RESPONSE OF SOYABEAN (*Glycine max.L.*)

TO HIGH LEVELS OF ALUMINIUM IN THE SOIL

BY

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DECLARATION

I, Patricia Mutale, declare that this dissertation represents my own work and that it has not been previously submitted for a Degree, Diploma or any other qualification at this or any other University.

Signature:……..

Date:……

APPROVAL

This dissertation of Patricia Mutale has been approved by the University of Zambia as partial fulfillment of the requirements for the award of the degree of Master of Science in Plant Breeding and Seed Systems

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DEDICATION

This work is dedicated to my adorable daughters Choolwe and Mapalo, and my lovely husband Chimuka Leo Haamukwanza.

ABSTRACT

Soyabean, *Glycine max.L,* is the most important oil crop in the Temperate and Sub-tropical regions. The grain consists of twenty percent oil, which makes it the most important crop for producing edible oil. Soyabean is sensitive to high levels of aluminium in the soil, a situation which limits its production. High rainfall areas of Zambia are characterized by acid soils. This study evaluated twenty varieties of soyabean from different seed companies, both in the laboratory and field. The laboratory experiment was done at the University of Zambia, School of Agricultural Sciences. The field experiment was done in the 2012/13 season at Seedco Lusaka West Farm and Liempe Farm. The Lusaka West Farm was treated as an optimum environment with pH 6.5 with aluminium content of 0.11 ppm, and Liempe Farm had 0.44 ppm levels of aluminium with pH 4.3. The laboratory experiment was in four phases: Determination of discriminatory level of aluminum, screening of genotypes, Hematoxylin test and Plant tissue analysis of aluminium content. The determination of discriminatory level of aluminium was carried out in order to determine the level of aluminum which was most injurious to soyabean so that the injurious level can be used to screen the rest of the genotypes. Five genotypes were used and the levels of aluminium used were 0,4,8,12,16 and 20mg/l in hydroponics. The results showed that as the aluminium levels increased, the parameters taproot length, shoot length, shoot biomass and root biomass decreased. The highest reduction was at 16mg/L and this was the discriminatory level that was identified. The level that was used in this experiment was 20mg/L for effective discrimination because it was noticed that even at that level, there was a decrease in all the parameters. The screening of all the genotypes was based on 0mg/L, as the control, and 20mg/L. The results showed highly significant differences, at probability ≤ 0.01, among genotypes. Semeki and Samba were chosen be the most tolerant varieties to Aluminium because their shoot biomass percentage decreases from level 0 to 20 mg/L were 5.77 and 6.74 respectively. While Spike, Hernon 147B and S810/6/10 were identified to be susceptible with shoot biomass percentage decrease of 74.42, 75.51 and 66.77 respectively. The Hematoxylin test was carried out to also select varieties tolerant to aluminium toxicity. The results from Hematoxylin helped group the genotypes into three: tolerant which included Samba, Semeki, Scribe, Squire, Sirocco, Score, Kaleya and Saga, moderate tolerant which included Sovereign, Satelite, Safari, Sequel, Soprano, Lukanga, Dina and Magoye, and susceptible varieties which included S810/6/10, Hernon 147B and Spike. Hematoxylin results confirmed the results found in the screening of genotypes which showed that Semeki and Samba were tolerant while Spike, Hernon 147B and S810/6/10 were susceptible. The plant tissue analysis to determine the amount of aluminium in the plant tissue was carried out to identify the mechanism of tolerance. It was noticed that the tolerant

varieties, Samba and Semeki had 97.33 and 108.61mg of Al/kg root sample respectively which was lower than in the susceptible varieties, Spike, S810/6/10 and Hernon 147B with 543.38, 508.21 and 549.22mg of Al/kg of root sample. The mechanism of tolerance was determined to be exclusion type. Single site analyses for both Liempe farm and Lusaka West farm data showed highly significant differences, at $p \leq 0.01$, among genotypes. Generally, genotypes performed better at Lusaka West Farm than at Liempe farm with the highest yield at Lusaka West Farm being 4.08t/ha from Squire and 3.17t/ha from Safari at Liempe farm. The genotypes selected to be tolerant in the laboratory, showed some stability in yield at both sites with Samba having a yield reduction percentage between Lusaka West and Liempe of 18.18 and Semeki 17.65 respectively. While the susceptible varieties Spike, Hernon 147B and S810/6/10 had their yield percentage decreases of 79.86, 82.61 and 76.95 respectively. In order to establish the most important parameter affecting yield at Liempe Farm, we determined the cause and effect relationship between yield and other parameters measured at that site. A stepwise multiple regression analysis was done where yield, as a response variable, was regressed on number of pods per plant, number of pink nodules per plant, root biomass, weight of 100 grains, plant height, days to flower, mature and pod shuttering. The results indicated the most important parameter that affected yield was number of pink nodules per plant because it contributed 51.3 %. A comparison of lboratory results to field results was done using and Orthogonal Contrast of the performance between the tolerant and susceptible varieties based on yield and number of pink root nodules per plant at Liempe farm. We discovered that highly significant differences appeared in the comparison between tolerant and susceptible genotypes at $p \leq 0.01$ for both yield and number of pink root nodules indicating that the Laboratory results were repeatable in the field. Hence laboratory screening can be used to select genotypes for aluminium tolerance.

