underlying Al^{3+} resistance is essential to speed up the development of new Al^{3+} resistant cultivars. The objectives of the study was to evaluate tolerant, sensitive sorghum lines and their F2 families for reaction to high Al^{3+} concentrations and to determine combining ability effects, genetic variability and heritability for shoot and root dry matter (DM) yield of sorghum seedlings grown in nutrient solutions containing different Al^{3+} concentrations.

Table 4. F_2 families and parental lines evaluated in nutrient solution for ten days in screen house.

Entry No	Entry Cross	Entry Name
1	G1xG6	L5xO2
2	G1xG7	L5xC1
3	G2xG9	UNGB2759xUNGB2672
4	G4xG6	UNGB2856xO2
5	G4xG7	UNGB2856xC1
6	G5xG7	UNGB2762xC1
7	G5xG9	UNGB2762xUNGB2672
Parental Lines		
1		L5
2		UNGB2759
3		UNGB2856
4		UNGB2762
5		O2
6		C1
7		UNGB2672

3.5.3.2 Experimental design, data assembly and analysis

The experiment was carried out in a split plot arrangement with four replications and plants allowed to grow in the solution for ten days. The pH of the solution was monitored and adjusted

to pH of 4.0 – 4.2. The daily light regimes was 14 hours of light and 10 hours of darkness and the growth room temperature set at 20 – 26 °C. In this experiment, both the shoot and root biomass were separately harvested, oven dried and weighed to determine the respective dry matter (DM) production.

Data from this experiment was subjected to analysis of variance and means separated by Fisher`s Protected Least Significant Difference at 5% probability level (Steel *et al.,* 1997), using Genstat version 14 (Lawes Experimental Trust: Rothamstead Experimental Station UK, 2007). The F_2 family was used to estimate genetic parameters in a Northern Carolina Design II analysis. This would allow estimations to be made for variance components and heritability. Heritability was estimated based on coefficient of genetic determination (CGD) as follows: NS-CGD = σ^2GCA(f) + σ^2GCA(m)/(σ^2GCA(f) + σ^2GCA(m) + SCA(f+m) + σ^2e and BS-CGD = σ^2GCA(f) + σ^2GCA(m) + SCA(f+m) /(σ^2GCA(f) + σ^2GCA(m) + SCA(f+m) + σ^2e

The linear model for the NCII design based on individual replication data used was

$Y_{ijk} = \overline{Y}\ldots + GCA_i + GCA_j + SCA_{ij} + B_k + e_{ijk}$

Y_{ijk} ---------- Mean performance of i^{th} parental mated to j^{th} parental line/Observed value

$\overline{Y}$.. ---------- Population mean (Grand Mean) common to all observations

GCA_i-------- General Combining Ability of i^{th} parental line/Effect of i^{th} parental line

GCA_j-------- General Combining Ability of j^{th} parental line/Effect of j^{th} parental line

SCA_{ij} -------- Specific Combining Ability of i^{th} andj^{th} parental lines (interaction of i^{th} and j^{th} parental lines)

B_k---------- Block effect

e_{ijk}---------- Random error

$\overline{Y}\ldots + GCA_i + GCA_j$ ---------- Predicted from parental GCA effects/values

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Study 1. Reaction of Ugandan sorghum accessions and their progeny to Al^{3+} toxicity

4.1.1 Reaction of accessions based on root length

Highly significant differences (P≤0.001) in tolerance to aluminium toxicity were found among the genotypes. Similarly, the interaction between the treatment levels and genotypes were highly significant (P≤0.001) (Table 5). The Al^{3+} levels were also significant (P≤0.05). The high concentration of 148 µM affected roots growth with susceptible genotypes exhibiting reduced root growth, with blackish tips, typical symptoms of Al^{3+} toxicity on the meristematic root tissue. On the basis of the reaction to Al^{3+} toxicity, genotypes were classified into tolerant, moderate tolerant and sensitive phenotypes (Table 6). The reduction of net root length at 148 µM to the control was computed and genotypes with larger values were tolerant to Al^{3+} toxicity; while those with very low ratios are very sensitive to Al^{3+} (Table 6).

Table 5. Mean squares for net root length of sorghum accessions subjected to varying levels of Al^{3+} when tested in a hydroponic system.

Source of variation	DF	SS	MS	VR	F pr
Replication	1	1.83	1.83	0.81	
Al^{3+} level	1	714.61	714.61*	316.93	0.036
Genotype	95	100.40	1.06***	7.42	<.001
Al^{3+} Level * Genotype	95	94.43	0.99***	6.98	<.001
Total	383	940.59			

***, * Significant at P≤0.001 and P≤0.05, respectively. CV% = 17.9, GM = 2.104, SED = 0.4055, SEM = 0.2867

Table 6. Effect of aluminium concentration on root length of selected Ugandan sorghum accessions when tested in a hydroponic system for five days.

Genotype	Net root length /Cm (0 µM Al^{3+})	Net root length /Cm (148 µM Al^{3+})	Net root length Ratio (148 Al^{3+}/0 Al^{3+})	Tolerance Classification
C1	2.55	1.25	0.49	T
K5e	3.83	0.60	0.16	S
L5	4.21	0.66	0.16	S
N140d	5.30	1.09	0.21	MT
O2	2.56	1.26	0.49	T
P3	4.80	0.54	0.11	S
P5	3.51	1.14	0.33	T
UNGB 2671	4.58	1.50	0.33	T
UNGB 2672	4.46	1.40	0.31	T
UNGB 2678	4.78	1.45	0.30	T
UNGB 2730	1.66	0.40	0.24	S
UNGB 2759	2.66	0.51	0.19	S
UNGB 2762	3.69	0.45	0.12	S
UNGB 2777	2.68	0.78	0.29	S
UNGB 2856	1.98	0.44	0.22	S
UNGB 2867	2.24	0.70	0.31	S
UNGB 2880	2.59	0.61	0.24	S
UNGB 2898	2.01	0.66	0.33	S
UNGB 3019	1.80	0.56	0.31	S
UNGB 3059	2.99	0.86	0.29	S
CV%	15.2	11.40	18.90	
SEM	0.37	0.06	0.03	
LSD (P≤0.05)	1.05	0.17	0.09	

Key: T = tolerant; MT = medium tolerant; S = sensitive to Al toxicity. Scale for classification at 148µM Al $^{3+}$: T > 1.10cm; MT 0.9 - 1.09 cm; S < 0.9 cm. – O2 and C1 and L5 were used as standard checks from ICRISAT for Al tolerance and sensitive respectively.

