INHERITANCE OF TOLERANCE TO ALUMINIUM TOXICITY IN COMMON BEANS (*Phaseolus vulgaris* L.)

BY

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DECLARATION

I, Prisca Mutale hereby declare that this dissertation represents my own work and that is has not been previously submitted for a degree, diploma or other qualification at this or any other University.

Signature: .....

Date: .....

APPROVAL

This dissertation of Prisca Mutale has been approved by the University of Zambia as partial fulfilment of the requirements for the award of the degree of Master of Science in Plant breeding and Seed Systems.

Examiner's name	Signature	Date
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DEDICATION

This work is dedicated to my father (Mr John Mutale) and my mother (Mrs Jennipher Mutale).

ABSTRACT

Common bean (*Phaseolus vulgaris*) is an important nutritional component in the diet of more than 300 million people around the world. Its production is limited by drought and aluminium toxicity among other abiotic factors. Correction of acidity-related soil constraints such as aluminium toxicity using lime and phosphate fertilizers are beyond the capacity of resource-poor farmers in Zambia. This situation, therefore, calls for the need to make use of the genetic variation existing for adaptation to acid soils and aluminium tolerance among common bean genotypes to improve tolerance to aluminium toxicity so that locally grown common beans may be successfully grown in acidic soils. The objectives of this study were (1) to characterize common bean genotypes for aluminium tolerance (2) to investigate the type of gene action conditioning aluminium tolerance in common beans. The study was carried out at the University of Zambia and involved; (i) evaluation of 20 bean genotypes in nutrient solution (0 and 12 mgL^{-1} aluminium) to assess genotypic response, to aluminium stress (ii) creation of experiment population in North Carolina Design II, (iii) advancement of F_1 to F_2 generation and (iv) evaluation of F_2 crosses for aluminium tolerance in the 12 mgL^{-1} aluminium nutrient solution. Data collected/derived were those associated with aluminium tolerance and included root length, shoot length, root biomass, shoot biomass, number of lateral roots and root/shoot ratio. Data were analysed using Genstat 14th version with means separated using Least Significant Difference ($LSD_{0.05}$). The ratio, $\sigma^2gca_m + \sigma^2gca_{f)} / (\sigma^2gca_m + \sigma^2gca_f + \sigma^2sca$, was used to determine the type of gene action conditioning inheritance of aluminium tolerance and the relative importance of General and Specific Combining Ability variances. Narrow-sense heritability estimates for each set were calculated. The results showed highly significant $P<0.001$) differences among genotypes, aluminium concentration and interaction between genotypes and aluminium concentration for all studied traits except number of lateral roots where non-significant aluminium concentration was observed. The hypothesis that there is enough genetic variation among common bean genotypes for acid soils adaptation and aluminium toxicity tolerance was validated in this study. Five genotypes (SZ 3-3-B-B2, Lukupa, VTTT918/15-4-4, CIM-RMOO-321LN02 and LY4-4-B) were found to be tolerant, four (SZ9-7-B-B, Kabulangeti, BF13607-12 and SZ9-B-B-B2) moderately tolerant and eleven (NUA 45, BF13607-6, VTTT915/2-2, VTTT924/12-4, Kalungu, Chambeshi, Lyambai, NUA91, Solwezi parent, BF13572-11 and ARA 4) sensitive to aluminium toxicity. Moreover, SZ3-3-B-B2 identified as aluminium tolerant, with good general combining abilities for root length, shoot length and root biomass, was identified as suitable for use in breeding for aluminium tolerance in common beans. The results obtained in this study further showed that inheritance of aluminium tolerance based on root length, shoot length, shoot biomass and root/shoot ratio, was controlled by additive gene action. Root biomass, on the other hand, was found to be controlled by non-additive gene action. Narrow sense heritability estimates ranged from 10%-55% for root length, 26%-74% for shoot length, 6%-17% for root biomass, 25%-48% for shoot biomass and 10%-13% for root/shoot ratio. Based on these heritability estimates, selection for root length, shoot length and shoot biomass could result in rapid gain in selection because of their medium heritability estimates, while selection for root biomass and root/shoot ratio would result in slow gain in selection because of their low narrow sense heritability estimates.

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LIST OF ABBREVIATIONS

ALMT	Aluminium-activated Malate Transporter
BCMV	Bean Common Mosaic Virus
BILFA	Bean Improvement for Low Fertility in Africa
CSO	Central Statistics Office
DTZ	Distal Transition Zone
EEOA	Economic Expansion in Outlying Areas
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistics
FoDiS	Food Crop Diversification Support
GCA	General Combining Ability
MACO	Ministry of Agriculture and Cooperatives
MAL	Ministry of Agriculture and Livestock
MATE	Multidrug and Toxic compound Extrusion
QTL	Quantitative Trait Loci
SCA	Specific combining Ability
ZARI	Zambia Agriculture Research Institute

CHAPTER ONE

1.0 INTRODUCTION

1.1 Global Importance of common beans

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption. It comprises 50% of the grain legumes consumed worldwide (McClean *et al.*, 2004). Diets in countries from Latin America, Eastern and Southern Africa often contain sufficient carbohydrates, through cereals such as maize, rice, and wheat, but are poor in proteins. Dietary proteins can be found in some animal products but are usually derived from legumes. In some countries, such as Mexico and Brazil, common bean is important as a primary source of dietary protein (Broughton *et al.*, 2003).

Common bean also provide vitamins and mineral nutrients such as zinc and iron (Miklas *et al*. 2006) for majority of the people in Central and eastern Africa. The common bean can supply all of the iron that humans require for metabolism (De Arunjo *et al*., 2003) and provides 25% of the daily requirements of magnesium and copper as well as 15% of the requirements for potassium and zinc. An adequate supply of iron and zinc helps to prevent iron deficiency, anaemia and zinc deficiency as well as other health problems of the developing world (Blair *et al*., 2009).

1.2 Global production and distribution of common bean

Although largely grown for subsistence, mainly by women, approximately 40 percent of the world common bean production is sold at a market value of $ 452 million (Xavery *et al.*, 2005). In recent years, crop production trends have not kept pace with the annual growth rate (estimated above 2 percent) in population in some countries due to a number of biotic, abiotic and socio-economic constraints (Xavery *et al.*, 2005).

According to FAOSTAT, (2006) data, the global production of common bean was approximately 19 million tons in 2005, of which around 6 million tons were produced in Latin America and in the Caribbean. Worldwide, Brazil is the second largest producer of common beans. In 2005, around 3 million tons of common beans were grown in an area of approximately 4 million hectares, with an annual increase estimated at 58.3 thousand tons of grains per year (Grisi *et al.*, 2007).

1.3 Importance and production of beans in Zambia

In Zambia, common beans are an important legume food crop which is widely consumed countrywide among most households, especially the low income households. The crop is a source of vegetable protein and can easily be a substitute for animal protein, which most small-scale households cannot afford yet critical for their nutritional welfare (FoDiS, 2009). Beans rank second to groundnuts among the food legume crops grown in Zambia in terms of economic importance, as this can be reflected from the total area under bean production and the number of households growing and consuming it (Figure 1).

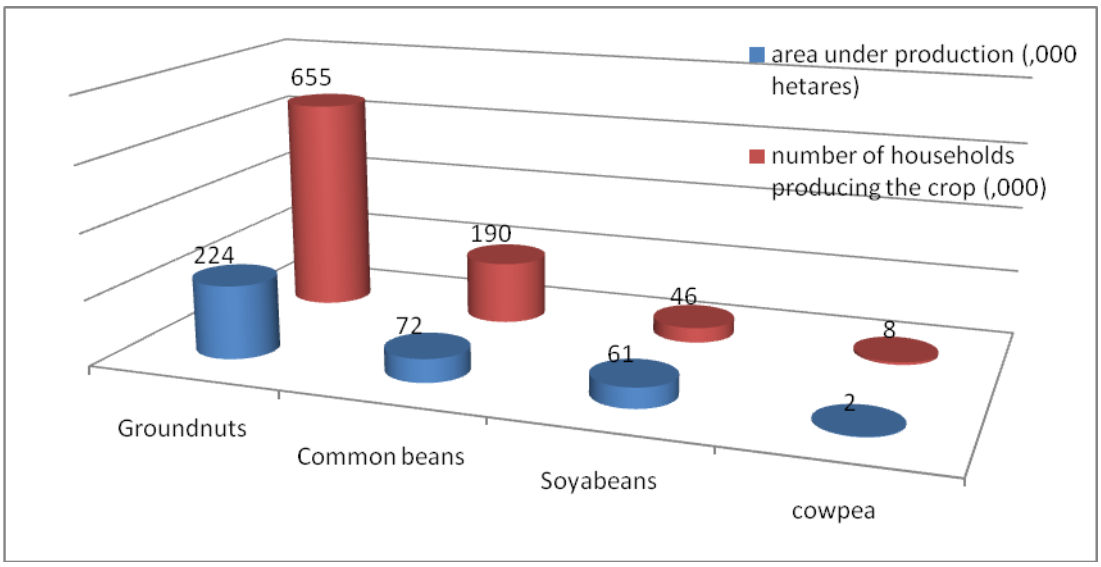

Figure 1: Food legume crops grown in Zambia, area under production and number of households growing the legume crops for 2010/2011 growing season

Source: MACO Crop Forecast Survey Report, 2011

In Zambia, the major areas of common bean production are Northern, North-Western, Muchinga, Luapula, Eastern and Central Provinces. Most of the bean crop is produced in the higher altitudes, cooler and high rainfall zones (Region III) followed by the medium warm zone (Region II). However, it is also possible to grow the bean in Region I if farmers can provide enough moisture (FoDis, 2009). Average yields of local cultivars are low, being in the range of 0.3- 0.5 tonnes/hectare. Improved varieties with an acceptable bean size, good coloration and taste, have yield potential up to 2 tonnes per hectare and resistance to pests and diseases have been developed, but seed is scarce (EEOA, 2000).

1.4 Factors affecting bean production

Common bean production in the tropical and subtropical countries is constrained by both biotic and abiotic factors reducing crop productivity and quality. Among the biotic factors are insect pests and diseases. In Zambia, Uganda, Zimbabwe and South Africa, common beans are widely susceptible to diseases such as Bean Common Mosaic Virus (BCMV), angular leaf spot (*Phaeoisariopsis griseola*), common bacterial blight (*Xanthomonas campestris*), anthracnose (*Colletotrichum lindemuthianum*) and halo blight (*Pseudomonas savastanoi*) (Zamani et al. 2011; Karavina et al. 2011). Important insect pests include; bean stem maggots (*Ophiomyia* spp.), especially at early growth stages and when plants are stressed by water and nutrient deficits. Others insect pests include; aphids (*Aphis fabae*), whitefly (*Bemisia tabaci)*, leaf miner and thrips (*Frankliniella spp*.) (Mwale *et al*., 2008).

Abiotic stresses that affect common bean production include drought and aluminium (Al) toxicity (Ishitani *et al*., 2004). Soil acidic conditions (pH less than 5.5) accumulate high concentrations of aluminium to levels that are toxic to plants. Plant roots are always exposed to aluminium in some form, fortunately, most of this aluminium occurs as harmless oxides and aluminnosilicates (Matos *et al*., 2005). Besides the natural occurrence of soil acidity, the extensive use of ammonia and amide containing fertilisers causes further soil acidification and aggravates aluminium toxicity that contributes to an increase in soil acidity and enhanced aluminium solubility in acid sensitive soils at low pH (less than 5.5) (Zhou *et al*., 2007a).

Approximately 70% of the soil in the world is affected by acidity, alkalinity and heavy metals among other factors of which soil acidity is the most frequently encountered limiting factor to production of most of the world's staples. It has been estimated that approximately 50% of the arable land is negatively impacted by the aluminium toxicity due to acidic soil (Panda *et al*. 2009).

Soils in high rainfall areas of Zambia are characterised by acid soils with pH less than 5.5 (Figure 2). This among other factors has led to the reduction in the productivity of the crop from a potential of 2 tonnes per hectare to lower yields ranging from between 0.2 and 0.8 tonnes per hectare (MAL, 2013). Correction of acidity-related soil constraints using lime and phosphate fertilizers are beyond the

capacity of resource-poor farmers. Furthermore, heavy application of lime may have adverse effects on some crops in the rotation or cause deficiencies of micronutrients such as iron, zinc, copper, manganese and boron.

Therefore the possible approach to deal with this abiotic problem is the genetic improvement of aluminium tolerance (Tahira and Salam, 2006; Witcombe *et al*., 2008; Dai *et al.*, 2009). This may be achieved through exploitation of the genetic variation that exist among common bean genotypes for adaptation to acid soils (Rao *et al*., 2004) and Al toxicity (Rangel *et al*., 2005).

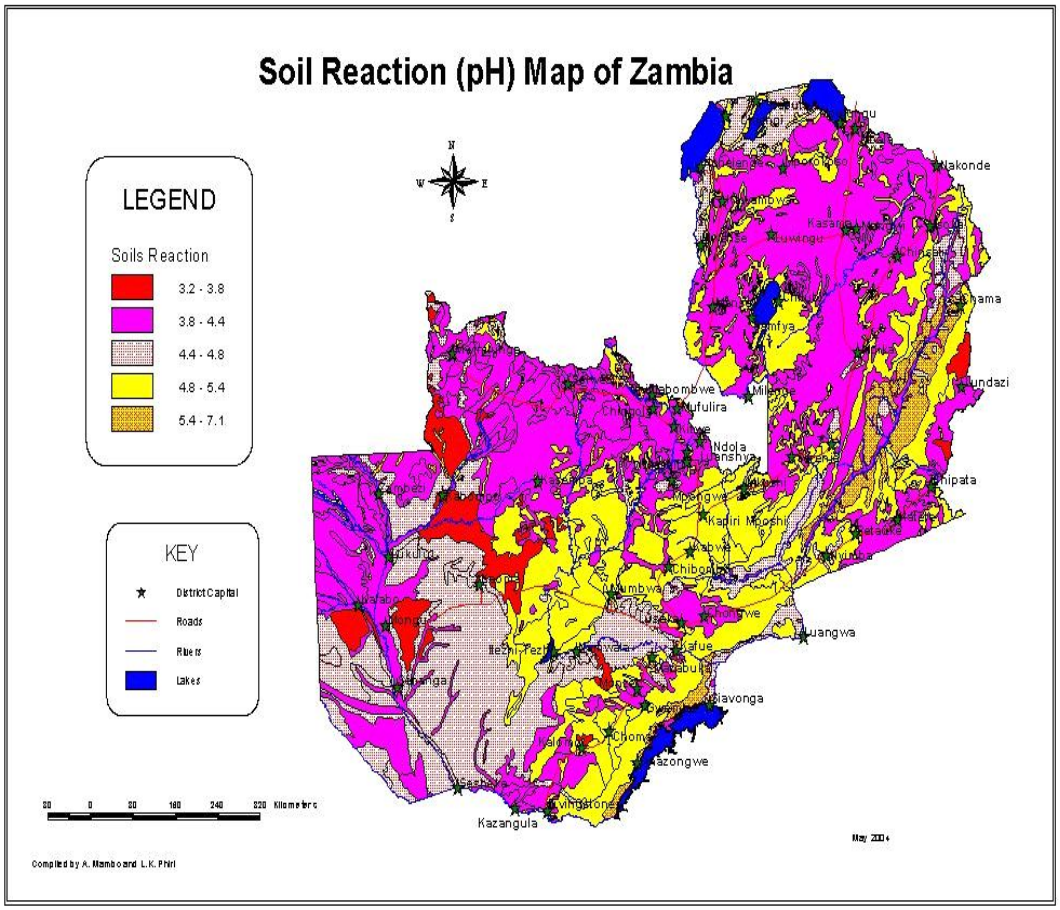

Figure 2: Soil reaction (pH) map of Zambia

Source: Zambia Research Institute, soil survey (2004)

An understanding of the genetic basis of aluminium tolerance in crop plants is a pre-requisite for the development of tolerant genotypes. The inheritance and genetics of aluminium tolerance has been assessed mostly in cereals like wheat, maize, rice and others. Some of the reports in these crops are inconclusive (Singh *et al.*, 2011).