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CHAPTER 1

1. INTRODUCTION

Soyabean, (*Glycine max.* L.), is the most important oil crop in the Temperate and Sub-tropical regions. It belongs to the family fabaceae, subfamily faboideae, genus Glycine and species G. max. This is a leguminous vegetable of the pea, which grows in tropical, subtropical, and temperate climates (Dugje *et al*., 2009).

The grain consists of more than 36% protein, 30% carbohydrates, and has excellent amounts of dietary fibre, vitamins and minerals. It also consists of 20% oil, which makes it the most important crop for producing edible oil (NewsEdge Corporation ©2012).

Malnutrition, particularly protein deficiency, is prevalent in many parts of Africa as animal protein is too expensive for most populations. Many leguminous crops provide some protein, but soybean is the only available crop that provides an inexpensive and high quality source of dietary protein comparable to meat, poultry and eggs.

A by-product from the oil production (soybean cake) is used as a high-protein animal feed in many countries. Soybean also improves soil fertility by adding nitrogen from the atmosphere. This is a major benefit in Africa farming systems, where soils have become exhausted by the need to produce more food for increasing populations, and where fertilizers are hardly and are expensive for farmers (Dugje *et al*., 2009).

It is a crop that can be grown in a wide range of soils, though it does very well in deep well drained sandy loam to clay loam soils. However, the soils should not have a pH less than 5.6. Preferably, soils are recommended to have a pH range of 5.5 to around 7.1 because that is the range which promotes most crop production.

The crop requires 500–850 mm water during the growing season. In areas where rainfall is not well-distributed and falls on the lower bracket, if irrigation is available, this can supplement its growth. The crop also needs a frost-free season and does not need to be grown in areas where temperatures go beyond 40^0C for a long time.

Soybeans can mature in three to four months (65–150 days) after planting depending on the varieties and their maturity group (Dugje et al., 2009).

Interest in soyabean production in Africa has increased considerably over the past decades. This is attributed to a number of factors such as increased utilisation of most commercially grown pulses as supplements in livestock feed (Uguru, 1996), usefulness as a source of cheap quality plant protein (Nnanyelugo *et al*., 1985) and the increasing prohibitive cost of animal protein. To satisfy the demand by producers and consumers, a number of soyabean varieties with excellent seed quality and agronomic characteristics have been released for cultivation by farmers in the tropical Africa (FAO, 1999). The other aim was to increase production and enhance protein intake of the low and middle income earners.

The production of soyabean in Zambia has risen from 70,000 metric tonnes in 2002 to 112,000 metric tonnes in 2010, (National Agriculture Marketing Council, 2011).

Farmers have shown increasing interest in soyabean production, as a result, soyabean production has extended to the high rainfall belts of Sub-Saharan Africa. Although there is a considerable potential for soyabean production in these belts (Mutsaere, 1991), yield has considerably varied in farmers' fields (Baten, 1991). This is attributed in part to continuous decline in soil fertility, due to deficiency in soil organic matter and other essential nutrients (Maduakor, 1991).

The high rainfall is associated with leaching of soil nutrients, low pH, erosion of mineralised and applied nutrients. Zeigler *et al*. (1995) reported that acid soils characterised by low pH and excess of aluminium and manganese. Unfortunately, 40% of the World's arable lands are acidic (Kochian 1995). Aluminium is the third most abundant element in the earth crust (Kochian, 1995). The main chemical reaction involved is alumnium hydrolysis (Sharma et al., 2007).

With respect to Zambia, soils of the large portion of high rainfall areas, designated as Region III, are acidic. Phiri (2008) reported that soil acidity is the most limiting constraint to crop production in highly leached soils of the high rain fall areas of Zambia. These soils are not suitable for growing most arable crops including soybeans (*Glycine max L*) if soil acidity is not ameliorated.

Foy (1992) reported that aluminium toxicity hamper crop production in tropical and subtropical areas. He further said that it is considered a primary factor in limiting plant growth in acid soils. Houde, *et al* in 2008 also reported that aluminium is considered

one of the most limiting factors for plant productivity in acidic soils and this restricts the production of soyabeans and other legumes. Herbert *et al.* in 2009 also reported that the major challenges in the adoption and productivity of soyabean culture, as is in Sub-Saharan Africa, are soil acidity (Al toxicity), diseases such as rust, red leaf blotch and frog eye and pace of adaptive.

The problem with soils that are very acid is that some elements that are important for soya beans growth will be in the unavailable form for the crop to access them. It also reduces nodulating potentials of the native Rhizobium strains affecting soyabean production especially in the high rainfall belts. Toxic aluminium levels retard root growth causing various root deformations, and discolorations (Blum, 1986; Villagarcia, 2001). The consequence of which is poor grain yield.

The most used technique of reducing soil acidity is lime application. Although, other farmers lime the soil to raise the pH, lime comes with its own challenges among them that lime is being bulky, messy when applying, takes long to be effective particularly where the acidity occurs at deeper layers of the soil and also cost of transportation is high. The only potential solution to combat the Al toxicity problem is through the use of genotypes that are tolerant to high aluminium levels in the soil.