4.1.2 Reaction of sorghum accessions based on Haematoxylin staining of roots

Highly significant differences in haematoxylin staining of roots was found. High variability in haematoxylin staining of the roots was found with some genotypes being more sensitive than the ICRISAT susceptible test line (L5). The tolerant lines were found to absorb no or relatively less Al^{3+} into the growing root tips (Plate 2 (a) meaning that they exuded more citric acid compared to the sensitive genotypes that showed an intense blue colour (Plate 2 (b)) together with epidermal degradation that extended from the root elongation zone to the root tips. However, the seedlings that were grown in 0 µM Al^{3+} did not show any staining with haematoxylin. A very

small proportion of the screened accessions including the standard checks, O2 and C1 were within the tolerance level of 3 (Table 7) thus, indicating the presence of some reasonable degree of tolerance to aluminium toxicity among the accessions. On the other hand, the majority of the accessions showed haematoxylin stain score of 4 (Table 7) just like the sensitive standard. However, very sensitive lines had score of 5). A significant but negative correlation between haematoxylin staining and net root length of test genotypes (r^2 = -0.94).

Table 7. Haematoxylin staining intensity on selected MUK improved sorghum accessions grown in solution at 148 µM Al^{3+} for three days when tested in a hydroponic system at MUARIK.

Genotype	Haematoxylin mean score
C1	1.4
K5e	4.3
L5	4.0
NI40d	2.5
O2	1.6
P3	4.5
P5	2.6
UNGB 2671	2.5
UNGB 2672	1.9
UNGB 2678	2.0
UNGB 2730	4.5
UNGB 2759	4.4
UNGB 2762	4.4
UNGB 2777	3.9
UNGB 2856	4.6
UNGB 2867	4.0
UNGB 2880	4.0
UNGB 2898	4.5
UNGB 3019	4.4
UNGB 3059	3.1
CV (%)	7.50
SEM	0.199
LSD ($p \leq 0.05$)	0.559

Figure 3. Haematoxylin staining of tolerant (a) and sensitive sorghum line (b) at 148 µM Al^{3+}

a) Tolerant sorghum line (non-stained) b) Sensitive sorghum line (Deeply stained)

4.1.3 Reaction of F_2 progeny to Al^{3+} toxicity based on root growth parameters

4.1.3.1 Results based on root length parameters

Very highly significant differences ($P \leq 0.001$) were observed for aluminium level, genotypes and interaction. Increasing Al^{3+} concentration from 0 to 222 µM in the nutrient basal solution had varying negative effects on seedling root growth (Table 8). Sensitive genotypes had stunted roots with severe root discoloration of meristematic tissue that turned black in color, coupled with epidermal degradation along the root length. The effect of Al^{3+} concentration was even more serious at 222 µM where the genotypes that appeared to tolerate the stress imposed at 148 µM aluminium were all sensitive at this concentration. Based on the net root length at 148 µM, the genotypes were classified as susceptible, or tolerant. The local accession UNGB2672 had longer roots (1.51) above the Al^{3+} tolerant standard checks, C1 and O2 that had net root lengths of 1.34 and 1.26 respectively (Table 8). The ratio of net root length at148 µM Al to net root length at 0 µM Al^{3+} an indicator of aluminium toxicity tolerance/sensitivity was also used to further characterize test lines. The higher the ratio, the more tolerant the genotype is, while the lower the ratio the more sensitive the genotype is to Al^{3+} toxicity. Using this approach, the crosses UNGB2856xC1, UNGB2856xO2, L5xO2 and UNGB2759xUNGB2672 and UNGB2762xC1 showed reasonable degree of tolerance to Al^{3+} toxicity at 148 µM while at 222 µM all the crosses become sensitive as shown by the low net root length ratio (Table 8).

Table 8. Effects of aluminum toxicity on root length of F_2 progeny and their parents of test sorghum genotypes grown in aluminium augmented nutrient solution culture for five days.

Genotype	Net root length/cm (0 µM)	Net root length/cm (148 µM)	Net root length/cm (222 µM)	148/0	222/0	Reaction Class
C1	2.97	1.34	0.55	0.45	0.19	T
L5xO2	3.58	1.16	0.51	0.33	0.14	T
L5xC1	4.92	1.18	0.51	0.24	0.10	T
UNGB2759xUNGB2672	3.73	1.16	0.43	0.31	0.12	T
UNGB2856xO2	2.89	1.13	0.46	0.39	0.16	T
UNGB2856xC1	2.33	1.11	0.47	0.48	0.20	T
UNGB2762xC1	3.64	1.14	0.47	0.31	0.13	T
UNGB2762xUNGB2672	3.90	1.05	0.44	0.27	0.11	MT
L5	4.08	0.69	0.43	0.17	0.10	S
O2	2.25	1.26	0.55	0.56	0.25	T
UNGB2672	5.55	1.51	0.56	0.27	0.10	T
UNGB2759	3.68	0.61	0.29	0.16	0.08	S
UNGB2762	4.03	0.49	0.21	0.12	0.05	S
UNGB2856	2.18	0.51	0.24	0.23	0.11	S
CV%	14.60	8.60	8.40	22.2	19.70	
SEM	0.26	0.04	0.02	0.04	0.01	
LSD (P≤0.05)	0.73	0.13	0.05	0.10	0.04	

4.1.3.2 Results based haematoxylin staining of progeny roots

The genotypes were highly significantly different ($P \leq 0.001$) in the level of haematoxylin stain from each other (Table 9). Tolerant F2 families and parents absorbed nothing or relatively less Al into the growing root tips.