This study sought to establish a genetic basis for developing Al tolerant varieties of common beans. Therefore, the specific objectives of the study were;

i. To characterize common bean genotypes for aluminium tolerance
ii. To determine the type of gene action conditioning aluminium tolerance in common beans

The study was carried out on the premise that there was enough genetic variation among common bean genotypes for acid soils adaptation and tolerance to aluminium toxicity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botanic description of common beans

Common bean (*Phaseolus vulgaris*) which belongs to the Family *Fabaceae* or *Leguminosae* is native to ancient Mesoamerica and is now grown all around the world. Five, of the 50 described *Phaseolus* species (*P. vulgaris, P. lunatus, P. coccineus, P. acutifolius*, and *P. polyanthus*) are grown for human consumption. *Phaseolus vulgaris* is the most cultivated worldwide and accounts for 75% of the food legumes traded in the world (Broughton *et al.*, 2003). Common Bean is an annual, herbaceous plant with a fibrous root system, and an erect, twining stem with small side branches. Leaves are large and trifoliate. Flowers are arranged in racemes, with long pods and they vary in colour: white, pink or yellow. Pods also vary in colour, size and shape. They grow from successfully pollinated flowers.

Common bean (*Phaseolus vulgaris*) includes many varieties and may be referred to as bush bean, dry bean, dwarf bean, field bean, French bean, snap bean, garden bean, haricot bean, kidney bean, pole bean or string bean. It shows variation in growth habits from determinate bush to indeterminate, extreme climbing types. The bushy type bean is the most predominant type grown in Africa (Buruchara, 2007).

2.2 Adaptation and climatic requirements of common beans

Common bean is a warm-season crop that does not tolerate frost or long periods of exposure to near-freezing temperatures at any stage of growth. High night temperatures ($>30^0$C) inhibit pollination and the crop may set little seeds or shed many flowers and buds, resulting in reduced yield (Gomez, 2004).

The crop requires moderate amounts of rainfall (300 – 600 mm) but adequate amounts are essential during and immediately after the flowering stage. Dry weather is desirable for maturation of the crop and for harvesting but late rains may discolour the beans and lower their grade and market value (Gomez, 2004).

Common bean does well under well drained loam-clay soils with a pH range of more than 5.5. Highly compacted clay soils result in poor germination; produce stout, stunted plants with restricted root development and having few nodules. Acidic soils inhibit development of nodules forming bacteria which result in reduction of fixing

atmospheric nitrogen (Fodis, 2009). Generally, common bean is considered a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturity (Buruchara, 2007). For climbers, maturity period can continue up to 200 days after planting (Gomez, 2004).

2.3 Chemical nature of aluminium

Aluminium is the most abundant light metal that makes up 7% of the earth's crust and is the third most common element after oxygen and silicon (Ma *et al.*, 2001). Plant roots are therefore almost always exposed to aluminium in some form. Dissolution of just a small fraction of the aluminium compounds in soil results in serious aluminium toxicity to susceptible plant species. Fortunately, not all forms of aluminium are phytotoxic. Those shown to be toxic include Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$ with Al^{3+} considered to be the major phytotoxic form, at low soil pH (< 5.5) (Delhaize, 2004).

The low pH contributes to solubilisation of aluminium making it available in the soil for plant uptake, causing severe damage to non-adapted genotypes. The main effect is a slow growth of the root system resulting in the development of surface roots. This hampers the use of soil nutrients and makes plants more drought susceptible (Piñeros *et al.*, 2005, Hartwig *et al.*, 2007)

If the pH is allowed to drop much below 5.5, the availability of manganese and aluminium increases to the point that they become toxic for plants (Delhaize, 2004; Panda and Matsumoto, 2007). According to Beebe *et al*. (2008), common bean is generally regarded as an aluminium and drought-sensitive crop.

2.4 Symptoms of aluminium toxicity

In physiological terms, aluminium toxicity can be observed in root apices, especially in the transition zone, lying 1 to 2 mm behind the root tip. This area, known as the elongation zone, is highly sensitive to aluminium (Sivaguru and Horst, 1998). In common bean, aluminium applied to this zone inhibits root growth (Rangel *et al.*, 2007).

The primary symptom of aluminium toxicity is reduced root development. Roots appear short and thickened, with short laterals, and may be discoloured yellow to brown. Root hair development is also suppressed. Where aluminium concentration

increases with soil depth, the downward extension of the roots may be restricted, resulting in a very shallow root system. In addition to poor growth and stunted appearance, a number of symptoms may appear as a result of poor root development, depending on which secondary factor is most limiting. The symptoms of high water stress are common. Aluminium toxicity considered to be a complex of nutritional disorders of growth and development of plants, may be manifested as a deficiency of essential nutrients like calcium, magnesium, iron, molybdenum, decreased availability of phosphorus or as toxicity of manganese and H^+ (Kamprath and Foy, 1985).

2.5 Effect of aluminium toxicity on plant growth and development

The primary target of aluminium toxicity is the root apex. Aluminium affects a host of different cellular functions. Affected root tips are stubby due to inhibition of cell elongation and cell division. The resulting restricted root system is impaired in nutrient and water uptake, making the plant more susceptible to drought stress. Plants sensitive to aluminium toxicity have greatly reduced yield and crop quality (Samac and Tesfaye, 2003; Jovanovic *et al*., 2006; Jovanovic *et al*., 2007).

Parker (1995) described two responses to aluminium: an initial acute inhibition of growth that is followed by a later chronic aluminium effect on root growth. About 40% of the world bean growing area is affected by aluminium toxicity, resulting in 30% to 60% decrease in grain yield. Acid soils inhibit development of nodule forming bacteria which result in reduction of the capacity to fix atmospheric nitrogen (Fodis, 2009). The low pH contributes to the solubilisation of aluminium, making it available in the soil for plant assimilation, causing severe damage to non-adapted genotypes. The main effect is a slow growth of the root system resulting in the development of surface roots. This hampers the use of soil nutrients and makes plants more susceptible to drought (Piñeros *et al*. 2005; Hartwig *et al*., 2007). Chemical interactions between aluminium and phosphorus within plant tissues are commonly considered an important secondary effect of aluminium toxicity.

2.6 Aluminium tolerance mechanisms

There are two main classes of tolerance mechanisms. Some are those that operate to exclude aluminium from the root apex (External tolerance mechanism) and others are those that allow the plant to tolerate aluminium accumulation within the root and

shoot cells (Internal tolerance mechanism) (Panda *et al.*, 2009). Organic acids play an important role in detoxifying plants from aluminium both internally and externally. Some organic acids can form stable complexes with aluminium, thereby preventing the binding of aluminium to cellular components, resulting in the detoxification of aluminium in plant species (Ma, 2005; Raman *et al*., 2006). Of the organic acids, citrate has the highest binding activity for aluminium followed by malate and succinate (Wang *et al*., 2006).

2.6.1 External tolerance mechanism

The exclusion mechanism enhances plant tolerance to aluminium stress by preventing excess uptake of aluminium ions from entering the root apex cells. Central to the exclusion mechanism is the root tips that secrete organic acids such as malate and citrate or oxalate to chelate aluminium in the rhizosphere that change the pH of the rhizosphere. Exclusion is often proceeded by either one or a combination of the following processes; exudation of chelating ligands, formation of pH barrier at the rhizosphere or at root apoplasm, cell wall immobilization, selective permeability of the plasma membrane, and Al efflux (Wang *et al*., 2006; Delhaize *et al*., 2007).

Among the above, exclusion of toxic aluminium through chelating ligands is, however, the dominant detoxification mechanism for many plant species (Maron *et al*., 2010). In fulfilling this, organic acids such as citrate, malate, and oxalate are reported to be exudated at the rhizosphere and the apoplast (Ryan *et al*., 2011). In fact, exudation of malate, citrate and/or oxalate by root tips is recognized to be the key factor in aluminium tolerance of many species due to their high affinity for binding of aluminium (Kochian *et al*., 2005).

Tolerant genotypes have been observed to exude more citrate than the sensitive genotypes with the degree of secretion dependent on the dosage of aluminium and on time (Shen *et al*., 2002). The differential response of tolerance to aluminium and exudation of citrate that exists among different types of common bean genotypes is related to phosphorus deficiency (Shen *et al*., 2002). Lower aluminium contents were observed in root tips of aluminium tolerant compared with aluminium sensitive common bean cultivars after 1 day or 3 days of aluminium treatment, respectively REF. The lower aluminium contents were related to a higher capacity to exude citrate in response to aluminium treatment (Shen *et al*., 2002).

Aluminium-enhanced citrate exudation has been previously related to aluminium tolerance of common bean (Shen *et al*., 2002) and soyabean (Yang *et al*., 2001). This citrate release requires the activation or expression of an organic anion permease in the plasma membrane and is initially mainly derived from the internal organic acid pool. Aluminium tolerant genotypes of wheat, maize, sunflower, soyabean, common bean and rice bean among other, exclude aluminium from root by exertion of organic acids that chelate aluminium (Lopez *et al.*, 2000; Watanabe *et al*., 2002).

Ma *et al*. (2001) proposed two patterns of aluminium stimulated organic acid release, on the basis of the timing of secretion. In Pattern I, no discernible delay is observed between the addition of aluminium and the onset of organic acid release. Aluminium ion (Al^{3+}) is able to rapidly activate efflux by interacting directly with the pre-existing proteins. For example, in wheat (*Triticum aestivum*) and buckwheat (*Fagopyrum esculentum*), the secretion of malate or oxalate was detectable within 15 to 30 minutes after exposure to aluminium (Ma *et al*., 2001; Delhaize, 2004; Ma, 2005).

Pattern II shows a delay between the addition of Al^{3+} and the start of organic anion efflux. It occurs in soyabean (*Glycine max* L.), rye (*Secale cereale*) and common bean (*Phaseolus vulgaris L.*), among others, with 12 to 24 hours lag phase between the addition of aluminium and the start of citrate release (Shen *et al*., 2002; Shen *et al*., 2004; Stass *et al*., 2007). This delay is interpreted as Al^{3+} first inducing the expression of the transport protein via a signal transduction pathway possibly involving a specific receptor. Once synthesized and inserted in the plasma membrane, Al^{3+} is thought to interact with the protein to activate efflux of organic anion (OA) (Delhaize, 2004; Ma, 2005; Yang *et al*., 2006).

Aluminium tolerance genes of several plant species have been identified and found to encode membrane proteins which mediate the exudation of organic acid anions from the root. These proteins belong to two families, Al-activated Malate Transporter (ALMT) and Multidrug and Toxic compound Extrusion (MATE). The ALMT facilitates malate efflux in plant species that depend on malate exudation as the aluminium tolerance mechanism (Sasaki *et al*., 2004; Hoekenga *et al*., 2006; Ligaba *et al*., 2006). On the other hand, the MATE proteins are citrate transporters which play a decisive role in Al-induced citrate exudation (Furukawa *et al*., 2007; Magalhaes *et al*., 2007; Ryan *et al*., 2009). On the basis of the results published by

Rangel *et al.* (2010), it was hypothesized that the expression of a citrate transporter and the enhanced synthesis of citrate are crucial for sustained aluminium tolerance in common bean.

Tolra *et al*. (2009) concluded that, in addition to aluminium exclusion from the roots, higher contents of phenolic substances with a high antioxidant and antiradical activities also contribute to aluminium tolerance in maize. Liu *et al.* (2009) also confirmed the aluminium-induced root tip exudation of flavonoid type phenolics in maize. However, due to high affinity for H^+, phenolics gain lower recognition compared to organic acids in acidic soils.

Another principal mechanism that has been correlated with tolerance to aluminium toxicity is extension of the root elongation zone. Variability for tolerance to aluminium toxicity between common bean genotypes may partly be related to differences in the extension of the root elongation zone. Results by Rangel *et al.* (2007) showed that a tolerant genotype such as ICA Quimbaya had a larger root elongation zone than a susceptible genotype such as VAX1. This suggested that the response to aluminium stress varies spatially in common bean.

Henderson and Ownby (1991) correlated the amount of mucilage produced by wheat root to aluminium tolerance and suggested that mucilage aided in the formation of a diffusion barrier to aluminium or concentrated organic acids that chelated aluminium. The mucilage from maize roots was shown to bind aluminium, (Li *et al.*, 2000) but did not give satisfactory protection of roots from aluminium toxicity. This lack of protection was attributed to the longer distance between site of formation of mucilage and the aluminium sensitive zone i.e., distal part of the transition zone (DTZ) (Li *et al*., 2000).

2.6.2 Internal tolerance mechanisms

The internal aluminium tolerance mechanisms are those which operate within the symplasm and are mediated at the cellular level either by detoxification or immobilisation of aluminium ions that have penetrated into plant cells (Ma, 2005; Wang *et al*., 2006; Guo *et al*., 2007).

The internal mechanism is characterized by the production of specific proteins capable of forming complexes with the toxic aluminium components. The possible mechanisms for internal tolerance are; chelatization of aluminium in the cytosol,

compartmentation of aluminium in the vacuole and detoxification of this metal when it penetrates the cells by the evolution of aluminium tolerance enzymes in the cells (Ma, 2005; Kikui *et al.,* 2007).

Some plant species, mostly woody species have the remarkable ability of accumulating aluminium in shoots and roots. These aluminium tolerant species have evolved mechanisms that maintain the aluminium in non-toxic forms within the plant as well as mechanisms that allow the aluminium to move through the plant and across a range of membranes to the rhizosphere (Delhaize, 2004; Ma, 2005).

Buckwheat is a species that also exudes oxalate in response to aluminium and its high level of aluminium tolerance may be as a result of both external and internal detoxification mechanisms (Delhaize, 2004). Wenzl *et al*. (2002) reported that aluminium tolerant signal grass and brachiaria grass accumulated high concentrations of Aluminium in the roots apices and that approximately two thirds of this aluminium was complexed by low-molecular-weight organic acids suggesting that it had been taken up into the root cells.

2.7 Screening methods

The screening of genotypes is a prerequisite for the selection and development of tolerant varieties (Ojo et al., 2010). Genetic improvement of crops for soil acid-tolerance has been accelerated by the availability of screening criteria for detecting aluminium tolerance. Laboratory and greenhouse based techniques are widely employed which are usually non destructive and can be applied in early developmental stages from seedlings only a few days old to flowering stage of the plants. Field-based screening techniques are more laborious, time consuming and expensive (Wang *et al*., 2006).

Laboratory screening methods include, nutrient solution culture methods (Naserian *et al.,* 2007), soil-petri dish method (Stass et al., 2007), and screening in pots in a greenhouse (Tazeem *et al*., 2009).

2.7.1 Laboratory screening method

2.7.1.1 Nutrient solution culture

Nutrient solution culture is the most common screening medium for aluminium tolerance. It provides easy access to root systems, tight control over nutrient availability and pH, and non-destructive measurement of tolerance. The most frequently measured effect of excess aluminium is inhibition of root elongation. Nutrient solution studies are better suited for determining more precisely the aluminium activity that is influencing root elongation, exclusive to other associated acid-soil-stress constraints (Rao, 2008).

There are two major criteria for evaluation of aluminium tolerance in nutrient solution culture. First, is the root length measurement which is the most suitable approach for genetic and molecular studies in which a precise quantitative response to aluminium stress is needed. It is also suitable for identifying genotypes with superior alleles for aluminium tolerance (Hede *et al*., 2002). Second, is the root staining method which is quicker, more efficient and suitable for screening a large segregating population derived from improved germplasm (Hede *et al*., 2002). Haematoxylin and Eriochrome cyanine R (Wang *et al*., 2006) stains have been used for evaluation of germplasm for aluminium tolerance.