The use of Al-tolerant germplasm complements liming practices that are aimed at neutralizing the acidity. Selection and breeding of crops for aluminium tolerance is a useful approach to increase production on acid soils. There is considerable variation within and between soyabean species in their ability to tolerate aluminium. Plant breeders can take advantage of this wide genetic base to develop genotypes able to grow on acid soils.

Screening of the genotypes for aluminium tolerance is a prerequisite for the selection and development of tolerant varieties. Various screening methods ranging from hydroponics, sand culture, to pot/field experiments have been adopted in searching for Al tolerant genotypes.

Therefore, this study was initiated to study the reaction of released varieties and elite advanced lines of the Zambian soyabean genotypes for tolerance to alumnium toxicity. The specific objectives of this study were;

1) To establish the mechanism by which soyabean plants tolerate aluminium toxicity.

2) To identify parameters that can be used in selecting for tolerance to high levels of soil aluminium.

3) To determine whether laboratory screening can be used in selecting soyabean genotypes tolerant to high levels of soil aluminium.

CHAPTER 2

2. LITERATURE REVIEW

Soyabean is a very important oil seed crop in both human and livestock nutrition.

2.1. Classification

Soyabean belongs to the kingdom plantae, order fabales, family fabaceae, subfamily faboideae, genus Glycine, and species G. max. The Bionomial name hence being *Glycine max* (L.) Merr.

The genus name *Glycine* was originally introduced by Carl Linnaeus (1737). The word glycine is derived from the Greek word– *glykys* (sweet) and refers to the sweetness of the pear-shaped (*apios* in Greek) edible tubers produced by the native North American twining or climbing herbaceous yambean legume, *Glycine apios*, now known as *Apios americana*. The cultivated soybean first appeared in *Species Plantarum*, by Linnaeus, under the name *Phaseolus max* L. The combination *Glycine max* (L.) Merr., as proposed by Merrill in 1917, has become the valid name for this useful plant.

The genus *Glycine* wild is divided into two subgenera, *Glycine* and *Soja*. The subgenus *Soja* (Moench) F.J. Herm. includes the cultivated soybean, *Glycine max* (L.) Merr., and the wild soybean, *Glycine soja* Sieb. & Zucc. Both species are annuals. *Glycine soja* is the wild ancestor of *Glycine max*, and grows wild in China, Japan, Korea, Taiwan and Russia (Singh, 2006). The subgenus *Glycine* consists of at least sixteen wild perennial species: for example, *Glycine canescens* F.J. Herm. and *G. tomentella* Hayata, both found in Australia and Papua New Guinea (Newell, 1983).

Like some other crops of long domestication, the relationship of the modern soybean to wild-growing species can no longer be traced with any degree of certainty. It is a cultural variety with a very large number of cultivars.

2.2. Botanic Description

2.2.1. Description and Physical Characteristics

Soyabean varies in plant morphology and growth habit. The height of the plant varies from less than 0.2 to 2.0 m (0.66 to 6.6 ft) (Hymowitz, 1995).

The pods, stems, and leaves are covered with fine brown, white or gray hairs. The hair density can be sparse, very sparse or dense. The leaves are trifoliate, having three to four leaflets per leaf, and the leaflets are six to fifteen cm (2.4–5.9 in) long and two to seven cm (0.79–2.8 in) broad. The leaflet can take the shapes lanceolate or ovate. Lanceolates have pointed leaflet ends, while ovate have round shaped ends. The crop shades its leaves before the seeds are matured.

The inconspicuous, self-fertile flowers are borne in the axil of the leaf. The flowers can be white, purple or pink.

The fruit is a hairy pod that grows in clusters of three to five. Each pod is three to eight cm long and usually contains two to four (rarely more) seeds whose size could be five to seven mm in diameter.

Soybeans seed coat colours can be black, brown, blue, yellow, green and mottled (Hymowitz, 1995). The hull of the mature bean is hard, water-resistant, and protects the cotyledon and hypocotyl (or "germ") from damage. If the seed coat is cracked, the seed will not germinate. The scar, visible on the seed coat, is called the hilum which also can be black, brown, buff, gray and yellow (Snow 1961). At one end of the hilum is the micropyle, or small opening in the seed coat which can allow the absorption of water for sprouting.

Remarkably, seeds such as soyabeans containing very high levels of protein can undergo desiccation, yet survive and revive after water absorption.

2.2.2. Growth Habit

Nearly all soybean varieties exhibit one of the two possible growth habits. These are the determinate and indeterminate growth habits.

Determinate varieties have rather distinct vegetative and reproductive development periods. Few stem nodes develop once flowering begins and the stem ends with a terminal raceme. Flowers and pods tend to develop at about the same time and rate for all stem nodes. They were developed for their better standability (they are less susceptible to lodging). These varieties are usually classified as semi-dwarf and are usually only 40-50% as tall as indeterminate varieties.