Table 9. Haematoxylin staining intensity on F_2 families and parental lines grown in aluminum augmented nutrient solution culture at 148 µM for three days.

Genotype	Mean haematoxylin staining score at 148 µM
L5xO2	2.9
L5xC1	3.1
UNGB2759xUNGB2672	2.9
UNGB2856xO2	3.3
UNGB2856xC1	3.1
UNGB2762xC1	3.0
UNGB2762xUNGB2672	3.0
Parental lines	
C1	1.2
L5	3.9
O2	1.4
UNGB2672	1.4
UNGB2759	4.3
UNGB2762	3.9
UNGB2856	3.8
Grand mean	2.7
CV%	8.47
SEM	0.16
LSD (P≤0.05)	0.48

4.1.3.3 Results based on root biomass

Analysis of variance revealed that there were high significant differences between genotypes ($P \leq 0.001$) and Al^{3+} concentration ($P \leq 0.001$) and a high significant interaction between aluminum and genotypes ($P \leq 0.01$). When the crosses were analyzed separately however, there was a non-significant interaction between aluminum and crosses ($P \leq 0.05$) showing that there was increase in aluminum tolerance in the crosses as shown by increase in mean root dry matter, thus making no significant difference in aluminium and crosses interaction (Table 10). In general, the mean root dry matter decreased with increase in Al^{3+} concentration. For example at Al^{3+} concentration of 148 µM, genotype UNGB2672 (tolerant parent) had the highest mean root dry matter of 3.42 mg followed by L5 (sensitive parent) of mean root dry matter of 2.37 mg and then by C1 (tolerant parent) of root mean root dry matter of 2.26 mg and least by UNGB2762 (sensitive parent) of mean root dry matter of 1.35 mg, while the rest of the genotypes had the mean root dry matter between the third highest and the least (Table 10). On the other hand, genotype

UNGB2672 had the highest mean root dry matter of 3.37 mg, followed by L5 (2.49 mg), C1 (2.20 mg) and least by UNGB2762 (1.38 mg) (Table 10). Among the crosses, L5xC1 had the highest mean root dry matter (3.35 mg), followed by UNGB2759xUNGB2672 (2.81 mg), L5xO2 (2.76 mg) and least was UNGB2762xUNGB2672 (1.61 mg) at Al^{3+} concentration of 148 µM. Most of progeny had lower mean root dry matter values than their parents L5xC1 (3.29 mg) which exhibited heterosis with a value greater than the parental values (L5 = 2.49 mg and C1 = 2.20 mg); L5xO2 (2.66 mg) against parental values of 2.49 mg and 1.88 mg and finally, UNGB2856xO2 (2.00 mg) compared to parental values of 1.67 mg and 1.88 mg respectively (Tables 10 & 11).

Table 10. Evaluation of seven F2 progeny and their parental lines for mean root dry matter at three Al^{3+} levels.

Genotype	**Al^{3+} concentration**			**MRDM across treatments**
	0 µM	**148 µM**	**222 µM**	
L5xO2	2.95	2.76	2.26	2.66
L5xC1	3.57	3.35	2.95	3.29
UNGB2759xUNGB2672	3.27	2.81	2.28	2.79
UNGB2856xO2	2.18	2.00	1.83	2.00
UNGB2856xC1	2.25	1.91	1.59	1.92
UNGB2762xC1	1.92	1.76	1.58	1.75
UNGB2762xUNGB2672	1.71	1.61	1.42	1.58
Parental Lines				
L5	3.30	2.37	1.79	2.49
UNGB2759	2.05	1.55	1.23	1.61
UNGB2856	2.01	1.65	1.36	1.67
UNGB2762	1.67	1.35	1.13	1.38
O2	2.16	1.92	1.57	1.88
C1	2.45	2.26	1.90	2.20
UNGB2672	3.85	3.42	2.84	3.37
Grand Mean (mg)	2.65	2.25	1.86	2.25
CV%	12.00	13.00	16.20	13.40
SEM	0.16	0.15	0.15	0.15
LSD (P≤0.05)	0.45	0.41	0.42	0.33

RDM = root dry matter, MRDM = Mean root dry matter, ** = Crosses with MRDM > parental value that were involved in cross.

Table 11. Evaluation of seven F2 progeny for mean root dry matter at three Al^{3+} levels.

Genotype	Al^{3+} concentration			RMDM across treatments
	0 µM	148 µM	222 µM	
UNGB2762xUNGB2672	1.71	1.61	1.42	1.58
UNGB2762xC1	1.92	1.76	1.58	1.75
UNGB2856xC1	2.25	1.91	1.59	1.92
UNGB2856xO2	2.18	2.00	1.83	2.00
L5xO2	2.95	2.76	2.26	2.66
UNGB2759xUNGB2672	3.27	2.81	2.28	2.79
L5xC1	3.57	3.35	2.95	3.29
Grand Mean (mg)	2.55	2.31	1.99	2.28
CV%	10.20	12.0	16.50	12.70
SEM	0.13	0.14	0.16	0.14
LSD (P≤0.05)	0.39	0.41	0.49	0.38

Root dry matter means in mg $plant^{-1}$. RDM = root dry matter, RMDM = root mean dry matter.