The haematoxylin staining method is an extremely powerful tool for observing tolerance without laborious quantitative measurements. The haematoxylin dye forms complexes with tissue aluminium that has been immobilized as $AlPO_4$ by phosphate on or immediately below the root surface (Ownby, 1993). There are different approaches to the haematoxylin method. Polle *et al.* (1978) used the haematoxylin-staining pattern of root tips as an indicator of aluminium tolerance. Haematoxylin binds aluminium to produce a purple complex (Delhaize, 2004; Zhou *et al*., 2007b). As the intensity of staining increases, reflecting a higher level of aluminium uptake, the level of tolerance decreases. The absence of colour in root tips of aluminium-tolerant genotypes indicates that these genotypes either exclude or bind the aluminium in complexes that are unavailable to haematoxylin (Delhaize, 2004).

Another procedure using haematoxylin, the modified-pulse method, evaluates aluminium tolerance based on the ability of aluminium tolerant seedlings to continue root growth after a short pulse treatment with high aluminium concentrations

(Hossain *et al*., 2005). Aluminium sensitive seedlings do not show root re-growth because of their damaged apical meristem.

2.7.2 Field screening

The most direct screening method for acid-tolerance is the measurement of yield of both grain and total biomass under field conditions. The result is an integrated measurement of tolerance expressed throughout development. The procedure is to conduct tests in unamended and lime added blocks to allow a direct measurement of tolerance and to ensure that acid soil tolerance is not related to low yield potential in the absence of stress. Soil management practices are otherwise equal between blocks (Wang *et al*., 2006).

Field screening for aluminium tolerance seems to be the most desirable approach, because it approximates the intended cropping environment. In practice, however, reliable ranking of genotypes in the field can be difficult. This is mainly because exchangeable aluminium levels may not be uniform and also environmental factors could interact with soil aluminium to mask the expression of aluminium tolerance (Naserian *et al.,* 2007). Thus it is necessary to combine field screening with greenhouse screening techniques based on physiological traits of aluminium tolerance (Shahinnia *et al*., 2005).

2.8 Genetics of Aluminium Tolerance

Genetic variation in response to aluminium toxicity has been found not only among plant species but also within species and among developed cultivars. Rao *et al*., (2004) and Rangel *et al*., (2005) reported that genetic variation exists for acid soils adaptation and aluminium tolerance among common bean genotypes. These plants differ significantly in their susceptibility to aluminium toxicity in acid soils and these differences are genetically controlled. While most cultivars are sensitive to aluminium, tolerant genotypes can be found in most plant species. When subjected to aluminium stress, the tolerant individuals would have more roots and produce greater shoot yield than the aluminium sensitive individuals (Tang *et al*., 2003; Gustafson, 2005; Ma, 2005).

The tolerance to aluminium toxicity exhibited by certain species, and cultivars within species depend on the prevention of aluminium uptake by roots or upon its detoxification on entering the cytosol. Over 20 genes induced by aluminium stress

have been isolated from a range of plant species, including wheat, rye, rice (*Oryza sativa*), soyabean (*Glycine max* L.), tobacco (*Nicotiana tabacum*), and Arabidopsis. Most of the aluminium-induced genes seem to be general stress genes that are induced by a range of different plant stresses (Fontecha *et al*., 2007).

A single gene controls the inheritance of aluminium tolerance in barley (*Hordeum valgare* L.). While barley cultivars exhibit a range of variation for aluminium tolerance, in many instances this appears to be due to the action of a single locus, with different alleles conferring different degrees of aluminium tolerance. Rye, barley and sorghum (*Sorghum bicolor* L.), like wheat, have an inheritance pattern with a single locus explaining the genotypic differences (Panda and Matsumoto, 2007).

Research has shown that the aluminium tolerance in common beans is under polygenic control. Results of the study conducted by Hernán *et al.,* (2009) show that common bean has polygenic inheritance of tolerance to aluminium and that some aluminium tolerance Quantitative Trait Loci (QTL) co-localize with QTL for tolerance to low phosphorus (P) (Yang *et al.* 2004; Beebe *et al.* 2006), suggesting cross-links between different mechanisms of abiotic stress adaptation in common beans. Louis et al., (2011) also reported that inheritance of aluminium tolerance and acid soil tolerance expressed by G35346-3Q (*Phaseolus coccineus*) is complex.

The inheritance of aluminium tolerance in maize, in nutrient solution, has been characterized as monogenic in some studies (Prioli *et al*. 2002). In contrast to the simple inheritance model, other studies indicated that aluminium tolerance is a quantitatively inherited trait, suggesting that tolerance is dominant over susceptibility (Kochian *et al.* 2004). Khatiwada *et al.* (1996b) found that aluminium tolerance in rice is controlled by a complex multigenic system but later in 1999, Ferreira *et al.,* concluded that aluminium tolerance is generally controlled by a single gene with tolerance determined by dominant alleles. In quantitative genetics, genetic components are divided into additive and dominance variance and epistasis. In the presence of additive gene action, characters of heterozygotes in the F_2 generations are the intermediate of the two parents, because additive variation is associated with the average effects of the particular alleles (Falconer, 1981).

The importance of additive component compared to dominant-additive portion of the genetic model was reported by Checa *et al.* (2006) to determine the inheritance of

climbing capacity based on plant height (PH) and internode length (IL) in common bean (*Phaseolus Vulgaris* L.). The additive portion reflects the degree to which progenies are likely to resemble their parents, which is reflected in the narrow sense heritability. Knowledge of heritability indicates to the breeder the possibility to which genetic improvement is possible through selection. Non-additive gene action is observed when the additive model cannot adequately explain the variation (Falconer, 1981).

Ojo and Ayuba (2013), in their combining ability analysis for aluminium stress tolerance, concluded that additive gene action was more predominant than dominance gene action indicating the possibility of selection of pure lines from the genotypes studied.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study was carried out in the laboratory, glasshouse and screen house of the Department of Plant Science of the School of Agricultural Sciences at the University of Zambia. It was carried out in four phases. Phase I involved screening common bean genotypes for tolerance to aluminium toxicity. Phase II was aimed at developing the experimental population and phase III involved advancing F_1 crosses to F_2 generation. Phase IV involved evaluation of the F_2 crosses for tolerance to aluminium toxicity. Phase I aimed at achieving the first specific objective (To characterize common bean genotypes for aluminium tolerance) and phases II, III and IV collectively aimed at achieving the second specific objective (To determine the type of gene action conditioning tolerance to aluminium toxicity).

3.1 Materials used

Table 1: List of common bean genotypes evaluated in nutrient solution for aluminium tolerance at the University of Zambia in 2012.

Entry No.	Genotype	Source
1.	SZ 3-3-B-B2	UNZA(mutation breeding)
2.	SZ PARENT	UNZA
3.	LY4-4-B	UNZA(mutation breeding)
4.	SZ 9-B-B-B2	UNZA (mutation breeding)
5.	SZ 9-7-B-B	UNZA (mutation breeding)
6.	NUA45	ZARI (BILFA programme)
7.	NUA91	ZARI (BILFA programme)
8.	VTTT 918/15-4-4	ZARI (BILFA programme)
9.	VTTT 915/2-2	ZARI (BILFA programme)
10.	VTTT 924/12-4	ZARI (BILFA programme)
11.	CIM-RMOO-321LN02	ZARI (BILFA programme)
12.	ARA 4	ZARI (BILFA programme)
13.	BF 13607-6	ZARI (BILFA programme)
14.	BF13572-11	ZARI (BILFA programme)
15.	BF 13607-12	ZARI (BILFA programme)
16.	Lukupa	ZARI
17.	Kabulangeti	ZARI
18.	Chambeshi	ZARI
19.	Lyambai	ZARI
20.	Kalungu	ZARI

KEY:BILFA= Bean Improvement for Low Fertility in Africa, ZARI= Zambia Agriculture Research Institute, UNZA= University of Zambia

3.2 Experimental Design and Treatments

3.2.1 Phase I: Screening of genotypes for tolerance to aluminium toxicity

The most discriminating level of aluminium used in the screening of common beans for tolerance to aluminium toxicity was pre-determined using five randomly selected common genotypes screened at varying levels of aluminium concentrations (0, 4, 8, 12, 16 and 20 mgL^{-1} aluminium). Based on the extent of aluminium-induced reduction in root length, 12 mgL^{-1} was found to be the most discriminating concentration and was therefore, used with 0 mgL^{-1} (control) for screening common bean genotypes for tolerance to aluminium toxicity in this study.

Twenty seeds of each bean genotype (Table 1) were surface sterilised with 1% sodium hypochlorite. The seeds were germinated on wet filter paper placed on separate petri dishes in the incubator at 25^{o}C for 72 hours. The nutrient solution was prepared as described by Kerridge *et al.*, 1971. It provided the following nutrients in mgL^{-1}: 48.1 Ca, 14.6 Mg, 42.61 N, 23.5 K, 0.02 Na, 0.08 Cl, 0.03 Mn, 0.06 Cu, 0.03 Mo, 0.16 Zn, 0.32 B, and 1.67 Fe added as $FeSO_4.7H_2O$. Aluminium was added to the nutrient solution in the form of $AlK(SO_4)_2 12H_20$ at 12 mgL^{-1}. The control treatment (0 mgL^{-1}) however, had no aluminium added. The pH of the nutrient solution was adjusted to 4.2 using 1N dilute HCL and 1N NaOH for it to include phyto-toxic species of Al, Al^{3+} and Al $(OH)^{2+}$, with Al^{3+} being more toxic than $Al(OH)^{2+}$.

Seedlings of uniform root length were selected and later transferred to nutrient solutions in 1.25 L plastic bowls covered with black polythene to prevent growth of algae. The seedlings were supported over the nutrient solution by stoppers. The experiment was laid out in a Completely Randomised Design with four replications per treatment. Seedlings were grown in the green house for 10 days with the nutrient solution replaced after 5 days. Aeration of the nutrient solution was done daily using an electric aeration pump.

3.2.2 Phase II: Creation of experimental population

Of the twenty genotypes screened, only fourteen were used as parents in the creation of the experimental population. Four tolerant genotypes (used as male parents) were crossed with ten sensitive genotypes (used as female parents) in a 4 x 10 North

Carolina Design II (Comstock and Robinson, 1948). To synchronise flowering of the male and female parents, the seeds were stagger planted five days apart. Two methods of cross pollination were used: (1) mechanical emasculation of the female parent using tweezers/pincers on flower buds one day before flowering followed by crossed pollination using ripe pollen from open flowers of the male parents (2) Hooking without emasculation as proposed by Freytag, (1977).

Of the forty (40) crosses made only seventeen (17) were successful recording 42.5% success for all crosses. Some flowers wilted after cross pollination due to high temperature (35-40^{o}C) during the time of hybridisation (November and December, 2012). The successful crosses were therefore grouped into three complete Sets (Appendix 1)

3.2.3 Phase III: Advancement of F_1 seed to F_2

F_1 generation in self pollinated crops, like common beans, shows no genetic variation. Therefore, the F_1 crosses were advanced to F_2 generation in order to create variation for aluminium tolerance testing. Therefore, data were collected and analysed from the F_2 generation seeds.

F_1 seeds from successful crosses were planted in 5 litre planting pots in the screen house. The pots were placed 45 cm apart and each had only one plant. The soil used was collected from the University of Zambia Field Station and pre-mixed with well decomposed compost using the ratio 3:1(soil to compost).

Watering was done once per day either early in the morning or late afternoon. Weeding was done manually as the weeds emerged and no chemical fertilizer was applied. Monochrotophos in the form of Phoskill was applied at the rate of 2 ml per litre to control aphids, white fly and leaf miner. Dithane M45 was applied at the rate of 1 g per litre as a preventive fungicide.

3.2.4 Phase IV: Evaluation of F_2 progeny for tolerance to aluminium toxicity

A total of fourteen (14) F_2 crosses, four male and ten female parental lines from the three sets, were evaluated in nutrient solution containing 12 mgL^{-1} aluminium. Seeds were germinated on wet filter paper soaked in distilled water in a dark germination chamber at 25^{o}C for three days. Seedlings of uniform root length, approximately 2.5 cm were transferred to 35 ml test tubes containing nutrient solution with 12 mg L^{-1} aluminium. The nutrient solution was aerated daily using an electric aeration pump

and on the sixth it was replaced with the fresh one. Seedlings were let to grow for ten days. The experiment was designated as a Completely Randomised Design with three replications.

3.2.5 Data collection

Data for phase I and II experiments were collected ten days after transplanting seedlings into the nutrient solution. Measurements taken/derived from the screening and evaluation experiments were; primary root length (cm), shoot (cm), root biomass (g) and shoot biomass (g). Number of lateral roots and root/shoot ratio were also measured/derived for the screening and evaluation experiments respectively. Screened genotypes were classified as tolerant, intermediate and sensitive and F_2 crosses were classified as either tolerant or sensitive.

3.2.5.1 Primary root length, shoot length and number of lateral roots

Each plant was removed from the nutrient solution and cut into two, separating the root from the shoot. Primary root and shoot lengths were measured using a 30 cm ruler and the lateral roots were physically counted for each genotype.

3.2.5.2 Root biomass, shoot biomass and root/shoot ratio

The plant parts (root and shoot) were each put into separate envelopes and then dried in an oven for two (2) days at 65^{o}C. Root and shoot biomass were then measured using analytical scale. The ratio of root to shoot biomass was calculated using the formula: root biomass (g)/shoot biomass (g).

3.2.6 Statistical analysis

Data were analysed using the 14^{th} version of Genstat statistical package (Payne *et al.*, 2011). Means were separated using Least Significant Difference ($LSD_{0.05}$). Data were subjected to analysis of variance using the linear model for analysis of North Carolina II for single environment:

$Y_{ij} = \mu + m_i + f_j + mf_{ij} + e_{ijk,}$ Where Y_{ij} = observed trait value, μ = mean effect M_i = effect of the i^{th} male parent, F_j = effect of the j^{th} female parent, mf_{ij} = effect of interaction between i^{th} male and j^{th} female parents, e_{ijk} = experimental error (Singh and Chaudhary, 1985). Data analysis was done with the assumption that there were no maternal effects and that there was no epistasis:

General combining ability (GCA) and Specific combining ability (SCA) effects were calculated for each parent and F_2 crosses respectively. The t-test was used to test whether GCA and SCA effects and were significantly different from zero. The calculations were done as follows:

GCA females, $g_i = (y_{i.} - y..)$, where, g_i = GCA of the i^{th} female parent, yi. = mean of the i^{th} female parent across jth male parents, y..= grand mean of female female parents. GCA males, $g_j = (y._j - y..)$, where, gj = General combining ability of the j^{th} male parent, y.j = mean of the j^{th} male parent across i^{th} female parents, y..= mean of j^{th} male.

$SCA_{males\ x\ females}$, $s_{ij} = (y_{ij} - y.. - g_i - g_{j)}$, where, s_{ij} = SCA of the cross between parent i and j, y_{ij} = mean of the cross, y.. = grand mean of the crosses, gi = GCA of the i^{th} female, gj = GCA of the j^{th} male.

Screened genotypes were grouped into three classes as tolerant, intermediate and sensitive using extent of inhibition of root elongation as proposed by Rangel et al. (2005). Genotypes with root elongation inhibited by more than 50%, between 30% and 50%, less than 30% were classified as sensitive, intermediate and tolerant respectively.

The root/shoot biomass ratio was calculated and used as an indicator of aluminium tolerance for the F_2 crosses. Ma et al., 2004, classified genotypes with root/shoot ratios greater than 0.24 as tolerant and those with root/shoot ratios less than 0.24 as sensitive.