The indeterminate varieties have overlapping vegetative and reproductive growth stages. Terminal growth bud on the main stem continues to grow after the first bloom and most of the pods are on the main stem. Flowers and pods develop at different times and rates depending on node locations. Nodes with the earliest flowers located near the bottom of stem; therefore, an indeterminate plant may contain pods with developing seed at lower nodes while upper nodes contain only small pods or flowers (University of Missouri, 2010).

With soybean development being driven by photoperiod, most varieties have vegetative growth limited by the season length. Short day length and warm temperatures control soybean flowering. Soybeans must reach at least the first trifoliolate in growth before they can be induced to flower. However, even within a variety, variations in time of flowering may occur from year to year with the same day length closely associated with temperature conditions. Planting a specific variety elsewhere than its adapted maturity range will extend the period of vegetative growth, delay flowering and delay maturity due to the extended summer day length and cooler temperatures.

2.3. Origin and Distribution of Soyabean

Soybeans were a crucial crop in eastern Asia long before written records. They remain a major crop in China, Japan, and Korea. Prior to fermented products such as soy sauce, tempeh, natto, and miso, soy was considered sacred for its use in crop rotation as a method of fixing nitrogen. The plants would be ploughed under to clear the field for food crops. Soybeans did not become an important crop outside of Asia until about 1910.

Soy was introduced to Africa from China in the late 19th century, and is now widespread across the continent.The first introduction of soybeans in Africa was in Algeria which was initiated by the many Frenchmen whose interest was acclimatization of the soybean. The next record of cultivation of soybeans in Africa dates from 1903, when they were grown in South Africa at Cedara in Natal and in the Transvaal. The maximum yield that year was 1,031 kg/ha (Burtt-Davy 1910; Sawer 1911a). In about 1907 soybeans were introduced to Mauritius, a tiny island and British colony east of Madagascar, by P. Boname (Moutia, in Whigham 1975), and to

Tanzania, at that time a German colony, by German agriculturalists (Mmbaga, in Whigham 1975).

Starting 1908 there was a dramatic increase of interest in growing soybeans in Africa, as Europe for the first time began to import large quantities of soybeans from Manchuria in response to severe shortages and high prices of oil in Europe. European nations turned to their African colonies as potential areas for soybean cultivation. English colonies were most actively involved. By 1908 soybeans were being grown on a small scale in Nigeria and in the Belgian Congo. Soybeans were first grown in Ghana in 1909 (Snow 1961). In the summer of 1910, Sir Alfred Jones shipped soybeans to West Africa for culture trials. A.G. Turner, who was entrusted with a special mission to encourage soybean cultivation in West Africa, later reported that they could be successfully grown throughout Gambia, Sierra Leone, Nigeria, and the Gold Coast (Ghana), but that the yields from the first experiments had been only 400-540 kg/ha (6-8 bu/a). Later results, however, were "phenomenally successful" (Sawer 1911a), as soybeans were grown in all these areas and in Mauritius. During World War I additional soybean trials were done in the Belgian Congo (today's Democratic Republic of Congo). During the 1920s soybeans were first introduced to Egypt, Zimbabwe (then Rhodesia), and Rwanda. In 1938 they were introduced to Uganda

The earliest known report of soy foods in Africa dates from the early 1930s, when Catholic missionaries organized soymilk production in Zaire (at that time the Belgian Congo). The earliest known commercial soy food in Africa was soy flour introduced in South Africa in 1937 by a well known milling company and used by a number of gold mines on the Rand to fortify the diets of mine workers.

Starting in about 1973 there was a rapid rise of interest in soybeans and soy foods in Africa, paralleling the new interest worldwide. The two major reasons for this strong interest in Africa were the sudden rise in world soybean prices and the work of the International Soybean Program, INTSOY, headquartered at the University of Illinois. INTSOY's soybean variety trials, starting in 1973, led to the rapid development of soybean varieties that yielded well under African growing conditions, as tested by co-operators in various African countries. For the first time in history, with yields and

prices high, and rising domestic interest in food uses, it made economic sense for African farmers to grow soybeans.

However, two major problems were encountered in trying to introduce it as a food at household level: the grain took too much time and fuel to cook and the taste was not well accepted.

2.4 Soyabean Nutrient Requirements

Soil fertility is the most critical aspect in soyabean production. Soyabean performs well in well drained sand-loamy soils. Usherwood (1998) described a highly productive soil as also a fertile soil. The macro nutrients needed by soyabean are nitrogen phosphorous and potassium. The micro nutrients include sulphur, boron, magnesium and calcium. Nitrogen can jump start soyabean seedlings. It is supplied by the symbiotic bacteria in the nodules at about two weeks from planting. Too much of nitrogen can reduce nodule effectiveness. Phosphorus is vital for high yield. While potassium needs are greatest during early pod filling. Potassuim might be applied with phosphorus at pre-plant or to the previous cereal crop, (Usherwood, 1998). Usher (1998) further reported that sulphur is essential for plant protection. Except for very sandy soils, sulphur need of soyabean could be applied to the previous small grain crop. Boron on the other hand promotes new tissue growth on the plants and also helps transfer sugars from leaves to seed storage. It can be applied at early pod set.