4.1.4 Discussions

Root length is one of the best indicators for reaction to aluminum toxicity. Early symptoms of aluminium toxicity occur in the roots because the roots are in direct contact with toxic Al^{3+} ions (Nguyen *et al*., 2003; Kochian *et al*., 2005). As a result, there was general decrease in root length with increase in aluminium level across all the sorghum entries (Table 6). This study revealed that there is genetic variability among sorghum genotypes in response to aluminum. The sensitive genotypes had stunted roots with reduced meristematic root growth manifested at 148 µM Al^{3+} treatment. Analysis of root sensitivity based on haematoxylin staining confirmed significant differences in reaction of genotypes (Table 7). Sensitive genotypes had an intense blue colour together with epidermal degradation that extended from the root elongation zone to the root tips, while the tolerant ones had no staining to moderate staining (Plate 1). Haematoxylin staining has been found to be an early indicator of aluminum toxicity effects on the apices of young developing roots grown in nutrient solution (Cancado *et al.*, 1999). The variation of the sorghum accessions to haematoxylin staining when subjected to 148 µM Al^{3+} treatment is an indication of phenotypic variability of the test sorghum genotypes for sensitivity to aluminum toxicity. Indeed, there was a wide variability for tolerance to Al^{3+} toxicity among these accessions with some accessions exhibiting greater tolerance to Al^{3+} toxicity than the ICRISAT tolerant genotype. This is an indication that breeding for tolerance to Al^{3+} toxicity using locally

available materials adapted to different agro-ecological zones is possible, particularly if their tolerance to aluminium toxicity can be established.

Assays of segregating progeny revealed significant differences in reaction to Al^{3+} toxicity. The hybrids involving tolerant parents exhibited hybrid vigour with respect to root length compared to the parents when tested in not Al^{3+} augmented nutrient media. However, when tested in augmented media, these test genotypes while significantly different amongst themselves had in general shorter and more damaged roots (Table 8). Haematoxylin staining confirmed variability in sensitivity to Al^{3+} among the F_2 progeny. Significant differences in the root biomass of segregating progeny was found with increase in Al^{3+} toxicity levels further confirming variability in sensitivity to Al^{3+}. This was consistent with the results obtained for the root length for non-segregating progeny. Since root length reduced with increase in the Al^{3+} toxicity level, the mean root dry matter was similarly reduced. Interestingly some progeny such as L5xC1, L5xO2 and UNGB2856xO2 had higher mean root dry matter than the parents involved in the cross in spite of having shorter roots following exposure to Al^{3+} (Tables 9, 10 &11).Tolerance to Al^{3+} toxicity as measured using root growth parameters (length and biomass) as well as the extent of damage through haematoxylin staining, tolerant genotypes were able to grow relatively longer roots at this higher level of Al^{3+} toxic concentration. These results are consistent with those obtained by Kochian *et al* (2005) and Tamas *et al* (2006), Magalhaes *et al* (2006), Rangel *et al* (2007), Ligeyo (2007) and Munyinda *et al* (2008) on similar studies of barley, sorghum, beans, maize and wheat respectively. Since growth is in general genetically conditioned, tolerance to Al^{3+} toxicity in these genotypes is conditioned by genetic mechanism (Kochian *et al.,* 2005; Rangel *et al.,*2007; Munyinda *et al.,* 2008). This heritable trait could be used for improving sorghum material to tolerance to Al^{3+} toxicity.

4.5 Study 2. Genetic analysis of tolerance to Al^{3+} toxicity in selected sorghum genotypes

4.5.1 Combining ability for tolerance to Al^{3+} toxicity based root parameters

4.5.1.1 General combining ability estimates based on root dry weight

The general combining ability (GCA) for the female parents (Al^{3+} sensitive parents) (GCA(S), were very highly significant ($P \leq 0.001$) at all levels of Al^{3+} while among the tolerant male parents

(GCA(T)) were significant only when Al^{3+} was absent. The specific combining ability (SCA) of the crosses were significant ($P \leq 0.05$) for all levels of Al^{3+} suggesting a wide genetic variability among the female parents, compared to the male group under investigation (Table 12). The GCA(S) was more important (at all Al^{3+} levels) than GCA(T) and thus contributed more to additive genetic effects of the female parents (as shown by the high Baker's ratio - Table 12). Significant GCA effects were observed for the all the female parents (Al^{3+} sensitive parents) for root dry matter (Table 12). The parent L5 had consistent significant GCA effects ($P \leq 0.001$) across treatments.

Table 12. Mean squares for combining ability for dry root weight at three Al^{3+} levels.

Source of variation	**DF**	**MS (0 µM)**	**MS (148 µM)**	**MS (222 µM)**	**ExpMS**	**V.C (0 µM)**	**V.C (148 µM)**	**V.C (222 µM)**
GCA(S)	3	0.942***	0.798***	0.491***	$\sigma^2+7/4*\sigma^2gca(S)$	0.529	0.445	0.265
GCA(T)	2	0.071*	0.036^{ns}	0.033^{ns}	$\sigma^2+7/3*\sigma^2gca(T)$	0.023	0.007	2.464E-03
SCA	1	0.075*	0.114*	0.218*	$\sigma^2+1\sigma^2sca$	0.059	0.095	0.191
Error	18	0.017	0.019	0.027	σ^2	0.017	0.019	0.027
BR						0.904	0.827	0.584
BS-CGD						0.973	0.966	0.945
NS-CGD						0.880	0.799	0.551

***, *, ^{ns} Significant at ≤0.001, ≤0.05 and non-significant at ≤0.05 probability levels respectively. V.C = variance components, GCA(S) and GCA(T) = General combining ability of female and male parents respectively, BR, BSC-GD & NSC-GD = Baker`s ratio, Broad-sense and narrow sense coefficients of genetic determination respectively.

However, the female parents UNGB2856 and UNGB2762 had negative GCA effects under Al^{3+} concentrations. All the male parents had non-significant GCA effects ($P \geq 0.05$) under all test concentrations (0 µM, 148 µM and 222 µM), except the Al^{3+} tolerant parent C1 that had a significant ($P \leq 0.05$) GCA with no application of aluminium (Table 13). The male parents O2 and UNGB2672 had negative GCA effects at 0 and 148 Al^{3+} but positive effects at 222 µM aluminum. While C1 had positive GCA effects across the Al^{3+} test levels.