The Baker ratio; $(\sigma^2 gca_m + \sigma^2 gca_{f)} / (\sigma^2 gca_m + \sigma^2 gca_f + \sigma^2 sca_{mf})$ was used to determine the type of gene action and the relative importance of GCA and SCA variances. Narrow-sense heritability estimates for each set were calculated using the formula;

$h^2 = (\sigma^2 A_f + \sigma^2 A_m) / (\sigma^2 A_f + \sigma^2 A_m + \sigma^2 D)$ where, h^2 = narrow sense heritability, $\sigma^2 A_f$ = additive variance for females, $\sigma^2 A_m$ = additive variance for males, $\sigma^2 D$ = dominance variance.

CHAPTER FOUR

RESULTS

4.1. Screening of genotypes for tolerance to Aluminium Toxicity

The analysis of variance for the studied traits (root length, shoot length, root biomass, shoot biomass and number of lateral roots) showed highly significant (P<0.001) differences among treatments (Table 2). Highly significant (P<0.001) differences were observed between aluminium concentrations (0 mgL^{-1} and 12 mgL^{-1}) for root length, shoot length, root biomass and shoot biomass. Different genotypes also displayed significant (P<0.001) differences in their response to aluminium treatment for all characters studied. In addition, highly significant (P<0.001) interactions between aluminium concentration and genotypes were observed for all characters studied (Table 2).

Table 2: Mean squares for root and shoot traits of 20 common bean genotypes grown at 2 levels of aluminium concentration (0 and 12 mgL^{-1} aluminium) in hydroponics for 10 days at the University of Zambia.

Source of variation	d.f.	RL	SL	RB	SB	#LR
Aluminium	1	454.78***	115.89***	0.027***	0.159***	5.333
Genotype	19	5.85***	31.11***	0.013***	0.090***	154.610***
Aluminium x genotype	19	4.70***	3.029***	0.001***	0.006***	192.669***
Error	120	0.25	0.69	0.0002	0.0014	9.2140
CV%		7.9	9.9	15.7	12.7	14.5

KEY: RL= Root length, SL=shoot length, RB= Root dry biomass, SB= shoot dry biomass, LR= number of lateral roots, ***=Significant at P≤0.001

4.1.1 Root Length

Averaged across aluminium concentrations, the mean root length was 6.3 cm with Lukupa recording the longest root length (8.4 cm) followed by SZ3-3-B-B2 (7.6 cm) and VTTT918/15-4-4 (6.9 cm). The shortest root length was exhibited in CIM-RMOO-321LN02 (4.9 cm) followed by ARA 4 (5.2 cm) and Chambeshi (5.4 cm) (Table 3).

A reduction in the mean root length was observed for all genotypes as aluminium concentration increased from 0 mgL^{-1} to 12 mgL^{-1} (Table 4). A Shift in the ranking of genotypes and magnitude of reduction in root length was also observed as aluminium concentration increased from 0 mgL^{-1} to 12 mgL^{-1} (Table 4). Genotypes

with the longest root length under the control (0 mgL^{-1}) did not show the longest root length under aluminium stress (12 mgL^{-1}). Under control (0 mgL^{-1} aluminium), Solwezi parent and NUA 91 had the longest root length (9.5 cm) followed by Lukupa and VTTT915/2-2 each recording 9.0 cm. The genotype with the shortest root length was CIM-RMOO-321LN02 (5.3 cm) followed by Kabulangeti (7.2 cm) (Table 4).

On the other hand, the genotype with the longest root length under aluminium stress (12 mgL^{-1}) was Lukupa (7.8 cm) followed by SZ3-3-B-B2 (7.2 cm) and VTTT918/15-4-4 (6.5 cm). The shortest root length was recorded for ARA 4 (3.0 cm) followed by Lyambai (3.3 cm), then Chambeshi (3.4 cm) (Table 4).

SZ3-3-B-B2 had the least percent reduction in root length (10%), followed by VTTT918/15-4-4 (11%) and then Lukupa (14%). The highest reduction in root length was observed in ARA 4 (59%) followed by Solwezi parent, BF13572-11 and NUA 91each with 57% reduction (Appendix 2).

4.1.2 Shoot Length

The mean shoot length of 8.4 cm was observed across aluminium concentration (Table 3). ARA 4 exhibited the longest shoot length (12.5 cm) followed by VTTT918/15-4-4 (11.6 cm) and Lukupa (10.7 cm). VTTT924/12-4 recorded the shortest shoot length of (5.7 cm), followed by NUA 91(5.9 cm) and BF13607-12 (6.1 cm) (Table 3).

Averaged across genotypes, a reduction in shoot length (from 9.2 cm to 7.5 cm) was observed as the concentration of aluminium increased from 0 mgL^{-1} to 12 mgL^{-1} (Table 5). A shift in the ranking of genotypes and magnitude of reduction in shoot length was observed as aluminium concentration increased from 0 mgL^{-1} and 12 mgL^{-1} aluminium (Table 5).

Table 3: Means of parameters measured from twenty common bean genotypes evaluated across aluminium concentrations (0 mgL^{-1} and 12 mgL^{-1})

Entry	Genotype	RL (cm)	SL (cm)	RB (g)	SB (g)	Number of LR
1	ARA 4	5.2	12.5	0.07	0.24	24
2	BF13572-11	6.3	8.2	0.05	0.13	17
3	BF13607-12	6.8	6.1	0.03	0.10	17
4	BF13607-6	5.7	7.8	0.06	0.13	28
5	Chambeshi	5.5	7.2	0.03	0.31	14
6	CIM-RMOO-321LN02	4.9	6.1	0.05	0.21	19
7	Kabulangeti	6.0	9.5	0.10	0.33	25
8	Kalungu	6.5	7.9	0.06	0.31	16
9	Lukupa	8.4	10.7	0.11	0.34	20
10	LY4-4-B	6.3	7.6	0.09	0.28	19
11	Lyambai	5.4	8.4	0.18	0.42	23
12	NUA 45	5.6	10.7	0.05	0.51	27
13	NUA 91	6.8	5.9	0.08	0.41	20
14	SZ3-3-B-B2	7.6	10.1	0.16	0.31	20
15	SZ9-7-B-B	6.8	9.1	0.10	0.39	32
16	SZ PRT	6.8	7.1	0.11	0.24	17
17	SZ9-B-B-B2	6.0	8.8	0.13	0.31	19
18	VTTT 915/2-2	6.7	6.9	0.04	0.26	18
19	VTTT918/15-4-4	6.9	11.6	0.11	0.40	24
20	VTTT 924/12-4	5.5	5.7	0.05	0.21	22
Grand mean		6.3	8.4	0.08	0.29	21
$LSD_{0.05}$		0.7	1.2	0.02	0.05	4

KEY: RL= Root length (cm), SL= Shoot length (cm), RB= Root Biomass (g), SB= Shoot Biomass (g), #LR= Number of Lateral Roots

Table 4: Effect of aluminium concentration on the mean root length of 20 common bean genotypes evaluated at 0 mgL^{-1} and 12 mgL^{-1} aluminium solutions after 10 days.

ENTRY	Genotype	Aluminium concentration (mgL^{-1})	
		0	12
1	ARA 4	7.3	3.0
2	BF13572-11	8.8	3.8
3	BF13607-12	8.6	5.0
4	BF13607-6	7.7	3.8
5	Chambeshi	7.6	3.4
6	CIM-RMOO-321LN02	5.3	4.5
7	Kabulangeti	7.2	4.7
8	Kalungu	8.9	4.2
9	Lukupa	9.0	7.8
10	LY4-4-B	7.4	5.2
11	Lyambai	7.5	3.3
12	NUA 45	7.6	3.6
13	NUA 91	9.5	4.1
14	SZ3-3-B-B2	8.0	7.2
15	SZ9-7-B-B	8.2	5.5
16	SZ PRT	9.5	4.1
17	SZ9-B-B-B2	7.7	4.3
18	VTTT 915/2-2	9.0	4.5
19	VTTT918/15-4-4	7.3	6.5
20	VTTT 924/12-4	7.4	3.7
Grand mean		**8.0**	**4.6**
$LSD_{0.05}$		**0.8**	**0.6**

At 0 mgL^{-1} aluminium, ARA 4 had the longest shoot length (14.7 cm) followed by VTTT918/15-4-4 (12.3 cm) and then Lukupa (12.0 cm) while VTTT924/12-4 and NUA 91exhibited the shortest shoot length (6.2 cm) (Table 5). At 12 mgL^{-1} aluminium, the longest shoot length (11 cm) was exhibited by VTTT918/15-4-4 followed by NUA 45 (10.6 cm) and then ARA 4 (10.4 cm).The shortest shoot length at this aluminium concentration (12 mgL^{-1}) was exhibited by Chambeshi (4.8 cm) followed by VTTT924/12-4 (5.3 cm) and CIM-RMOO-321LN02 (5.3 cm) (Table 5).

Reduction in shoot length as aluminium concentration increased from 0 mgL^{-1} to 12 mgL^{-1} was lowest for NUA 45 (1%) followed by Solwezi parent (5%) and SZ3-3-B-B2 (8%) and highest for Chambeshi (50%) followed by Kabulangeti (32%) and ARA 4 (29%) (Appendix 3).

Table 5: Effect of aluminium concentration on the mean shoot length of 20 common bean genotypes evaluated at 0 mgL^{-1} and 12 mgL^{-1} aluminium solutions after 10 days.

Entry	Genotype	Aluminium concentration mgL^{-1}	
		0	12
1	ARA 4	14.7	10.4
2	BF13572-11	8.8	7.5
3	BF13607-12	6.7	5.5
4	BF13607-6	8.7	7.0
5	Chambeshi	9.6	4.8
6	CIM-RMOO-321LN02	6.8	5.3
7	Kabulangeti	11.3	7.6
8	Kalungu	8.5	7.3
9	Lukupa	12.0	9.4
10	LY4-4-B	8.6	6.6
11	Lyambai	9.0	7.8
12	NUA 45	10.7	10.6
13	NUA 91	6.2	5.5
14	SZ3-3-B-B2	10.4	9.9
15	SZ9-7-B-B	9.5	8.8
16	SZ PRT	7.8	6.5
17	SZ9-B-B-B2	9.7	8.0
18	VTTT 915/2-2	7.5	6.2
19	VTTT 918/15-4-4	12.3	11.0
20	VTTT 924/12-4	6.2	5.3
Mean		**9.2**	**7.5**
$LSD_{0.05}$		**0.3**	**1.0**

4.1.3 Root Biomass

Averaged across aluminium concentrations, the mean root biomass was 0.08 g. Lyambai recorded the highest root biomass (0.18 g) despite having a relatively short root length. It was followed by SZ3-3-B-B2 (0.16 g). The lowest root biomass was exhibited by BF13607-12 (0.03) followed by Chambeshi (0.03g) (Table 3).

With the increase in aluminium concentration from 0 mgL^{-1} to 12 mgL^{-1}, a reduction in root biomass was observed for all genotypes (Table 6). The results obtained showed shifts in the ranking of genotypes and magnitude of reduction in root biomass (Table 6). At 0 mgL^{-1} aluminium, Lyambai showed the highest root biomass (0.18 g) followed by SZ3-3-B-B2 (0.17 g) and SZ9-B-B2 (0.15 g). The least root biomass was displayed by BF13607-12 (0.03 g) followed by Chambeshi (0.04 g) and VTTT915/2-2 (0.06 g) (Table 6). However, at 12 mgL^{-1}, the genotype with the highest root biomass was SZ3-3-B-B2 (0.15 g), followed by SZ9-B-B-B2 (0.11 g) and VTTT918/15-4-4 (0.09 g). The least root biomass was displayed by BF13607-12, Chambeshi and VTTT915/2-2 each with root biomass of 0.02 g followed by BF13572-11, VTTT924/12-4 and CIM-RMOO-321LN02 each recording a root biomass of 0.04 g (Table 6).

As aluminium concentration increased from 0 mgL^{-1} to 12 mgL^{-1}, reduction in root biomass was least for SZ9-7-B-B (10%) followed by SZ3-B-B-B2 (12%) and ARA 4 (13%) and highest for VTTT915/2-2 (67%) followed by Lyambai (56%) and Chambeshi (50%) (Appendix 4).

Table 6: Effect of aluminium concentration on the mean root biomass of 20 common bean genotypes evaluated at 0 mgL^{-1} and 12 mgL^{-1} aluminium solutions after 10 days.

Entry	Genotype	Aluminium concentration mgL^{-1}	
		0	12
1	ARA 4	0.08	0.07
2	BF13572-11	0.06	0.04
3	BF13607-12	0.03	0.02
4	BF13607-6	0.07	0.06
5	Chambeshi	0.04	0.02
6	CIM-RMOO-321LN02	0.07	0.04
7	Kabulangeti	0.12	0.08
8	Kalungu	0.07	0.05
9	Lukupa	0.14	0.08
10	LY4-4-B	0.10	0.07
11	Lyambai	0.18	0.08
12	NUA 45	0.06	0.05
13	NUA 91	0.09	0.07
14	SZ3-3-B-B2	0.17	0.15
15	SZ9-7-B-B	0.10	0.09
16	SZ PRT	0.14	0.08
17	SZ9-B-B-B2	0.15	0.11
18	VTTT 915/2-2	0.06	0.02
19	VTTT918/15-4-4	0.13	0.09
20	VTTT 924/12-4	0.07	0.04
Mean		**0.10**	**0.07**
$LSD_{0.05}$		**0.02**	**0.02**

4.1.4 Shoot Biomass

Averaged across aluminium concentrations, the mean shoot biomass was 0.29 g. NUA 45 exhibited the highest shoot biomass (0.51 g) followed by Lyambai (0.42 g) and NUA 91 (0.41 g). The lowest shoot biomass (0.10 g) was exhibited by BF13607-12 (Table 3).

Averaged across different genotypes the mean shoot biomass reduced from 0.32 g to 0.26 g as the concentration of aluminium increased from 0 mgL^{-1} to 12 mgL^{-1} (Table 7). Shifts in the ranking of genotypes and magnitude of reduction in shoot biomass. At 0 mgL^{-1} the genotype with the highest shoot biomass was NUA 45 (0.54 g)

followed by VTTT918/15-4-4 (0.50 g), Lyambai and NUA 91 (0.44 g). However, under aluminium stress (12 mgL^{-1}) NUA 45 had the highest shoot biomass (0.48 g) followed by Lyambai (0.40 g) and NUA 91 (0.39 g). BF13607-12 (0.07 g) had the lowest shoot biomass followed by BF13572-11 (0.11 g) and BF13607-6 (0.12 g) (Table 7).

Reduction in shoot biomass was least for Lukupa (3%), followed by SZ9-7-B-B (5%) and LY4-4-B (7%) and highest for Chambeshi (51%), BF13607-12 (46%) and VTTT918/15-4-4 (38%) (Appendix 5).

Table 7: Effect of aluminium concentration on the mean shoot biomass of 20 common bean genotypes evaluated at 0mgL^{-1} and 12mgL^{-1} aluminium solutions after 10 days.

Entry	Genotype	Aluminium concentration mgL^{-1}	
		0	12
1	ARA 4	0.26	0.23
2	BF13572-11	0.16	0.11
3	BF13607-12	0.13	0.07
4	BF13607-6	0.13	0.12
5	Chambeshi	0.41	0.20
6	CIM-RMOO-321LN02	0.24	0.19
7	Kabulangeti	0.39	0.27
8	Kalungu	0.34	0.27
9	Lukupa	0.35	0.34
10	LY4-4-B	0.29	0.27
11	Lyambai	0.44	0.40
12	NUA 45	0.54	0.48
13	NUA 91	0.44	0.39
14	SZ3-3-B-B2	0.33	0.30
15	SZ9-7-B-B	0.40	0.38
16	SZ PRT	0.28	0.20
17	SZ9-B-B-B2	0.33	0.29
18	VTTT 915/2-2	0.27	0.24
19	VTTT918/15-4-4	0.50	0.31
20	VTTT 924/12-4	0.24	0.17
Mean		**0.32**	**0.26**
$LSD_{0.05}$		**0.06**	**0.05**

4.1.5 Number of lateral roots

Averaged across aluminium concentration the number of lateral roots ranged from 14 to 32 with the overall mean of 21. SZ9-B-B-B2 had the highest number of lateral roots (32) followed by BF13607-6 (28) and NUA 45 (27). Chambeshi had the least number of lateral roots (14) followed by Kalungu (16) and Solwezi parent (17) (Table 3).