However, the availability of these nutrients to the plants is limited by many factors and Al is one of the most limiting factors. Excess soluble/available aluminum (Al^{+++}) is toxic to plants and causes multiple other problems. Some of the more important problems include direct toxicity, primarily seen as stunted roots, reduces the availability of phosphorus (P), through the formation of Al-P compounds,reduces the availability of sulfur (S), through the formation of Al-S compounds and reduces the availability of other nutrient cations through competitive interaction, (Agronomic Library, 2004).

2.5 Chemical Nature of Aluminium

In the high rainfall areas, the soils get leached. In most instances, the soil becomes acidic. In such soils aluminium phytotoxicity becomes one of the major problems.

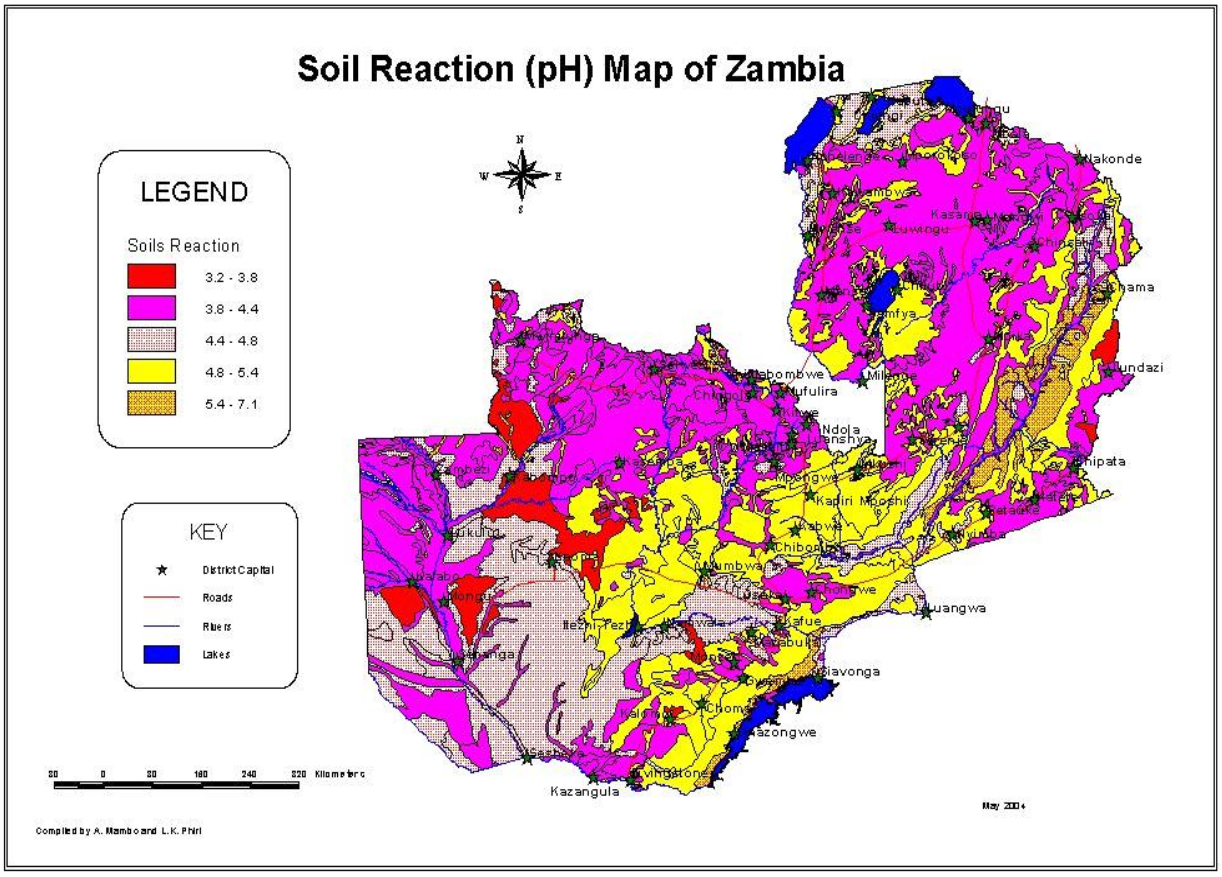


Figure 1: Map of Zambia Showing Soils' reaction

As shown in the map in Figure 1, a large portion of Zambian land has acid soils.

Al is a major constituent of the soil. Most aluminium in the soil is present as solids in non-toxic forms at pH >5.5, but only when it becomes soluble or goes into its exchangeable form can it affect the plant. Exchangeable aluminium concentration maybe high in soils with pH below 5.5, but may occur at pH values as high as 6.0 in heavy textured soils (Matsumoto *et al*., 2001). Factors such as predominance of clay soils, organic matter levels, concentration of cations, anions and salts, species of the plant being considered, affect the critical pH at which aluminium becomes exchangeable in toxic concentrations.