Table 13. Estimates for GCA effects for seven parents (4 female and 3 male) based on dry root weight when screened for sensitivity to Al^{3+} in nutrient solution.

Female	GCA effects (0 µM)	GCA effects (148 µM)	GCA effects (222 µM)
L5	0.72***	0.76***	0.62***
UNGB2759	0.78***	0.52**	0.35*
UNGB2856	-0.32**	-0.35**	-0.27*
UNGB2762	-0.79***	-0.67***	-0.52***
Male			
O2	-0.19^{ns}	-0.14^{ns}	0.12^{ns}
C1	0.16*	0.11^{ns}	0.11^{ns}
UNGB2672	-0.05^{ns}	-0.03^{ns}	0.05^{ns}
ErrMS	0.02	0.02	0.03
t-Critical	2.10	2.10	2.10
Avg (SEM_f)	0.10	0.11	0.13
Avg (SEM_m)	0.09	0.09	0.11

***, **, *, ^{ns} Significant at ≤0.001, ≤0.01, ≤0.05 and non-significant at ≤0.05 probability levels respectively.

4.5.1.2 Specific combining ability (SCA) estimates for dry root biomass

Whereas no significant SCA effects were observed under all Al^{3+} test levels use in the study, there was variation in SCA effects among the crosses (Table 14). High positive SCA estimates for dry root weight were obtained for following hybrids: L5xC1 and UNGB2856xO2 across treatment. The hybrids L5xO2 and UNGB2856xC1 had negative SCA effects across the Al^{3+}concentrations. However, the remaining hybrids had very small SCA values across treatment levels.

Table 14. Estimates for SCA effects for seven F_2 hybrid populations derived by crossing 3 male and 4 female parents assess for dry root weight under three Al^{3+} levels.

Crosses	SCA effects (0 µM)	SCA effects (148 µM)	SCA effects (222 µM)
L5xO2	-0.14^{ns}	-0.17^{ns}	-0.23^{ns}
L5xC1	0.14^{ns}	0.17^{ns}	0.23^{ns}
UNGB2759xUNGB2672	0.00^{ns}	0.00^{ns}	0.00^{ns}
UNGB2856xO2	0.14^{ns}	0.17^{ns}	0.23^{ns}
UNGB2856xC1	-0.14^{ns}	-0.17^{ns}	-0.23^{ns}
UNGB2762xC1	0.00^{ns}	0.00^{ns}	0.00^{ns}
UNGB2762xUNGB2672	0.00^{ns}	0.00^{ns}	0.00^{ns}
SEM	0.13	0.14	0.16
LSD (P≤0.05)	0.39	0.41	3.00
ErrMS	0.02	0.02	0.03
t-Critical	2.10	2.10	2.10

^{ns} = Non-significant at ≤0.05 probability level

4.5.2 Combining ability for tolerance to Al^{3+} toxicity based shoot parameters

4.5.2.1 Variability for shoot biomass production

Analyses of variance revealed highly significant differences between genotypes (P≤0.001) and between Al^{3+} treatments (P≤0.001) for mean shoot dry matter (Table 15). The mean shoot was inversely related to aluminium concentrations with genotypic differences observed between hybrids (Table 15). The results also showed that genotypes that inherently had large shoot biomass such as L5 (sensitive parent) and UNGB2672 (tolerant parent) through affected by Al^{3+} toxicity remained with relatively high biomass (Table 16). Among the hybrids, progeny involving these two parents (L5 and UNGB2762) consistently had larger shoot biomass. At 148 µM, the hybrid L5xC1 had the highest mean shoot dry matter value of 13.99 mg, followed by UNGB2762xC1 with biomass of 10.97 mg. In some cases crosses involving these parents with high shoot biomass had relatively low dry matter such as UNGB2762xUNGB2672 with shoot dry matter of 8.91 mg (Table 16). In relation to their parents, most of the crosses had mean shoot dry matter values between the parental values except L5xC1 (13.97 mg) and UNGB2762xC1 (10.87 mg) which had values greater than the parental values (L5 = 10.7 mg, C1 = 9.84 mg and UNGB2762 = 8.41 mg) across the aluminium levels. Similar results were obtained when only the hybrids were analysed for mean shoot dry matter (Table 17).

Table 15. Mean squares for sorghum parents and their F_2 progeny evaluated for dry shoot weight under varying Al^{3+} concentrations.

Source of variation	DF (F_2 and Parents)	MS (F_2 and Parents)	DF (F_2)	MS (F_2)
Replication	3	0.38**	3	0.28*
Al^{3+}level	2	60.68***	2	12.51***
Genotype	21	13.74***	6	30.99***
Al^{3+}level*Genotype	42	0.59***	12	0.03^{ns}
Residual	195	0.07	60	0.10
Total	263	1.71	83	2.63
CV%	2.64		CV% = 2.91	

***, **, *, ^{ns} Significant ($p \leq 0.001$), ($p \leq 0.01$), ($p \leq 0.05$), and non-significant ($P \leq 0.05$) respectively.

Table 16. Evaluation for seven population and parental lines for mean shoot dry matter at three Al^{3+} levels in nutrient solution.