Averaged across genotypes, the mean number of lateral roots reduced from 24 to 18 as aluminium concentration increased from 0 mgL^{-1} to 12 mgL^{-1}. Observed, were shifts in the ranking of genotypes and magnitude of reduction in number of lateral roots (Table 8). Under the control (0 mgL^{-1} aluminium), the genotype with the highest number of lateral roots was Lyambai and VTTT918/15-4-4 each with 36 lateral roots followed by NUA 45 and SZ9-7-B-B each with 33 lateral roots. Kalungu had the least number of lateral roots (17) followed by Solwezi parent and BF13607-12 each with 18 lateral roots (Table 8). Under aluminium stress (12mgL^{-1}), SZ9-7-B-B had the highest number of lateral roots (30) followed by BF13607-6 (26) and Kabulangeti (21). The least number of lateral roots was exhibited by Lyambai and Chambeshi (10) followed by VTTT918/15-4-4 (13) and VTTT915/2-2 (14).

The magnitude of reduction in the number of lateral roots was least for SZ3-3-B-B2 and SZ9-B-B-B2 (5%), followed by Kalungu (6%) and SZ9-7-B-B (9%) and highest for Lyambai (72%), VTTT918/15-4-4 (64%) and Chambeshi (47%) (Appendix 6).

Table 8: Effect of aluminium concentration on the mean number of lateral roots of 20 common bean genotypes evaluated at 0 mgL^{-1} and 12 mgL^{-1} aluminium solutions after 10 days.

Entry	**Genotype**	**Aluminium concentration mgL^{-1}**	
		0	**12**
1	ARA 4	30	17
2	BF13572-11	19	16
3	BF13607-12	18	16
4	BF13607-6	29	26
5	Chambeshi	19	10
6	CIM-RMOO-321LN02	20	18
7	Kabulangeti	29	21
8	Kalungu	17	16
9	Lukupa	22	18
10	LY4-4-B	20	17
11	Lyambai	36	10
12	NUA 45	33	21
13	NUA 91	23	18
14	SZ3-3-B-B2	20	19
15	SZ9-7-B-B	33	30
16	SZ PRT	18	15
17	SZ9-B-B-B2	20	19
18	VTTT 915/2-2	22	14
19	VTTT918/15-4-4	36	13
20	VTTT 924/12-4	24	19
Mean		**24**	**18**
$LSD_{0.05}$		**4**	**4**

Figure 3, shows the effect of aluminium concentration on root length for one of the screened genotypes, ARA 4. Reduction in root length was observed in seedlings grown in nutrient solution containing 12 mgL^{-1} aluminium. The extent of reduction in root length and classification of genotypes for aluminium tolerance was as illustrated in Figure 4. Five genotypes namely; SZ3-3-B-B2, VTTT918/15-4-4, Lukupa, CIM-RMOO-321LN02 and LY4-4-B had their root length reduced by less than 30%. On the other hand, four genotypes, SZ9-7-B-B, Kabulangeti, BF13607-12

and SZ9-B-B-B2 had root length reduced between 30% and 50% and eleven genotypes (NUA 45, BF13607-6, VTTT915/2-2, VTTT924/12-4, Kalungu, Chambeshi, LY4-4-B, NUA91, Solwezi parent, BF13572-11 and ARA 4by more than 50%. The mean reduction in root length across genotypes was 25%.

Figure 3: Effect of aluminium concentration on root length for ARA 4 after three days of growth in nutrient solution.

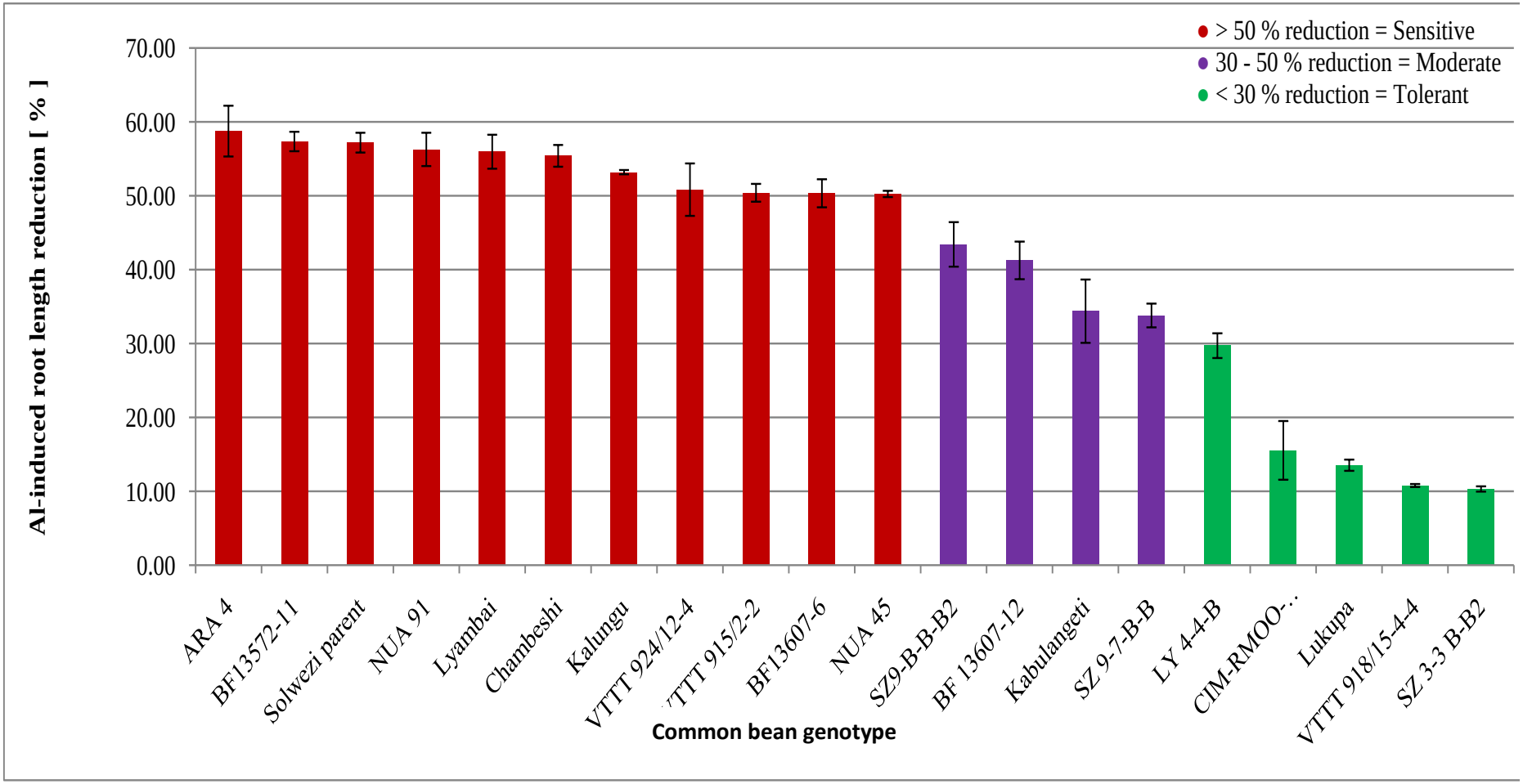

Figure 4: Classification of 20 common bean genotypes based on aluminium-induced reduction in root length. Bars represent standard error from the means (n = 4).

4.2 Determination of gene action conditioning aluminium tolerance

4.2.1 Evaluation of F_2 crosses for aluminium tolerance

4.2.1.1 Set I

Significant differences were observed among crosses for root length, shoot length and shoot biomass at $P<0.05$, $P<0.01$ and $P<0.05$ respectively (Table 9). Variation due to GCA_{female} (between female half-sib family groups) was significant for root length, shoot length ($P<0.01$)) and shoot biomass ($P<0.05$). The values of baker ratio were greater than 0.5 for all studied traits (root length, shoot length, shoot biomass and root/shoot ratio) except root biomass (Table 9).

The mean performance of parents and their F_2 crosses were presented in Table 10.

4.2.1.1.1 Root Length

Among the parental genotypes, SZ3-3-B-B2 had the longest root length (7.3 cm) followed by VTTT918/15-4-4 (6.6 cm). BF13572-11 displayed the shortest root length (3.7 cm). The mean root length among the crosses ranged from 8.2 cm to 10.6 cm with the grand mean of 7.5 cm for all genotypes (parents and crosses). The cross with the longest root length was SZ3-3-B-B2 x BF13572-11(10.6 cm) and SZ3-3-B-B2 x VTTT915/2-2 recorded the shortest root length (8.1 cm). Comparison of performance of F_2 crosses to the corresponding highest parents revealed that all the F_2 crosses had longer root length than any of their respective parental genotypes (Table 10).

4.2.1.1.2 Shoot Length

Parental genotypes, VTTT918/15-4-4 (10.1 cm) and Kalungu (7.1 cm) had the longest and shortest shoot lengths respectively. Two of the five parental genotypes, SZ3-3-B-B2 and VTTT918/15-4-4, had longer mean shoot length (10.1 cm and 8.4 cm respectively) than the overall mean for parents and crosses (7.8 cm) (Table 10). The cross, SZ3-3-B-B2 x Kalungu, showed significant ($P<0.05$) longer shoot length (9.3 cm) than both of its parental genotypes, SZ3-3-B-B2 and Kalungu (7.4 cm and 3.2 cm respectively). On the other hand VTTT918/15-4-4 x BF13572-11 exhibited significantly shorter shoot length than both of its parents (Table 10).

2.2.1.1.3 Root Biomass

The highest root biomass among the parents was exhibited by SZ3-3-B-B2 and VTTT918/15-4-4 (0.11g) and BF13572-11 showed the lowest (0.04g). Differences among crosses were non-significant for root biomass (Table 10).

4.2.1.1.4 Shoot Biomass

Among the parents Kalungu had the highest shoot biomass (0.31 g) despite having the shortest shoot length. BF13572-11 had the lowest shoot biomass (0.12 g). SZ3-3-B-B2 x VTTT915/2-2 surpassed the performance of both its parental genotypes; SZ3-3-B-B2 and VTTT915/2-2 by 21% and 48% respectively (Table 10).

4.2.1.1.5 Root/Shoot Ratio (R/S)

SZ3-3-B-B2 and VTTT918/15-4-4 had higher ratios than 0.24 (0.37 and 0.40 respectively). BF13572-11, Kalungu and VTTT915/2-2 had root/shoot ratios less than 0.24 (0.22, 0.16 and 0.20 respectively) (Table 10). The analysis of variance showed non-significant differences among crosses for root/shoot ratio. However, all the crosses showed root/shoot ratios which were greater than 0.24 except SZ3-3-B-B2 x VTTT915/2-2 (Table 10).

Table 9: Mean squares for root and shoot parameters of F_2 common bean crosses evaluated in 12 mgL^{-1} aluminium, nutrient solution (Set I).

Source of variation	**d.f.**	**RL(cm)**	**SL (cm)**	**RB (g)**	**SB (g)**	**R/S**
Crosses	5	3.0857*	5.250**	0.001	0.0097*	0.0211
GCA_{male}	1	0.0467	3.751	0.001734	0.0079	0.0161
GCA_{female}	2	6.2973**	10.538**	0.0001	0.0160*	0.0259
SCA	2	1.3936	0.711	0.0004	0.00459	0.0190
Error	12	0.8087	1.015	0.0012	0.0039	0.0201
CV%	**-**	**9.6**	**13**	**41.7**	**23.1**	**43.0**
Baker's ratio $(\sigma^2 gca_m + \sigma^2 gca_f)/(\sigma^2 gca_m + \sigma^2 gca_f + \sigma^2 sca)$	**-**	**0.83**	**0.95**	**0.43**	**0.90**	**0.80**

KEY: RL = Root Length, SL = Shoot Length, RB = Root Biomass, SB = Shoot Biomass, R/S = Root/Shoot ratio *, ** Significant at P<0.01and P<0.05 respectively

Table 10: Mean performance of the parents and their F_2 crosses evaluated at 12mgL^{-1} aluminium after 10 days (Set I)

ENTRY	**RL(cm)**	**SL (cm)**	**RB (g)**	**SB (g)**	**R/S**
SZ3-3-B-B2[a]	7.3	8.4	0.11	0.28	0.38
VTTT918/15-4-4 [a]	6.6	10.1	0.11	0.26	0.40
BF13572-11 [b]	3.7	7.3	0.04	0.20	0.22
KALUNGU[b]	4.3	7.2	0.05	0.31	0.16
VTTT915/2-2[b]	4.6	7.2	0.05	0.24	0.20
SZ3-3-B-B2 x BF13572-11	10.6	7.2	0.10	0.20	0.49
SZ3-3-B-B2 x KALUNGU	9.3	9.5	0.10	0.33	0.31
SZ3-3-B-B2 x VTTT915/2-2	8.2	7.9	0.08	0.34	0.23
VTTT918/15-4-4 x BF13572-11	10.5	5.6	0.07	0.22	0.31
VTTT918/15-4-4 x KALUNGU	8.5	8.5	0.07	0.27	0.27
VTTT918/15-4-4 x VTTT915/2-2	9.3	7.7	0.08	0.26	0.31
Grand Mean	**7.5**	**7.9**	**0.08**	**0.26**	0.30
$LSD_{0.05}$	**1.6**	**1.8**	**0.06**	**0.11**	0.25

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root/Shoot Ratio, a = male parent b = female parent

4.2.1.2 Set II

Variation due to GCA_{male} (between male Half-Sib family groups), was significant for root length, root biomass ($P<0.001$), Shoot length ($P<0.05$), and Root/ shoot ratio ($P<0.01$). GCA_{female} (between female half-sib family groups) showed highly significant differences for root biomass ($P<0.001$) and Shoot Biomass ($P<0.01$). Significant SCA (between full-sib family groups) was observed for root length ($P<0.01$), shoot length ($P<0.05$) and Root biomass ($P<0.001$). Baker ratio values for root length shoot length, root biomass, shoot biomass and root/shoot ratio were 0.53, 0.44, 0.12, 0.78, 0.88 and 0.74 respectively (Table 11).

4.2.1.2.1 Root Length

Among the parental genotypes, Lukupa had the longest root length (7.4 cm) and ARA 4 had the shortest root length (3.3 cm). All the crosses had longer root length than either of their parental genotypes except Lukupa x ARA 4 whose mean root length was shorter than its male parent. The cross, SZ3-3-B-B2 x ARA 4, had the longest mean root length (11.9 cm) and the shortest was exhibited by Lukupa x ARA 4 (6.4 cm). All the parents and two of the crosses Lukupa x ARA 4 and Lukupa x Kalungu had shorter mean root length than the overall mean (7.3 cm) (Table 12).

4.2.1.2.2 Shoot Length

Among the parental genotypes ARA 4 had the longest and Kalungu had the shortest shoot length (9.2 cm and 7.2 cm respectively). In addition, ARA 4 exhibited longer mean shoot length (9.2 cm) than the overall mean for parents and crosses (8.6 cm) (Table 12). The shoot length for Lukupa x ARA 4 (8.8 cm), was less than its female parent but more than its male parent. On the other hand, SZ3-3-B-B2 x Kalungu had longer shoot length than both of its parents. SZ3-3-B-B2 x ARA 4 and Lukupa x Kalungu had shorter shoot length than the female and male parents respectively (Table 12).