In water solutions, alumnium always forms octahedral coordination with some combination of the water molecules and hydroxyl ions, much as it does with six oxygen or hydroxyls in clay minerals. aluminium's natural coordination is with six of

these size molecules (octahedral coordination) because the size of hydroxyls, oxygen, and water in this bonding is similar. If the soil is not too strongly acid, one or more of the water molecules ionize, releasing hydrogen (H^+) to the solution, increasing the solution acidity. According to Pineros *et al.*, 2002, this soluble form of aluminium is the one which influences biological systems.

Critical levels of Al in alfalfa pot experiment done by Kollmeir *et al.*, 2000, increased as the soil organic matter level increased from 6.6 to 81.6 Kg/l. This shows that organic matter in the soil can reduce the toxicity of aluminium to the plants.

Certain crops such as tea, pineapple, blueberries tolerate a strong acidity and grow well. In contrast, certain plant crops such as sugar beet, barley, beans and cowpea only do well in slightly acidic to moderately basic soils because of a high calcium demand or inability to tolerate soluble aluminium.

There are various forms of potentially available aluminium, such as the relatively non-phytotoxic amorphous precipitates [Al_2SiO_5,$Al(OH)^{3+}$,and $AlPO_4$] and organically complexed forms of aluminium or the potentially toxic inorganic monomers [Al^{3+},$Al(OH)^{2+}$,$Al(OH)^{2+}$ that occur in the various mineral components of the soil, (Kinride, 1991).

The degree of soil acidification caused by leaching of alkaline metals such as Na^+, K^+, Ca^{2+} and Mg^{2+} from the soil and a decrease in pH of soil solution, intensifies the process of aluminium compounds solubilisation. Ma, 2000 reports that alumnium ions translocate very slowly to the upper part of the plant.

2.6 Effects of Aluminium on Plants

2.6.1 Negative Effects

2.6.1.1 Alumnium Toxicity Symptoms in Plants

Al toxicity is considered to be a complex of nutritional disorders of growth and development of plants, which may be manifested as a deficiency of essential nutrients like calcium, magnesium, iron or molybdenum; decreased availability of phosphorus or as toxicity of Mn and H+ (Alam and Adams, 1980; Foy, 1984; 1988; 1992;

Kamprath and Foy, 1985; IRRI, 1974; Clark *et al*., 1981; Furlani and Clark, 1981; Foy and Fleming, 1982).

The primary response to aluminium stress in plants occurs in roots, as reduced elongation at the tip, followed by swelling and distortion of differentiated cells, as well as root discolouration (Foy *et al*., 1978; Bergmann, 1992; Hossain *et al*., 2005). Aluminium toxicity inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poorer plant growth and yield (Alam, 1981; Clarkson, 1966; Foy, 1983; Foy *et al*., 1967; Gauthier, 1953; Reid *et al*., 1969; 1971).
Shoots of soyabean plants are also inhibited due to limiting supply of water and nutrients. Aluminium toxicity caused Ca deficiency or reduced Ca transport within the plant by curling or rolling of young leaves, inhibited growth of lateral branches or a collapse of growing points or petioles. Young seedlings are affected more than older plants (Thaworuwong and van Diest, 1974).

Aluminium toxicity is associated with an increased vacuolation and turnover of starch grains (de Lima and Copeland, 1994), as well as disruption of dictyosomes and their secretory function (Bennet *et al*., 1985; Puthota *et al*., 1991). Relative shoot and root dry weights in tolerant barley cultivars were two-fold and three-fold respectively compared to susceptible cultivars (Foy, 1996).
Aluminium toxicity decreases drought-tolerance and the use of subsoil nutrients Gallardo *et al*.(1999) reported 50% and 30% reduction of grain yield, respectively for sensitive and tolerant cultivars of barley when they were grown in naturally acid soil (pH 4.9) with a large amount of extractable aluminium compared to that grown in non acid soils.

2.6.2 Beneficial Effects of Aluminium

In as much as aluminium has many negative effects on plant growth, it has some beneficial effects if in low quantities. Some of these benefits are growth stimulation and inhibition of pathogens.

Not surprisingly, aluminium addition has a growth stimulatory effect on aluminium accumulators. In tea, addition of aluminium and phosphorus increased phosphorus absorption and translocation as well as root and shoot growth (Konishi *et al*., 1985 and

1992). Similarly, Osaki *et al*., 1997, reported that the Al-accumulating shrub, Melastoma malabathricum L., exhibited increased growth of leaf, stem, and roots as well as increased phosphorus accumulation when alumnium was added to culture solutions.

Low levels of aluminium sometimes stimulate root and shoot growth of non accumulators. Kinraide *et al*.,1990, reported that Turnip (Brassica rapa L. subsp. campestris A.R. Clapham) root lengths were increased by increasing aluminium levels up to 1.2 µM at pH 4.6. In Soybean, Rufty *et al*, 1995, observed that root elongation and $^{15}NO_3^-$ uptake increased with increasing aluminium concentrations up to 10 µM, but were reduced when aluminium levels increased further to 44 µM. Keltjens *et al*, 1990, also reported on Douglas fir (Pseudotsuga menziesii Franco) seedlings that the shoot and root growth of seedlings were stimulated by increasing aluminium levels up to 150 µM but were reduced at higher aluminium levels. Root elongation of an aluminium-tolerant race of silver birch (Betula pendula Roth) increased as solution aluminium increased up to 930 µM aluminium but then decreased at 1300 µM Aluminium (Kidd *et al*,.2000). Several researchers (Kinraide *et al*., 1990, Keltjens *et al*., 1990 Kinraide *et al*., 1992 and Lazof *et al*.,1999) have hypothesized that low levels of Al^{3+} ameliorated the toxic effects of H^+ on cell walls, membranes, or nutrient transport, but aluminium-toxic effects predominated at higher Al levels.