Genotype	**Al^{3+} concentration**			**MSDM across treatments**
	0 µM	**148 µM**	**222 µM**	
L5xO2	10.86	10.07	9.43	10.12
L5xC1	14.62	13.99	13.28	13.97
UNGB2759xUNGB2672	11.07	10.42	9.70	10.40
UNGB2856xO2	10.40	9.81	9.18	9.79
UNGB2856xC1	10.46	9.85	9.32	9.88
UNGB2762xC1	11.47	10.97	10.16	10.87
UNGB2762xUNGB2672	9.76	8.91	8.23	8.97
Parental line				
L5	12.33	10.52	9.27	10.70
UNGB2759	10.58	9.54	8.69	9.60
UNGB2856	9.70	8.59	7.64	8.64
UNGB2762	9.54	8.33	7.36	8.41
O2	10.48	9.95	9.37	9.93
C1	10.41	9.90	9.20	9.84
UNGB2672	10.51	10.09	9.58	10.06
Grand Mean (mg)	10.98	10.10	9.32	10.13
CV%	2.20	2.70	2.90	2.50
SEM	0.12	0.14	0.13	0.13
LSD(P≤0.05)	0.34	0.39	0.38	0.36

Mean shoot dry matter in mg $plant^{-1}$. SDM = Shoot dry matter, MSDM = Mean shoot dry matter. ** = Crosses with MSDM > parental value that were involved in cross.

Table 17. Evaluation of seven F_2 populations for mean shoot dry matter at three Al^{3+} levels.

Cross	0 µM	148 µM	222 µM	MSDM Across treatment
UNGB2856xC1	10.46	9.85	9.32	9.88
UNGB2762xUNGB2672	9.76	8.91	8.23	8.97
UNGB2856xO2	10.40	9.80	9.18	9.79
L5xO2	10.86	10.07	9.43	10.12
UNGB2759xUNGB2672	11.07	10.42	9.70	10.40
UNGB2762xC1	11.47	10.97	10.16	10.87
L5xC1	14.62	13.99	13.28	13.97
Grand Mean (mg)	11.24	10.58	9.90	10.57
CV%	3.40	3.40	2.00	3.00
SEM	0.19	0.18	0.10	0.15
LSD (P≤0.05)	0.57	0.53	0.30	0.43

Mean shoot dry matter in mg $plant^{-1}$. SDM = Shoot dry matter, MSDM = Mean shoot dry matter.

4.5.2.2 Combining ability for shoot dry weight

Analyses of variance of the progeny and their parents indicated highly significant differences for both GCA and SCA of all parental groups (aluminium sensitive female and tolerant parents) (Table 18).

Table 18. Mean squares for combining ability for dry shoot weight at three Al^{3+} levels.

Source of variation	DF	MS (0 µM)	MS (148 µM)	MS (222 µM)	ExpMS	V.C 0 µM	V.C 148 µM	V.C 222 µM
GCA(S)	3	2.213***	2.063***	2.040***	$\sigma^2+7/4*\sigma^2gca(S)$	1.244	1.161	1.160
GCA(T)	2	2.556***	3.022***	2.920***	$\sigma^2+7/3*\sigma^2gca(T)$	1.080	1.282	1.247
SCA	1	3.422***	3.746***	3.448***	$\sigma^2+\sigma^2sca$	3.386	3.715	3.438
Error	18	0.036	0.032	0.010	σ^2	0.036	0.032	0.010
BR						0.407	0.397	0.412
BS-CGD						0.994	0.995	0.998
NS-CGD						0.404	0.395	0.411

*** Significant at ≤0.001 probability level, V.C and A.V.C = variance components and average variance component respectively, GCA(S) and GCA(T) = General combining ability of female and male parents respectively, BR, BS-CGD & NS-CGD = Baker`s ratio, Broad-sense and narrow sense coefficients of genetic determination respectively.

Significant GCA effects for mean shoot dry matter (P≤0.001) were observed for the all the female parents (aluminum sensitive parents) across the Al^{3+} concentration but only GCA effect

for UNGB2759 was significant at (P≤0.05) at 0 Al^{3+} (Table 19). However, the female parents UNGB2856 and UNGB2762 had negative GCA effects across the Al^{3+} concentration. All the male parents had statistically high significant (P≤0.001) general combining ability (GCA) effects for aluminum tolerance across the Al^{3+} concentration. The male parents O2 and UNGB2672 had negative GCA effects while C1 had a positive GCA effects across the Al^{3+}concentration respectively. This means that C1 was the best general combiner across the Al^{3+}concentration.

Table 19. Estimates for GCA effects for seven parental lines (3 male and 4 female) for dry shoot weight at three Al^{3+} levels.

Female	**GCA effects (0 µM)**	**GCA effects (148 µM)**	**GCA effects (222 µM)**
L5	1.43***	1.30***	1.33***
UNGB2759	0.51*	0.75***	0.61***
UNGB2856	-0.88***	-0.91***	-0.77***
UNGB2762	-0.80***	-0.76***	-0.86***
Male			
O2	-0.88***	-0.83***	-0.87***
C1	1.03***	1.15***	1.12***
UNGB2672	-0.67***	-0.90***	-0.81***
ErrMS	0.04	0.03	0.01
t-Critical	2.10	2.10	2.10
Avg (SEM_f)	0.15	0.14	0.08
Avg (SEM_m)	0.13	0.12	0.07

***, * Significant at ≤0.001 and ≤0.05 probability levels respectively.

There was a variation in SCA effects among the hybrids (Table 20). L5xC1 and UNGB2856xO2 crosses had high significant positive (p≤0.001) SCA effects of equal magnitude at each level of aluminum across the Al^{3+} concentration. Hybrids UNGB2759xUNGB2672, UNGB2762xC1 and UNGB2762xUNGB2672 had non-significant (p≤0.05) zero (0) SCA effects at all the three Al^{3+} levels. L5xO2 and UNGB2856xC1 had significant negative and positive SCA effects (p≤0.001) respectively across aluminium concentration but of equal magnitude at each level of aluminium.

Table 20. Estimates for SCA effects for seven F_2 populations derived from crosses involving 3 male and 4 female for dry shoot weight at three Al^{3+} levels.