4.2.1.2.3 Root Biomass

SZ3-3-B-B2 accumulated the highest root biomass (0.11 g) among the parental genotypes and ARA 4 had the lowest (0.045g). Among the crosses, Lukupa x Kalungu accumulated the highest root biomass (0.33 g) and also surpassed performance of both of its parents. The least among the crosses was Lukupa x ARA 4 which accumulated 0.08 g root biomass (Table 12).

4.2.1.2.4 Shoot Biomass

Among the parental genotypes Lukupa displayed the highest shoot biomass while ARA 4 had the lowest shoot biomass (0.32 g and 0.20 g respectively). Lukupa x Kalungu had the highest, and Lukupa x ARA 4 had the lowest shoot root biomass among all the crosses (Table 12). Lukupa x Kalungu and SZ3-3-B-B2 x Kalungu, each, surpassed the performance of both of their parental genotypes and Lukupa x ARA 4 accumulated less biomass than both of its parents (Table 12).

4.2.1.2.5 Root/Shoot Ratio

Two of the parental genotypes, Lukupa and SZ3-3-B-B2, had root/shoot ratios greater than 0.24 and two others ARA 4 and Kalungu had ratios less than 0.24. All the four crosses had root/shoot ratios greater than 0.24 (Table 12).

4.2.1.3 Set III

Significant differences ($P<0.01$) were observed among crosses for root biomass, shoot biomass and root/shoot ratio ($P<0.001$, 0.05 and 0.01 respectively) (Table 13). Variation due to GCA_{male} (between male Half-Sib family groups), was significant for root biomass ($P<0.001$), shoot biomass and root/shoot ratio ($P<0.05$). GCA_{male} (between female half-sib family groups) was significant for root biomass ($P<0.001$) and root/shoot ratio ($P<0.05$). Significant SCA (cross combination between male and female) was also observed for root biomass ($P<0.001$) and root/shoot ratio ($P<0.05$). The values of baker ratio for root length, shoot length, root biomass, shoot biomass and root/shoot ratio were 0.30, 0.64, 0.29, 0.48 and 0.41respectively (Table 13).

4.2.1.3.1Root Length

Significant differences ($P<0.001$) were observed among parental genotypes for root length. The male parents Lukupa (7.4 cm) and VTTT918/15-4-4 (6.6 cm) had longer mean root length than the female parents Kalungu (4.3 cm) and VTTT924/12-4 (3.7 cm). Lukupa had the longest mean root length and Kalungu had the shortest. All the crosses had longer root length than either of their respective parental genotypes. Only VTTT918/15-4-4 x VTTT924/12-4, had the higher mean root length (9.0 cm) than the overall mean (8.6 cm) for parental genotypes and crosses (Table 14).

Table 11: Mean squares for root and shoot parameters of F_2 common bean crosses grown in 12 mgL^{-1} aluminium, nutrient solution (Set II).

Source of variation	**d.f.**	**RL**	**SL**	**RB**	**SB**	**R/S**
Crosses	3	15.781***	2.2166*	0.0432***	0.0608**	0.1081*
GCA_{Male}	1	31.905***	2.4601*	0.0367***	0.0033	0.2544**
GCA_{Female}	1	0.549	0.0675	0.0486***	0.1511**	0.0002
SCA	1	14.889**	4.1223*	0.0444***	0.0280	0.0698
Error	8	1.033	0.4474	0.001	0.0070	0.0267
CV%	**-**	**11.4**	**7.8**	**21.1**	**30.1**	**26.3**
Baker's ratio =						
$(\sigma^2gcam + \sigma^2gca_{m)} / (\sigma^2gca_m + \sigma^2gca_f + \sigma^2sca)$	**-**	**0.53**	**0.44**	**0.12**	**0.78**	**0.74**

KEY: RL = Root Length, SL = Shoot Length, RB = Root Biomass, SB = Shoot Biomass, RB/SB=Root to shoot biomass ratio, ***, ** and * = significant at P<0.001, P<0.01 and P<0.05 respectively

Table 12: Mean performance of the parents and their F_2 crosses evaluated at 12 mgL^{-1} aluminium after 10 days (Set II)

Entry	**RL(cm)**	**SL (cm)**	**RB (g)**	**SB (g)**	**R/S**
Lukupa[a]	7.4	8.5	0.099	0.319	0.31
SZ3-3-B-B2[a]	7.3	8.4	0.106	0.280	0.38
ARA 4[b]	3.3	9.2	0.046	0.199	0.23
Kalungu [b]	4.3	7.2	0.050	0.308	0.16
Lukupa x ARA 4	6.4	8.8	0.083	0.136	0.61
Lukupa x Kalungu	8.2	7.4	0.332	0.457	0.73
SZ3-3-B-B2 x ARA 4	11.9	8.5	0.094	0.199	0.47
SZ3-3-B-B2 x Kalungu	9.3	9.5	0.100	0.327	0.31
Mean	**7.3**	**8.4**	**0.11**	**0.28**	**0.40**
$LSD_{0.05}$	**1.9**	**1.3**	**0.061**	**0.158**	0.13

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root/Shoot Ratio, a = Male parent b = Female parent

4.2.1.3.2 Shoot Length

The parental genotypes, Lukupa (8.5 cm) and VTTT918/15-4-4 (10.1 cm), had longer mean shoot length (7.8 cm) than the overall mean for parents and crosses (8.6 cm) (Table 14). Non-significant differences were observed among the crosses for shoot length. However, all the crosses had longer and shorter shoot lengths than their female and male parents respectively (Table 14).

4.2.1.3.3 Root Biomass

VTTT918/15-4-4 accumulated the highest root biomass (0.11g) among the parental genotypes and VTTT924/12-4 accumulated the least (0.04 g). All the parents had lower root biomass than the overall mean (0.14 g). Among the crosses, Lukupa x Kalungu had the highest root biomass (0.33 g) followed by VTTT918/15-4-4 x VTTT924/12-4 (0.10 g). In addition, Lukupa x Kalungu surpassed the performance of all the parental genotypes and the overall mean root biomass. The root biomass for Lukupa x VTTT924/12-4, VTTT918/15-4-4 x Kalungu and VTTT918/15-4-4 x VTTT924/12-4 were higher than their specific female parents but lower than their male parents (Table 14).

4.2.1.3.4 Shoot Biomass

Significant differences ($P<0.001$) were observed for shoot biomass among the parents with Lukupa showing the highest (0.32 g) and VTTT924/12-4 having the least (0.17 g). The cross with the highest shoot biomass was Lukupa x Kalungu (0.46 g) while VTTT918/15-4-4 x VTTT924/12-4 had the lowest shoot biomass (0.27 g). In comparison to parental genotypes Lukupa x Kalungu surpassed the performance of both of its parental genotypes Lukupa and Kalungu. Lukupa x VTTT924/12-4 and VTTT918/15-4-4 x VTTT924/12-4 had higher shoot biomass than their female parents but lower than their male parents.

4.2.1.3.5 Root/Shoot Ratio

The male parents Lukupa and VTTT918/15-4-4 had ratios greater than 0.24 (0.31 and 0.40 respectively) and female parents VTTT924/12-4 and Kalungu had ratios less than 0.24 (0.18and 0.164 respectively). Of the four crosses in set III, three (Lukupa x Kalungu, VTTT918/15-4-4 x Kalungu and VTTT918/15-4-4 x VTTT924/12-4) had root/shoot ratios greater than 0.24 (0.758 and 0.273

respectively) and one (Lukupa x VTTT924/12-4) had a ratio which was less than 0.24 (Table 14).

In Summary, the analysis of variance showed significant differences among the crosses in all the three sets (Tables 9, 11 and 13). Variation due to GCA_{male} (between male half-sib families) was significant for root length ($P<0.001$) and shoot length ($P<0.05$) in set II, root biomass ($P<0.001$) and root/shoot ratio ($P<0.05$) in sets II and III, shoot biomass ($P<0.05$) in set III. GCA_{female} (between female half-sib family) was significant for root length ($P<0.01$) and shoot length ($P<0.01$) in set I, root biomass ($P<0.001$) in Sets II and III, shoot biomass in sets I and II ($P<0.05$) and root/shoot ratio in set III ($P<0.05$). Significant SCA (cross combinations between males and females) was observed for root length ($P<0.01$) and shoot length (0.05) in set II, root biomass ($P<0.001$) in sets II and III and root/shoot ratio ($P<0.05$) in set III.

The parents that were common in two or three sets were; SZ3-3-B-B2, VTTT918/15-4-4, Lukupa and Kalungu. SZ3-3-B-B2 was a male parent in sets I and II. VTTT918/15-4-4 was common in sets I and III as a male parent. Lukupa was a common male parent in sets II and III. Kalungu was a common female parent in all the three sets (I, II and III). There were no differences in the mean performance of all the common parents in all the sets where they appeared for all the studied traits (Tables 10, 12 and 14).

Tables 10, 12 and 14 show that crosses SZ3-3-B-B2 x Kalungu, VTTT918/15-4-4 x Kalungu and Lukupa x Kalungu were common in at least two sets. SZ3-3-B-B2 x Kalungu was common in sets I and II, VTTT918/15-4-4 x Kalungu in sets I and III and Lukupa x Kalungu in Sets II and III. There were no differences in the performance of these crosses for all the traits studied in all the sets where they appeared. All the three crosses (SZ3-3-B-B2 x Kalungu, VTTT918/15-4-4 x Kalungu and Lukupa x Kalungu) had root/shoot ratio greater than 0.24.

Table 13: Mean squares for root and shoot parameters of common bean F_2 crosses grown in nutrient solution containing 12 mgL^{-1} aluminium (Set III).

Source of variation	**d.f**	**RL**	**SL**	**RB**	**SB**	**R/S**
Crosses	3	0.324	0.9549	0.0486***	0.0247*	0.1753**
GCA_{Male}	1	0.428	2.1112	0.0378***	0.0378*	0.0772*
GCA_{Female}	1	0.507	0.549	0.0416***	0.0187	0.1331*
SCA	1	0.037	0.2045	0.0665***	0.0176	0.3156**
Error	8	1.060	0.8279	0.0009	0.0061	0.0144
CV%		**12.0**	**11.7**	**21.1**	**24.2**	**29.3**
Baker's ratio = $\sigma^2gca_m + \sigma^2gca_f / (\sigma^2gca_m + \sigma^2gca_f + \sigma^2sca)$	**-**	**0.46**	**0.78**	**0.45**	**0.65**	**0.58**

KEY: RL = Root Length, SL = Shoot Length, RB = Root Biomass, SB = Shoot Biomass, R/S= Root/Shoot ratio***, ** and * significant at P< 0.001, P<0.01 and P< 0.05 respectively

Table 14: Mean performance of the parents and progeny grown in nutrient solution containing 12 mgL^{-1} aluminium for 10 days (Set III)

Entry	RL(cm)	SL (cm)	RB (g)	SB (g)	R/S
Lukupa[a]	7.4	8.5	0.099	0.319	0.31
VTTT918/15-4-4[a]	6.6	10.1	0.106	0.263	0.40
Kalungu[b]	4.3	7.2	0.050	0.308	0.16
VTTT924/12-4[b]	3.7	5.4	0.039	0.170	0.23
Lukupa x Kalungu	8.2	7.4	0.332	0.457	0.73
Lukupa x VTTT924/12-4	8.5	7.3	0.066	0.301	0.22
VTTT918/15-4-4 x Kalungu	8.5	8.5	0.071	0.268	0.27
VTTT918/15-4-4 x VTTT924/12-4	9.0	7.9	0.102	0.266	0.38
Mean	7.0	7.8	0.108	0.294	0.34
$LSD_{0.05}$	1.9	1.7	0.057	0.147	0.23

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root/Shoot Ratio, a = Male parent, b = Female parent

4.2.2 Analysis of general and specific combining ability effects

4.2.2.1 Set I

Individual General Combining Ability (GCA) and Specific Combining Ability (SCA) effects are presented in Table 15. Significant GCA effects were observed for all traits studied except root biomass and root/shoot ratio (Table 15). BF13572-11 showed significant ($P<0.01$) positive GCA effects for root length (1.2) and significant negative GCA effects for shoot length (-1.3) and shoot biomass (-0.060) at $P<0.01$ and $P<0.05$ respectively. Kalungu exhibited significant ($P<0.01$) positive GCA effects (1.3) for shoot length. All crosses showed non- significant SCA effects for all traits studied (Table15).

4.2.2.2 Set II

Individual GCA and SCA effects are presented in Table 16. Significant GCA effects were observed for root length, shoot length, root biomass, shoot biomass and root/shoot ratio at $P<0.001$, $P<0.05$, $P<0.001$, $P< 0.01$ and $P<0.01$ respectively (Table 16). Lukupa showed significant positive GCA effects for root/shoot ratio (0.08) and significant negative GCA effects for root length (-1.6), shoot length (-0.5) and root biomass (-0.06). SZ3-3-B-B2 on the other hand displayed significant positive GCA effect for root length (1.6), shoot length (0.5) and root biomass (0.06) and negative effects for root/shoot ratio (-0.08). ARA 4 exhibited positive (0.055) and negative (-0.112) GCA effects for root biomass and shoot biomass respectively. On the other hand, Kalungu had positive and negative GCA effects for shoot biomass (0.112) and root biomass (-0.055) respectively (Table 16).

Significant SCA effects were observed for root length, shoot length and root biomass in all the four crosses (Table 16). Significantly positive and negative SCA effects were observed for root length, shoot length and root biomass at $P<0.01$, $P<0.05$ and $P<0.001$ respectively. The crosses, Lukupa x Kalungu and SZ3-3-B-B2 x ARA 4 exhibited positive SCA effects for root length (1.1) and root biomass (0.061) and negative SCA effects for shoot length (-0.6). Lukupa x ARA 4 and SZ3-3-B-B2 x Kalungu showed positive SCA effects for shoot length (-0.6) and negative effects for root length (-1.1) and root biomass (-0.061) (Table 16).

Table 15: Set I estimates of general and specific combining Ability effects on root length shoot length, root biomass, shoot biomass and root/shoot biomass ratio for parental genotypes and crosses evaluated in 12 mgL^{-1} aluminium.

	RL	SL	RB	SB	R/S
Parent GCA effects					
SZ3-3-B-B2[a]	-0.1	0.5	0.010	0.021	0.03
VTTT918/15-4-4[a]	0.1	-0.5	-0.010	-0.021	-0.03
BF13572-11[b]	1.2**	-1.3**	0.001	-0.060*	0.08
Kalungu[b]	-0.5	1.3**	0.003	0.028	-0.04
VTTT915/2-2[b]	-0.7	0.0	-0.003	0.032	-0.04
F2 Crosses SCA affects					
SZ3-3-B-B2 x BF13572-11	0.1	0.3	0.005	-0.031	0.06
SZ3-3-B-B2 x Kalungu	0.4	0.0	0.005	0.009	-0.01
SZ3-3-B-B2 x VTTT915/2-2	-0.5	-0.4	-0.009	0.022	-0.05
VTTT918/15-4-4 x BF13572-11	-0.1	-0.3	-0.005	0.031	-0.06
VTTT918/15-4-4 x Kalungu	-0.4	0.0	-0.005	-0.009	0.01
VTTT918/15-4-4 x VTTT915/2-2	0.5	0.4	0.009	-0.022	0.05
SE_{gi}	0.3	0.3	0.012	0.021	0.05
SE_{gj}	0.4	0.4	0.014	0.025	0.06
SE_{sij}	0.5	0.6	0.020	0.036	0.08

KEY: RL=Root Length (cm), SL=Shoot Length (cm), RB=Root Biomass (g), SB=Shoot Biomass (g), R/S=Root to Shoot Ratio, * and ** = significant at P<0.05 and P<0.01 respectively, a=male parent, b=female parent, SE_{gi} = standard error for GCA male parents, SEg_j = standard error for GCA female parent, SE_{sij} = standard error for SCA of the crosses

Table 16: Set II estimates of general and specific combining ability effects on root length shoot length, root biomass, shoot biomass and root/shoot biomass ratio for parental genotypes and crosses evaluated in 12 mgL^{-1} aluminium.