Aluminium can be toxic to pathogenic microorganisms, thus helping plants to avoid disease. Meyer *et al*., 1994, reported that spore germination and vegetative growth of the black root rot pathogen, *Thielaviopsis basicola* Ferraris, were inhibited by 350 µM aluminium at pH 5. Similarly, mycelial growth and sporangial germination of potato late blight pathogen, *Phytophthora infestans*, were inhibited by 185 µM aluminium, and Andrivon (Andrivon, 1995) speculated that amendment of soils with Al might be used as a means of disease control.

2.7 Aluminium Tolerance Mechanism

Jones and Ryan, 2003, reported that plant species can vary in their ability to grow in acid soils with severe Al phytotoxicity. Aluminium injury to barley was characterized by a decrease in root length with a reduction in the number of lateral roots as aluminium concentration in the nutrient solution increased Reid *et al* (2001). Rangel *et*

al. (2007) also reported that the reduction in root length exposed to different Al concentrations varies among varieties of the same species depending on level of tolerance to aluminium concentration. A recent study by Munyinda, *et al.,* 2008 also showed a reduction in the root length of common beans as well as number of lateral roots as aluminium concentration was increased.

Rangel *et al* 2007 carried out a study on beans which showed that a reduction in root growth when sown in high aluminium concentration. A study by Munyinda *et al.,* 1986 on wheat also showed that Al treatment reduced the taproot length but increased the lateral roots as the aluminium concentration increased in the nutrient solution. However, there was no differential effect with shoot length across aluminium concentrations.

These several strategies that various plants use to tolerate aluminium phytotoxicity can be grouped into two categories: (1) external resistance mechanisms, by which aluminium is excluded from plant tissues, especially the symplastic portion of the root meristem; and (2) internal tolerance mechanisms, conferring the ability of plants to tolerate Al ion in the plant symplasm where aluminium that has permeated the plasmalemma is sequestered or converted into an innocuous form (Kochian, 1995).

2.7.1 External Tolerance Mechanism (Exclusion)

The possible external resistance or exclusion mechanisms of aluminium tolerance are: immobilization of aluminium at the cell wall or low cell wall cation exchange capacity, selective permeability of the plasma membrane, formation of a plant induced pH barrier in the rhizosphere or root apoplasm, exudation of chelate ligands, exudation of phosphate, and Al efflux (Kochian, 1995; Taylor, 1991), Al^{3+}-induced changes in the membrane protein, and ATPase activity of the microsomal membrane function (Matsumoto *et al*., 1992; Wagatsuma *et al*., 1995). A metabolism-dependent exclusion of aluminium from root apical meristem has been described, which involves inhibition of aluminium accumulation in root tips (Rincon and Gonzales, 1992). Foy (1996) also reported that when barley plants were grown at pH 4.4, the accumulation of aluminium and phosphorus in shoots of susceptible cultivars were three times and two times higher, respectively, than in that of the tolerant cultivars. Accumulation of such high

concentrations of aluminium and P in the aerial parts of the plants are considered to be toxic to growth and development of the plants.

2.7.2 Internal Tolerance Mechanisms (Inclusion)

The internal resistance mechanisms are those where the plant takes up the aluminium, but the aluminium is converted in a form which is not harmful to plants. They operate within the symplasm and are mediated at the cellular level either by detoxification or immobilization of alumnium ions that have penetrated into plant cells (Taylor, 1995). Some organic acids form a stable complex with Al^{3+}, thereby preventing binding of aluminium with intra- and intercellular compounds in roots. Ishikawa *et al*., 2000 and Kochian *et al*., 2005 suggested that the release of various di- and tricarboxylic acids can form strong complexes with aluminium has lead to various studies attempting to show that plants use this as a defense mechanism against aluminium toxicity (Ishikawa *et al*., 2000; Kochian *et al*., 2005). Exudation of organic acids, mainly citric and malic acids appears to be one of the main mechanisms for aluminium-tolerance (Carver and Ownby, 1995; Gallardo *et al*., 1999; Ishikawa *et al*., 2000).

2.8 Screening Methods

Genetic improvement of crops for acid soil tolerance has been accelerated by the availability of screening criteria for detecting aluminium-tolerance. These include field and laboratory techniques.

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. This involves the use of solution culture method and use of soil bioassay. Nutrient solution culture is the most common screening medium for aluminium tolerance, which provides an easy access to root systems, tight control over nutrient availability and pH, and non-destructive measurement of tolerance (Carver and Ownby, 1995). The use of soil media has received less attention than solution media because of the complications of creating a soil environment with a specific type and amount of phyto-toxicity (Foy, 1976). Mitigating effects of other nutrients (e.g., Ca, P, or Mg) or organic matter must be considered as well as other factors like variability at the soil collection site, time of collection, and soil storage condition (Scott and Fisher, 1989).