Crosses	SCA effects (0 µM)	SCA effects (148 µM)	SCA effects (222 µM)
L5xO2	-0.93***	-0.97***	-0.93***
L5xC1	0.93***	0.97***	0.93***
UNGB2759xUNGB2672	0^{ns}	0^{ns}	0^{ns}
UNGB2856xO2	0.93***	0.97***	0.93***
UNGB2856xC1	-0.93***	-0.97***	-0.93***
UNGB2762xC1	0^{ns}	0^{ns}	0^{ns}
UNGB2762xUNGB2672	0^{ns}	0^{ns}	0^{ns}
ErrMS	0.04	0.03	0.01
SEM	0.19	0.18	0.10
LSD (p≤0.05)	0.57	0.53	0.30
t-Critical	2.10	2.10	2.10

*** Significant at ≤0.001, ns = non-significant at ≤0.05 probability level

4.6 Discussions

4.6.1 Combining ability for root dry weight

In this study, highly significant differences general combining ability GCA effects among the male and female parents compared to specific combining ability (SCA) were found. These results suggest that additive gene effects (Cruz and Regazzi, 1994), especially from the female group influence tolerance to Al^{3+} toxicity especially for dry root weight which also had high Baker`s ratio (Table 12). Indeed the large value of GCA variance to SCA variance for dry root weight indicates the importance of additive genetic variance in conditioning tolerance to aluminum toxicity in sorghum. A low GCA, whether positive or negative, indicates that the mean of a parent in the cross with the other, does not differ greatly from the general mean of the crosses (Cruz and Regazzi, 1994). Conversely, a high GCA estimate shows that the parental mean is superior or inferior to the general mean thus indicating their good combining abilities as they can easily transfer their tolerance genes to their progeny (Dabholker, 1992).

Among the female parents, UNGB2759 was the best combiner and UNGB2762 was the poorest combiner. At 148 and 222 µM Al^{3+} respectively L5 was the best combiner compared to UNGB2762 and UNGB2759. The male parent C1 was a good combiner though it had a non-

significant GCA effect. At 148 μM Al^{3+} level, O2 was the worst combiner with a negative non-significant GCA effects. At the highest Al^{3+} test concentration (222 μM), O2 was the best combiner among the male parents. It should be noted that whereas some inbred parents were poor general combiners, some positive GCA had relatively higher variance perhaps as a result of exuding citrate from the roots (Magalhaes *et al*., 2004). However, parents L5, UNGB2759 and C1 produced hybrids with positive GCA effects in line with the additive nature of such a quantitative trait across the Al^{3+} concentration. Therefore, parents with high GCA effects for dry root weight can be selected and may be of immediate usefulness to the sorghum breeders using high Al^{3+} saturation stress conditions. This study suggests that GCA effects are more important than SCA effects and thus in these populations, tolerance to aluminum in sorghum is controlled by additive gene action (Pitta *et al*, 1978).

As with GCA effects, the estimates of SCA effects provide an important indication about hybrid performance in relation their parents with respect to a definite trait. This shows the importance of non-additive effects due to large or minor gene effects in a particular hybrid combination. The specific combining abilities of all crosses were all non-significant across Al^{3+} concentration. However, some crosses had non-significant positive SCA effects suggesting that in such cases careful selection of parents could be useful in selection of robust hybrids, albeit this is very limited compared to the effects of GCA. Overall, all this study suggests that selection for Al^{3+} tolerance in sorghum could be done in early generations (F_2 or F_3), improving the rate at which variety development could be done. However, at high concentrations all genotypes are susceptible. Therefore, assessment of tolerance in sorghum may not be possible at extremely high Al^{3+} concentration.

4.6.2 Combining ability for shoot dry matter

There was not a reduction in mean shoot dry matter with increase in Al^{3+} concentration for parents and crosses. Plants with reduced shoot biomass were similar to those with extensive root damage suggesting that root damage invariably reduced mean shoot dry matter. This relationship is perhaps due to damage of xylem transport vessels (Raman *et al*., 2002; Zheng and Yang, 2005). Interestingly, when only the crosses were analyzed for mean shoot dry matter, a non-significant interaction between Al^{3+} and crosses was registered showing that there was increase

in aluminum tolerance in the crosses than the parents as shown by increase in mean shoot dry matter, thus making no difference in Al^{3+} and hybrids interaction (Table 20).

In this study both GCA and SCA were significant at all test levels of $Al^{3+,}$ suggesting that both additive and non-additive genetic effects influenced biomass. The implication is that good combiners especially those with high GCA can easily be used to improve tolerance to Al^{3+}. The variation in the GCA effects among the parental lines is an indication of a broad genetic base among the Uganda sorghum accessions. This suggests that among the collections, there is sufficient genetic variability for aluminium toxicity. The female parent L5 was the best combiner across the Al^{3+}concentration therefore, can be used to develop new tolerant varieties and hybrids. Similarly, the male parent C1 is the best combiner across all the Al^{3+} concentration, thus can also be selected for crossing with the female parents in order to produce hybrids with high mean shoot biomass compared to O2 and UNGB2672 that had negative though significant GCA effects.

The hybrids L5xC1 and UNGB2856xO2were the most tolerant to Al^{3+} toxicity with highly significant and positive SCA effects across Al^{3+} concentration. This hybrid albeit had relatively low mean shoot dry matter (Table 14) compared to the tolerant parent, O2. The hybrid L5xC1 appears to have benefited from the high root growth traits inherited from C1and high mean shoot dry matter of L5. The hybrid Cross L5xO2 appears to have inherited high net root growth from the parent O2 and high mean shoot dry matter from L5. Another hybrid UNGB2759xUNGB2672 benefited from the high net root length and high mean shoot dry matter of parent UNGB 2672. Whilst UNGB2856xO2 benefited from a high net root growth and a high mean shoot dry matter of O2 while UNGB2856xC1 benefiting from a high net root growth and high mean shoot dry matter of parent C1. UNGB2762xC1 benefited from a high net root growth and a high mean shoot dry matter of C1. The hybrid UNGB2762xUNGB2672 benefited from the high net root growth and a high mean shoot dry matter of UNGB2672. Overall, the hybrids benefited from the high net root length of the tolerant parents and high mean shoot dry matter from either parents, hence strong plant growth and aluminium tolerance. The parents involved in these crosses would be of great use in breeding programmes for Al^{3+} tolerance in sorghum as their off-springs would perform better than the average performance of other parents in a range of crosses.