	RL	SL	RB	SB	R/S
Parents					
Lukupa[a]	-1.6***	-0.5*	-0.060***	0.017	0.08**
SZ3-3-B-B2[a]	1.6***	0.5*	0.060***	-0.017	-0.08**
ARA 4[b]	0.2	0.1	0.055***	-0.112**	0.00
Kalungu[b]	-0.2	-0.1	-0.055***	0.112**	0.00
SCA Effects for Crosses					
Lukupa x ARA4	-1.1**	0.6*	-0.061***	-0.048	0.03
Lukupa x Kalungu	1.1**	-0.6*	0.061***	0.048	-0.03
SZ3-3-B-B2 x ARA 4	1.1**	-0.6*	0.061***	0.048	-0.03
SZ3-3-B-B2 x Kalungu	-1.1**	0.6*	-0.061***	-0.048	0.03
SE_{gi}	0.4	0.3	0.013	0.034	0.03
SE_{sij}	0.3	0.2	0.011	0.028	0.09

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root to Shoot Biomass Ratio, ***, **,* Significant at P<0.001, P< 0.01 and P<0.05 respectively, [a] =male parent, [b]= female parent, SE_{gij} = standard error for GCA male and female parents, SE_{sij} = standard error for SCA of the crosses

4.2.2.3 Set III

Individual General Combining Ability (GCA) and Specific Combining Ability (SCA) estimates were presented in Table 17. Significantly positive and negative GCA effects were observed for root biomass, shoot biomass and root/shoot ratio at $P<0.001$, $P<0.05$ and $P<0.05$ respectively (Table 17). All parents exhibited significant GCA effects for root biomass and root/shoot ratio but in different directions (Table 17). Lukupa and VTTT918/15-4-4, respectively, exhibited significant positive and negative GCA effects root biomass ($P<0.001$), shoot biomass and root/shoot ratio ($P<0.05$). Kalungu and VTTT924/12-4 respectively exhibited significant positive and negative GCA effects for root biomass and root/shoot ratio. VTTT924/12-4 had a relatively higher negative GCA effect (-0.11) for root/shoot ratio than that exhibited by VTTT918/15-4-4 (-0.08).

Significant ($P<0.01$) SCA effects were also observed for root biomass and shoot/shoot ratio for all crosses (Table 17). Lukupa x Kalungu and VTTT918/15-4-4 x VTTT924/12-4 exhibited significant positive SCA effects for root biomass (0.0744) and root/shoot ratio (0.16), at $P<0.001$ and $P<0.01$ respectively. On the other hand, Lukupa x VTTT924/12-4 and VTTT918/15-4-4 x Kalungu had significantly negative SCA effects for root biomass (-0.074) and root/shoot ratio (-0.16) at $P<0.001$ and $P<0.01$ respectively.

In summary, the GCA effects of parents and SCA effects of crosses that were common in two or three sets are shown in tables 15, 16 and 17. SZ3-3-B-B2 exhibited significant positive GCA effect for root length, shoot length and root biomass and a negative GCA effect for root/shoot ratio in set II (Table 16). VTTT918/15-4-4 showed significant negative effects for root biomass shoot biomass and root/shoot ratio in set III (Table 17). Lukupa had significant positive GCA effects for root/shoot ratio in sets II and III (Tables 16 and 17), root biomass and shoot biomass in set III (Table 17). Lukupa also exhibited significant negative GCA effects for root length, shoot length and root biomass in set II. Kalungu was the only female parent common in all the three sets. Significant positive GCA effects were observed for shoot length (set I), shoot biomass (set II), root biomass and root/shoot ratio (set III). Significant negative GCA effects were observed for root biomass in set II.

The Specific combining ability (SCA) was significant in sets II and III. SZ3-3-B-B2 x Kalungu exhibited significant positive SCA effects for shoot length ($P<0.05$) and significant negative effects for root length ($P<0.01$) and root biomass in set II ($P<0.001$) (Table 16). Lukupa x Kalungu common in sets II and III exhibited significant positive SCA effects for root length ($P<0.01$) in set II (Table 16), root biomass ($P<0.001$) in sets II and III (Table 17) and root/shoot ratio ($P<0.01$) in set III. Significant negative SCA effects were observed for this cross for shoot length ($P<0.05$). VTTT918/15-4-4 x Kalungu common in sets II and III only showed significant negative SCA effects for root biomass ($P<0.001$) and root/shoot biomass ($P<0.01$) in set III.

Table 17: Set III estimates of general and specific combining ability effects on root length shoot length, root biomass, shoot biomass and root/shoot biomass ratio for parental genotypes and crosses evaluated in 12 mgL^{-1} aluminium.

	RL	SL	RB	SB	R/S
Parent GCA effects					
Lukupa[a]	-0.2	-0.4	0.056***	0.056*	0.08*
VTTT918/15-4-4[a]	0.2	0.4	-0.056***	-0.056*	-0.08*
Kalungu[b]	-0.2	0.2	0.059***	0.040	0.11*
VTTT924/12-4[b]	0.2	-0.2	-0.059***	-0.040	-0.11*
F2 Crosses SCA effects					
Lukupa x Kalungu	0.1	-0.1	0.074***	0.039	0.16**
Lukupa x VTTT924/12-4	-0.1	0.1	-0.074***	-0.039	-0.16**
VTTT918/15-4-4 x Kalungu	-0.1	0.1	-0.074***	-0.039	-0.16**
VTTT918/15-4-4 x VTTT924/12-4	0.1	-0.1	0.074***	0.039	0.16**
SE_{gij}	0.4	0.4	0.012	0.032	0.02
SE_{sij}	0.6	0.3	0.010	0.026	0.07

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root to Shoot Biomass Ratio, ***, ** and *= Significant at P<0.001, P<0.01 and P<0.05 respectively, [a]=male parent, [b]=female parent, SE_{gij} = standard error for GCA male and female parents, SE_{sij} = standard error for SCA of the crosses

4.3 Narrow sense heritability estimates

Variation due to genotype, $\sigma^2 g$, contributed more to total phenotypic variation than environmental variation for all characters studied except root/shoot ratio in Set II (Appendix 7-9). Narrow sense heritability estimates (h^2) were classified as high (>0.50), medium (0.30- 0.50), and low (<0.30) according to Bhateria et al. (2006). Narrow sense heritability across sets ranged from 10% to 55% for root length, 26% to 74% for shoot length, 6% to 17% for root biomass, 25% to 58% for shoot biomass, and 10% to 31% for root/shoot ratio (Table 18). In set III narrow sense heritability was not estimated for root/shoot ratio because the additive genetic variance was negative (Appendix 9).

Table 18: Heritability estimates across sets

SET	RL	SL	RB	SB	R/S
I	0.55	0.74	0.15	0.48	0.13
II	0.35	0.26	0.06	0.58	0.10
III	0.10	0.31	0.17	0.25	-

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S = Root/Shoot Ratio

CHAPTER FIVE

5.0 DISCUSSION

5.1. Classification of genotypes for tolerance to aluminium toxicity

The study aimed at looking at the inheritance of tolerance to aluminium toxicity in common beans and hence its objectives were; to characterize common bean genotypes for aluminium tolerance and to determine the type of gene action conditioning aluminium tolerance in common beans.

The significant differences observed in response to aluminium treatments (0 mgL^{-1} and 12 mgL^{-1}) for all studied traits, except number of lateral roots, suggested the presence of aluminium induced differential response in common bean genotypes. The highly significant differences observed among genotypes for all the traits studied (root length, shoot length, root biomass, shoot biomass and number of lateral roots) showed the existence of a genetic variability among common bean genotypes in response to Al stress. Rangel et al., (2005) in their study on, interference of proton toxicity with the screening of common bean (*Phaseolus vulgaris* L.) genotypes for aluminium tolerance in nutrient solution, observed similar variation for these root parameters and inferred that a potential for plant breeders to select the tolerant genotypes was established based on such variability. These results are important because presumably genotypes that grew vigorously in aluminium solution as observed in this study would have the ability to grow in acidic soils with high aluminium saturation. Large root systems are known to have a greater capacity for absorbing water and minerals, as they are able to explore a larger rhizosphere (Osmont *et al.,* 2007).

The highly significant genotype x aluminium interaction observed for all traits (root length, shoot length, root biomass shoot biomass and number of lateral roots) is an indication of the variation in the response of genotypes to a similar change in aluminium concentration. The differential genotype responses allow for characterization of the genotypes for aluminium tolerance. Root length and root biomass have been highly associated with aluminium tolerance as they represent the ultimate product of growth and development of roots (Nguyen *et al.,* 2003; Kochian *et al.,* 2005).

In the current study, parental genotypes were classified for aluminium tolerance based on the extent of reduction in root length (Rangel *et al*., 2005) with tolerant ones showing less than 30% reduction, moderate 30-50% and sensitive ones above 50% reduction in root length (Figure 4). SZ3-3-B-B2, VTTT918/15-4-4 and Lukupa were classified as aluminium tolerant because they showed the least reduction (<30%) in root length to increased aluminium concentration. This result indicates that these genotypes can be grown in high aluminium soils and their growth will still be sustained. On the other hand, ARA 4, Solwezi parent, NUA 91 and Lyambai showed the highest reduction in root length (>50%) were hence classified as sensitive to aluminium toxicity. Tolerance in this regard was exhibited by least reduction in root length with increased aluminium concentration. This result implied that cell division in the root tip meristem of aluminium sensitive genotypes, was inhibited by aluminium thus preventing root elongation and consequently shorter root length (Anderson and Furlani, 2005).

The classification of genotypes for tolerance to aluminium toxicity based on the extent of reduction in root length (Rangel *et al.*, 2005), could not be used for crosses because they were only evaluated in nutrient solution containing 12 mgL^{-1} aluminium without the control. Therefore, classification of crosses for tolerance to aluminium toxicity was based on root/shoot ratio (Ma *et al.*, 2004). Genotypes (crosses) with root/shoot ratio greater than 0.24 were classified as tolerant and those less than 0.24 were classified as sensitive to aluminium toxicity. In this study, all crosses (Lukupa x ARA 4, SZ3-3-B-B2 x ARA 4, SZ3-3-B-B2 x Kalungu, SZ3-3-B-B2 x BF13572-11, Lukupa x Kalungu, VTTT918/15-4-4 x BF13572-11, VTTT918/15-4-4 x VTTT915/2-2, VTTT918/15/4-4 x VTTT924/12-4 and VTTT918/15-4-4 x Kalungu) except Lukupa x VTTT924/12-4 and SZ3-3-B-B2 x VTTT915/2-2 showed greater root/shoot ratios than 0.24 and were therefore classified as aluminium tolerant. The results implied that the tolerant crosses could be grown in high aluminium soils. On the other hand the results suggest that aluminium tolerance trait was transferred from parents to the offspring.

In addition to root length, other root traits; root biomass and number of lateral roots, also classified SZ3-B-B-B2 as aluminium tolerant and Lyambai as aluminium sensitive. On the basis of root biomass and number of lateral roots SZ9-7-B-B was classified as aluminium tolerant and Chambeshi as aluminium sensitive. NUA 45,

Solwezi parent and SZ3-3-B-B2 were classified as aluminium tolerant on the basis of shoot length and the genotypes, Chambeshi, Kabulangeti and ARA 4 as aluminium sensitive. On the other hand shoot biomass classified Lukupa, SZ9-7-B-B and LY4-4-B as aluminium tolerant and Chambeshi, BF13607-12 and VTTT918/15-4-4 as aluminium sensitive.

Variability for tolerance to aluminium toxicity observed among common bean genotypes may partly be related to differences in the extension of the root elongation zone. Rangel *et al.* (2007) reported that a tolerant common bean genotype (ICA Quimbaya), presented a larger elongation zone than did a susceptible genotype (VAX1). This suggested that the dynamics of perceiving and responding to aluminium stress varies spatially. Another principal mechanism that has been correlated with tolerance to aluminium toxicity in common beans is the exudation of organic acids. Tolerant genotypes have been observed to exude more citrate than the sensitive genotypes with the degree of secretion dependent on the dosage of aluminium and on time (Shen et al., 2002). However, a differential response of tolerance to aluminium and exudation of citrate exists among different common bean genotypes.

5.2 Gene action conditioning aluminium tolerance

The significant GCA and SCA effects observed for root length, shoot length, root biomass and root/shoot ratio (Tables 15, 16 and 17) suggested that inheritance of these traits, as measures of aluminium tolerance, was controlled by both additive and non-additive gene action. Significant GCA and non-significant SCA effects observed for shoot biomass (Tables 15 and 16) pointed to the importance of additive gene action in the inheritance of aluminium tolerance. Baker (1978) suggested that the progeny performances could be predicted using the ratio of combining ability variance components. He indicated that the closer the ratio is to unity, the greater the predictability of progeny performance based on GCA alone and the more important the additive gene action. The Baker ratio values for root length, shoot length, shoot biomass and root/shoot ratio were closer to unity, an indication that additive gene action was more important than non- additive gene action in the inheritance of aluminium tolerance based on these traits. On the other hand, the Baker ratio value for root biomass was low (< 0.5), an indication that non-additive gene action was

more important in the inheritance of root biomass, as a measure of aluminium tolerance.

These results suggest the possibility of pure line selection for aluminium stress tolerance, based on root length, shoot length, and root/shoot ratio from the common bean genotypes studied (Ojo and Ayuba, 2013). The predominance of additive gene action also suggests the opportunity to attain genetic improvement by accumulating favourable alleles through selection (Adefris and Becker, 2005). The results further suggest that inheritance of aluminium tolerance in common beans, on the basis of these traits (root length, shoot length, and root/shoot ratio), is controlled by many genes. These results were consistent with the report by Khatiwada *et al.* (1996a) which revealed that root length, as a measure of aluminium tolerance, is governed by both additive and non-additive gene effects with the preponderance of additive effects. The results obtained in this study were, however, in contrast with the report by Ojo and Ayuba (2013), which showed that additive gene action was more important than non-additive gene action for expression of root biomass. The differences in the gene action controlling root biomass could be attributed to the differences in materials used.

A related study on GCA for aluminium tolerance conducted by Hernán *et al.*, (2009) showed that common bean has polygenic inheritance of tolerance to aluminium and that some aluminium tolerance Quantitative Trait Loci (QTL) co-localize with QTL for tolerance to low phosphorus (P) ,suggesting cross-links between different mechanisms of abiotic stress adaptation in common bean. Louis *et al*. (2011) also reported that inheritance of aluminium tolerance and acid soil tolerance expressed by a runner bean (*Phaseolus coccineus* L), G35346-3Q, is complex.

The medium to high narrow sense heritability estimates (30% to 50%) obtained for root length, shoot length and shoot biomass was an indication that selection for these traits should be fairly easy as the genotype corresponds closely with the phenotype. The results suggest that selection for aluminium tolerance based on this trait could be possible in early generations. The low narrow sense heritability estimates (< 30%) obtained for root biomass and root/shoot ratio indicated that selection for these traits could be achieved in advanced generation. The low heritability further implied little transmissibility of characters between generations, and thus prompts the need for

efficient methods of selection to achieve better genetic gains (Adefris and Becker, 2005).

In this study, significant positive combining ability effects were desirable for all the studied traits (root length, shoot length and root biomass, shoot biomass and root/shoot ratio) because they indicated contribution of the genotype towards aluminium tolerance, while negative combining ability effects indicated contribution towards aluminium sensitivity. The significant positive GCA effects exhibited by SZ3-3-B-B2 (for root length, shoot length and root biomass), BF13572-11 (for root length), Kalungu (for shoot length, shoot biomass and root/shoot ratio), ARA 4 (for root biomass) and Lukupa (for root biomass and root/shoot ratio) was an indication that these parents were adding to aluminium tolerance on the basis the specific traits. These results implied that these genotypes were suitable for incorporation in the breeding programme for improvement of aluminium tolerance. These results suggested that selection from SZ3-3-B-B2 and Lukupa (aluminium tolerant genotypes) and crosses involving them would enhance a rapid progress in the breeding of aluminium tolerant genotypes of common beans.