CHAPTER FIVE

5.1 General Discussions

Sorghum is an increasingly robust cereal being targeted for investments in semi-arid and marginal environments. Indeed, within East and southern Africa, the crop is of major importance in areas classified as having moderate agricultural potential requiring investments (Omamo *et al*., 2006; Tabo *et al*., 2007; Twomlow, 2008). In this thesis, investigations aimed at elucidating the nature of resistance in selected sorghum accessions for tolerance to Al^{3+} toxicity a common problem in most of mid-western East Africa (Kochian *et al.*, 2004; Obura, 2008). A number of genotypes selected from accessions collected from the region were assayed for tolerance to Al^{3+} toxicity using a hydroponic system designed to culture plants in augmented nutrient culture. These studies actually show that some of the accessions were more tolerant to Al^{3+} than the commonly used tolerant check C1. Taken together, these results suggest that there is local genetic variability for tolerance to aluminium toxicity among accessions obtained from the region that could be harnessed for the crop improvement. These materials are locally available and in principle adapted to existing agro-ecological conditions, and as such would provide suitable breeding parents with less linkage drag than introduced genotypes.

These studies also found highly significant differences for general combining ability GCA effects among the male and female parents compared to specific combining ability (SCA). These results suggest that additive gene effects (Cruz and Regazzi, 1994), especially from the female group influence tolerance to Al^{3+} toxicity particularly for dry root weight which also had high Baker`s ratio (Table 12). Given that in most cases the effort is to improve the female parent, the results are a good indication for improvement of local material for tolerance to Al^{3+} toxicity. Indeed the large value of GCA variance to SCA variance for dry root weight indicates the importance of additive genetic variance in conditioning tolerance to aluminium toxicity in sorghum. Selection under such circumstances would be faster and more reliable. In the case of aluminium toxicity, however, under very high levels, (222 μM) almost all plants were susceptible implying that the best selection limit for sorghum genotypes is at 148 μM.

In sorghum to screen for tolerance to aluminium toxicity, root length measurement, haematoxylin staining score, root biomass and shoot biomass production have been used

(Magnavaca *et al.,* 1987; Cancado*et al.,* 1999; Nguyen *et al*., 2003; Kochian *et al*., 2005). However, root length and root biomass are the best indicators for reaction to aluminium concentrations as they represent the ultimate product of growth and development of roots (Nguyen *et al*., 2003; Kochian *et al*., 2005). Haematoxylin staining is also an early indicator of Al^{3+} toxicity effects on the apices of young developing roots grown in nutrient solution (Cancado *et al.,* 1999). In this study root length and root biomass and haematoxylin staining were all good indicators of susceptibility of tolerance to Al^{3+}.

The experiment also show the advantage of hybrid vigor for tolerance to Al^{3+} in some specific hybrids for example the cross L5xC1 (3.29 mg-MRDM), L5xO2 (2.66 mg-MRDM) and UNGB2856xO2 (2.00 mg) well above the mean root dry matter for the parents involved in the crosses (across aluminium concentration). The hybrid vigor is also shown by improved mean shoot dry matter is some hybrids across aluminium concentration. These hybrids are L5xC1 (13.70 mg), UNGB2759xUNGB2672 (10.4 mg), UNGB2856xC1 (9.88 mg) and UNGB2762xC1 (10.87 mg). This means that the hybrids benefited from the high root growth of the tolerant parents hence, able to absorb enough nutrients for root growth and plant development. Overall, this different studies conducted support the null hypothesis under which this thesis was conducted. Novel genotypes have been found and suitable parental crosses that can support new variety development also identified.

5.2 Conclusion

The study revealed that there is a wide variability among the Ugandan sorghum accessions with regard to their aluminium response. A good number of accessions showed sensitivity to aluminium toxicity. This shows that aluminium toxicity could be one the major contributing factors to low yield of sorghum in acidic soils. It is therefore, probably time that use of aluminium tolerant lines to improve sorghum yield in toxic soils is encouraged. This large difference in aluminium tolerance can be exploited in an attempt to develop aluminium tolerant sorghum varieties and hybrids as tolerant lines such as C1 and tolerant hybrids and sensitive sorghum lines such as L5 with great potential can be identified. In this respect therefore, a more thorough screening of sorghum germplasm collected from all sorghum growing areas of Uganda be conducted in order to address the aluminium toxicity in the country`s sorghum.

Other methods could also be deployed for screening tolerant materials such as field trials and potted soil experiments using traits identified in this study such as root length measurement. This method was adopted from studies in maize and is just as effective explain why it is being used in cereal breeding (Magnavaca *et al*., 1978; Marschner, 1995, Raman *et al*., 2002; Zheng and Yang, 2005, Kochian *et al*., 2005, Rangel *et al.*, 2007 and Munyinda *et al.*, 2008). Moreover, the high positive contribution of the variables in the laboratory analysis suggests that laboratory attributes can be used to predict high grain yielding genotypes suitable for soil with low pH and aluminium toxicity.

5.3 Recommendations

The genotypes and/or hybrids that performed predictably well in the screening experiment and biomass production should be rescreened to validate their performance before the field trial is conducted. This is because few seedlings were used in the evaluation process as the experimental set-up and some reagents may have also not been very efficient. It is also very important to validate the screening experiment in the field since most acid soils are deficient in various nutrients as well as having toxic levels of elements like aluminium and manganese (Sanchez and Salinas, 1981). Sorghum markers (marker association) for tolerance to aluminium toxicity in sorghum (acidity) can be used to hasten the breeding programme and deliver varieties and hybrids in a shorter time. More variables that relate directly to tolerance to aluminium such as number of lateral roots and shoot length should be studied in order to establish a more comprehensive and effective combinations of selection criteria.

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