Ojo and Ayuba (2013) reported similar results where, two aluminium tolerant soyabean genotypes, TGX 1896- 3F and TGX 1844-18E, were contributing to aluminium tolerance because of the positive GCA effects, thereby making them possible candidates in selection work for aluminium tolerance. Khatiwada et al. (1996b) in their study on variability and genetics of tolerance for aluminium toxicity in rice (*Oryza sativa* L), reported that tolerant parents, Azucena, IRAT104, and Moroberekan were good general combiners and therefore suitable as sources for aluminium tolerance in breeding programmes.

The significant negative GCA effects displayed by Lukupa (for root length and shoot length), BF13572-11 (for shoot length and shoot biomass), Kalungu (for root biomass), VTTT918/15-4-4 (for root biomass and root/shoot ratio), VTTT924/12-4 (for root biomass and root/shoot ratio), ARA 4 (for shoot biomass) and SZ3-3-B-B2 (for root/shoot ratio) was an indication that these parents were contributing to aluminium sensitivity based on the specific traits. The results further implied that these genotypes were undesirable in breeding programmes for aluminium tolerance

improvement because of their undesirable negative GCA effects (Daniel *et al.*, 2006).

The significant positive SCA effects displayed by the crosses, Lukupa x Kalungu, SZ3-3-B-B2 x ARA 4 (for root length and root biomass), Lukupa x ARA 4, SZ3-3-B-B2 x Kalungu (for shoot length) and VTTT918/15-4-4 x VTTT924/12-4 (for root biomass and root/shoot ratio) signified the desirable complementarity of the alleles controlling aluminium tolerance of the specific parents. The cross, VTTT918/15-4-4 x VTTT924/12-4, exhibited good SCA effects for root biomass, though derived from parents that were poor general combiners. This suggested the presence of non additive gene action and an indication of genetic interaction between favourable alleles contributed by both parents (Adeniji and Kehinde, 2003). Large and positive SCA effects for a trait have also been reported by Ojo (2003) to suggest the possibility for transgressive segregation for the trait in later generation of selfing.

SZ3-3-B-B2 x ARA 4 and Lukupa x Kalungu, derived from parents both with positive GCA effects, also displayed significant positive SCA effects for root biomass. This was an indication that a good performing cross can be obtained by crossing parents with the highest GCA estimates (Ogunbodede *et al*., 2000). Similarly, Everina *et al.* (2008) from 7 x 7 diallel crosses noted that parents NTA 93-21, Delcot-344, Aubrun-56 and MZ-561 which had high and positive GCA also manifested high SCA for same traits, thus suggested that some of the parents could be identified to improve majority of the traits. The SCA effects are however not very important for a self pollinated crops like common beans making it difficult to produce commercial hybrids (Kimani and Derera, 2008).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

The study was set out to characterize common bean genotypes for tolerance to aluminium toxicity, determine the type of gene action conditioning tolerance to aluminium toxicity and hence establish the genetic basis for developing aluminium tolerant varieties of common beans. The results of this study showed existence of genetic variation among common bean genotypes for adaptation to acid soils and tolerance to aluminium toxicity. Highly significant differences were observed among the common bean genotypes in response to aluminium stress.

Based on the extent of reduction in root length, all the twenty screened genotypes were classified into three categories: Five (5) genotypes were classified as aluminium tolerant, (SZ3-3-B-B2, VTTT918/15-4-4 Lukupa, CIM-RMOO321-LN02 and LY4-4-B), four intermediate (SZ9-7-B-B, Kabulangeti, BF13607-12 and SZ9-B-B-B2) and eleven aluminium sensitive (ARA 4, Solwezi parent, BF13572-11, NUA 91, Lyambai, Chambeshi, NUA 45, Kalungu, VTTT924/12-4, VTTT915/2-2 and BF13607-6). The five identified aluminium tolerant varieties could, therefore, be explored in the development of improved high yielding common bean genotypes for production on acid soils in the high rainfall areas of Zambia.

Furthermore, the identification of aluminium tolerant genotypes adds to the knowledge regarding the potential ability of common beans varieties to yield optimally on acid soils of high rainfall areas in Zambia. Moreover, SZ3-3-B-B2 identified as aluminium tolerant, with good general combining abilities for root length, shoot length and root biomass, was identified as suitable for use in breeding for common beans with high yielding capacity on aluminium prone acid soils.

The results obtained in this study showed that inheritance of aluminium tolerance is controlled by additive gene action as observed for all traits studied (root length, shoot length, shoot biomass and root/shoot ratio) except root biomass for which non-additive gene action plays an important role in the inheritance of aluminium-tolerance. Therefore, additive gene action forms the basis for genetic improvement of aluminium tolerance in common beans.

Narrow sense heritability estimates ranged from 10%-55% for root length, 26%-74% for shoot length, 6%-17% for root biomass, 25%-48% for shoot biomass and 10%-

13% for root/shoot ratio. Based on these heritability estimates, selection for root length, shoot length and shoot biomass could result in rapid gain in selection because of their medium heritability estimates, while selection for root biomass and root/shoot ratio would result in slow gain in selection because of their low narrow sense heritability estimates.

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APPENDICES

Appendix 1: Sets of successful F2 crosses

Set I

	Male Parents	
Female Parents	SZ3-3-B-B2	VTTT918/15-4-4
BF 13572-11	x	x
KALUNGU	x	x
VTTT915/2-2	x	x

Set II

	Male Parents	
Female Parents	SZ3-3-B-B2	LUKUPA
KALUNGU	x	x
ARA 4	x	x

Set III

	Male Parents	
Female Parents	VTTT918/15-4-4	LUKUPA
VTTT924/12-4	x	x
KALUNGU	x	x

Appendix 2: Genotype ranking by percent reduction in root length (cm)

Genotype	0 mgL^{-1} Al	Rank	12 mgL^{-1} Al	Rank	% reduction	Rank
SZ3-3-B-B2	8.0	7	7.2	2	0.10	1
VTTT918/15-4-4	7.3	13	6.5	3	0.11	2
Lukupa	9.0	2	7.8	1	0.14	3
CIM-RMOO-321LN02	5.3	15	4.5	8	0.14	4
LY4-4-B	7.4	11	5.2	5	0.30	5
SZ9-7-B-B	8.2	6	5.5	4	0.34	6
Kabulangeti	7.2	14	4.7	7	0.34	6
BF13607-12	8.6	5	5.0	6	0.41	7
SZ9-B-B-B2	7.7	8	4.3	9	0.43	8
BF13607-6	7.7	8	3.8	14	0.50	9
VTTT 915/2-2	9.0	2	4.5	8	0.51	9
VTTT 924/12-4	7.5	10	3.7	13	0.51	10
Kalungu	8.9	3	4.2	10	0.53	11
NUA 45	7.6	9	3.6	14	0.53	11
Chambeshi	7.6	9	3.4	15	0.55	12
Lyambai	7.5	10	3.3	16	0.56	13
NUA 91	9.5	1	4.1	11	0.57	14
SZ PRT	9.5	1	4.1	11	0.57	14
BF13572-11	8.8	4	3.8	12	0.57	14
ARA 4	7.3	12	3.0	17	0.59	15

Appendix 3: Genotype ranking by percent reduction in shoot length (cm)

Genotype	0 mgL^{-1} Al	Rank	12 mgL^{-1} Al	Rank	% reduction	Rank
NUA 45	10.7	5	10.6	2	0.01	1
SZ PRT	10.4	6	9.9	4	0.05	2
SZ3-3-B-B2	9.5	9	8.8	6	0.08	3
VTTT918/15-4-4	12.3	2	11.0	1	0.11	4
NUA 91	6.2	19	5.5	15	0.11	4
Lyambai	9.0	10	7.8	8	0.14	5
BF13572-11	8.8	11	7.6	9	0.14	5
Kalungu	8.5	14	7.3	10	0.14	5
VTTT 924/12-4	6.2	19	5.3	16	0.15	6
SZ9-7-B-B	7.8	15	6.5	13	0.16	7
VTTT 915/2-2	7.5	16	6.2	14	0.17	8
SZ9-B-B-B2	9.7	7	8.0	7	0.18	9
BF13607-12	6.7	18	5.5	15	0.19	10
BF13607-6	8.7	12	7.0	11	0.20	11
Lukupa	12.0	3	9.4	5	0.21	12
CIM-RMOO-321LN02	6.8	17	5.3	16	0.22	13
LY4-4-B	8.6	13	6.6	12	0.23	14
ARA 4	14.7	1	10.4	3	0.29	15
Kabulangeti	11.3	4	7.6	9	0.32	16
Chambeshi	9.6	8	4.8	17	0.50	17

Appendix 4: Genotype ranking by percent reduction in root biomass (g)

Genotype	0 mgL^{-1} Al	Rank	12 mgL^{-1} Al	Rank	% reduction	Rank
SZ9-7-B-B	0.1	7	0.09	3	0.10	1
SZ3-3-B-B2	0.17	2	0.15	1	0.12	2
ARA 4	0.08	9	0.07	5	0.13	3
BF13607-6	0.07	10	0.06	6	0.14	4
NUA 45	0.06	12	0.05	7	0.17	5
NUA 91	0.09	8	0.07	5	0.22	6
SZ9-B-B-B2	0.15	3	0.11	2	0.27	7
Kalungu	0.07	10	0.05	7	0.29	8
LY4-4-B	0.1	7	0.07	5	0.30	9
VTTT918/15-4-4	0.13	5	0.09	3	0.31	10
Kabulangeti	0.12	6	0.08	4	0.33	11
BF13572-11	0.06	11	0.04	8	0.33	11
BF13607-12	0.03	14	0.02	9	0.33	11
Lukupa	0.14	4	0.08	4	0.43	12
SZ PRT	0.14	4	0.08	4	0.43	12
CIM-RMOO-321LN02	0.07	10	0.04	8	0.43	12
VTTT 924/12-4	0.07	10	0.04	8	0.43	12
Chambeshi	0.04	13	0.02	9	0.50	13
Lyambai	0.18	1	0.08	4	0.56	14
VTTT 915/2-2	0.06	11	0.02	9	0.67	15

Appendix 5: Genotype ranking by percent reduction in shoot biomass (g)

Genotype	0 mgL^{-1} aluminium	Rank	12 mgL^{-1} aluminium	Rank	% reduction	Rank
Lukupa	0.35	7	0.34	5	0.03	1
SZ9-7-B-B	0.4	5	0.38	4	0.05	2
LY4-4-B	0.29	10	0.27	9	0.07	3
BF13607-6	0.13	16	0.12	17	0.08	4
Lyambai	0.44	3	0.40	2	0.09	5
SZ3-3-B-B2	0.33	9	0.30	7	0.09	5
NUA 45	0.54	1	0.48	1	0.11	6
NUA 91	0.44	3	0.39	3	0.11	6
VTTT 915/2-2	0.27	12	0.24	10	0.11	6
ARA 4	0.26	13	0.23	11	0.12	7
SZ9-B-B-B2	0.33	9	0.29	8	0.12	7
CIM-RMOO-321LN02	0.24	14	0.19	13	0.21	8
Kalungu	0.34	8	0.27	9	0.21	8
SZ PRT	0.28	11	0.20	12	0.29	9
VTTT 924/12-4	0.24	14	0.17	14	0.29	9
BF13572-11	0.16	15	0.11	15	0.31	10
Kabulangeti	0.39	6	0.27	9	0.31	10
BF13607-12	0.13	16	0.07	16	0.46	12
VTTT918/15-4-4	0.5	2	0.31	6	0.38	11
Chambeshi	0.41	4	0.20	12	0.51	13

Appendix 6: Genotype ranking by percent reduction in number of lateral roots

Genotype	0 mgL^{-1} Al	Rank	12 mgL^{-1} Al	Rank	% reduction	Rank
SZ3-3-B-B2	20	8	19	4	0.05	1
SZ9-B-B-B2	20	8	19	4	0.05	1
Kalungu	17	11	16	7	0.06	2
SZ9-7-B-B	33	2	30	1	0.09	3
BF13607-6	29	4	26	2	0.10	4
CIM-RMOO-321LN02	20	8	18	5	0.10	4
BF13607-12	18	10	16	7	0.11	5
LY4-4-B	20	8	17	6	0.15	6
BF13572-11	19	9	16	7	0.16	7
SZ PRT	18	10	15	8	0.17	8
Lukupa	22	7	18	5	0.18	9
VTTT 924/12-4	24	5	19	4	0.21	10
NUA 91	23	6	18	5	0.22	11
Kabulangeti	29	4	21	3	0.28	12
NUA 45	33	2	21	3	0.36	13
VTTT 915/2-2	22	7	14	9	0.36	13
ARA 4	30	3	17	6	0.43	14
Chambeshi	19	9	10	11	0.47	15
VTTT918/15-4-4	36	1	13	10	0.64	16
Lyambai	36	1	10	11	0.72	17

Appendix 7: Set I Components of genetic variation and heritability for root length, shoot length, root biomass, shoot biomass and root/shoot ratio

Variance component	RL	SL	RB	SB	R/S
σ^2p	3.5224	5.3716	0.0027	0.0093	0.0203
σ^2g	2.7137	4.3566	0.0015	0.0055	0.0002
σ^2a	1.9339	3.9512	0.0004	0.0045	0.0029
σ^2d	0.7799	0.4053	0.0011	0.0011	-0.0015
σ^2e	0.8087	1.015	0.0012	0.0039	0.0201
h^2_{ns}	0.55	0.74	0.15	0.48	0.13

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root/Shoot Ratio, σ^2p = total phenotypic variation, σ^2g = genotypic variation, σ^2a = additive variation, σ^2d = Dominance variance, σ^2e = environmental variance

Appendix 8: Set II Components of genetic variation and heritability for root length, shoot length, root biomass, shoot biomass and root/shoot ratio

	RL	**SL**	**RB**	**SB**	**R/S**
σ^2p	29.9596	7.2529	0.0628	0.0843	0.0937
σ^2g	28.9266	6.8055	0.0618	0.0772	0.0669
σ^2a	10.452	1.9057	0.0039	0.0493	0.0096
σ^2d	18.4746	4.8999	0.0578	0.0280	0.0574
σ^2e	1.033	0.4474	0.001	0.0070	0.0267
h^2ns	0.3489	0.2627	0.0631	0.5845	0.1023

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S=Root/Shoot Ratio, σ^2p = total phenotypic variation, σ^2g= genotypic variation, σ^2a= additive variation, σ^2d= Dominance variance, σ^2e= environmental variance

Appendix 9: Set III Components of genetic variation and heritability for root length, shoot length, root biomass, shoot biomass and root/shoot ratio

	RL	SL	RB	SB	R/S
σ^2p	2.711	2.4095	0.1062	0.0285	0.2757
σ^2g	1.651	1.5816	0.1053	0.0225	0.2612
σ^2a	0.287	0.7504	0.0179	0.0071	-0.1403
σ^2d	1.364	0.8312	0.0875	0.0154	0.4015
σ^2e	1.06	0.8279	0.0009	0.0061	0.0145
h^2ns	0.10	0.31	0.17	0.25	-

KEY: RL = Root Length (cm), SL = Shoot Length (cm), RB = Root Biomass (g), SB = Shoot Biomass (g), R/S = Root/Shoot Ratio, σ^2P = total phenotypic variation, σ^2g= genotypic variation, σ^2a = additive variation, σ^2d = Dominance variance, σ^2e = environmental variance