The hematoxylin stain has also proved to be useful in determining the aluminium tolerance of plants (Polle *et al.*, 1978). It requires less time and simpler pH management than other methods and is very useful for selection or screening a relatively large population in breeding program. In this case, roots grown in aluminium solution for a period of a day or more are stained and those whose root tips develop a purplish color are designated as being sensitive to the particular concentration of aluminium used in the growth solution. Hematoxylin binds Al to produce a purple complex and the absence of the color in root tips of the aluminium-tolerant genotypes indicates that these genotypes either exclude the Al or bind the aluminium in complexes that are unavailable to hematoxylin.

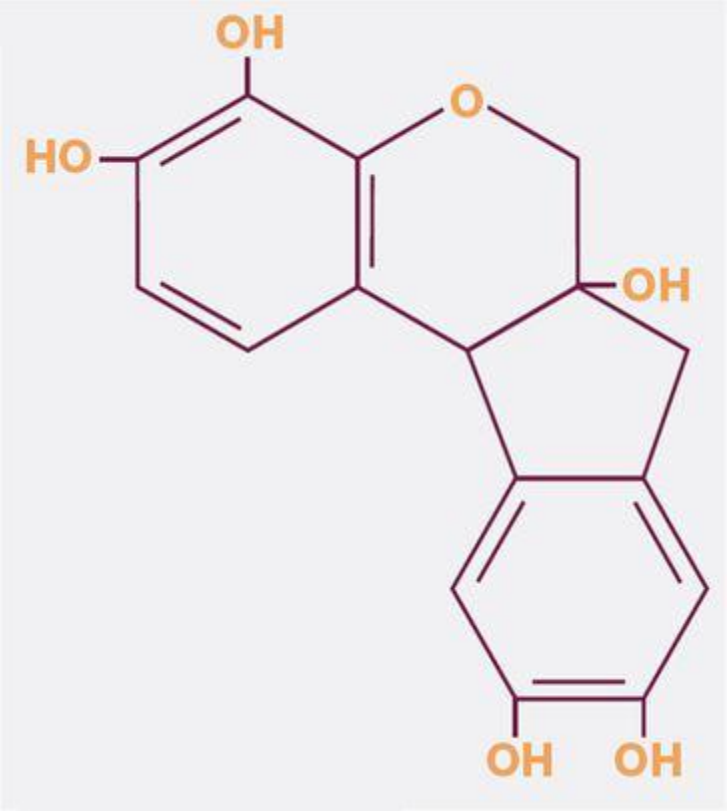


Figure 2: Chemical structure of Hematoxylin

Russel Myers reported in 2011 that to produce a functional dye, hematoxylin is oxidized to hematein and subsequently is bound to one of several metal ions including aluminum (Al^{+3}), iron (Fe^{+3}) and chromium (Cr^{+3}). He further said that the change in structure results in the conversion of the relatively colorless hematoxylin to the

reddish/brown hematein. In addition, the oxidation to hematein is necessary in order to bind metal ions such as aluminum.

Most evidence, however, supports the concept that hematein-Al^{+3} complexes are positively charged or "cationic" at most staining conditions.

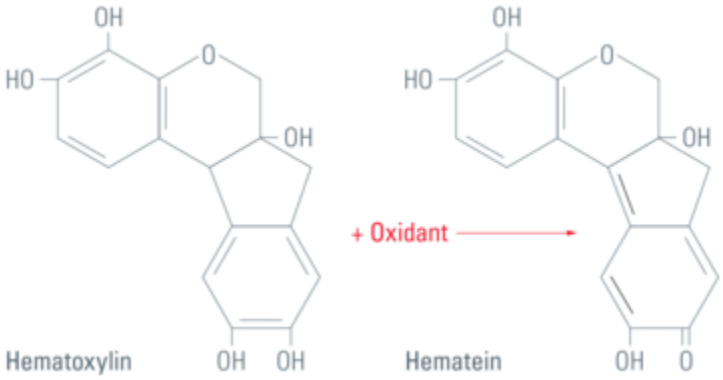


Figure 3: Oxidation of Hematoxylin

Species where screening for Aluminium under defined conditions in the Laboratory or glasshouse has successfully resulted in the development of genotypes with increased tolerance to acid soils include wheat, maize, soybean, phalaris, and barley.

Field-based screening techniques are more laborious, time consuming and expensive. Despite these demerits, it would be the best approximate for selecting aluminium-tolerant plants. In practice, however, reliable ranking of tolerance in the field screening is difficult because the aluminium concentration in soil may not be uniform because environmental factors interact with soil aluminium to mask expression of aluminium-tolerance (Naserian *et al.*, 2007). Screening by using the growth response to aluminium added to the soil in pots in greenhouse (referred to as growth response method hereafter) maybe superior to this respect.

Choice of a particular screening test is influenced by the kind of material under selection, i.e., germplasm collections for identifying suitable parents, segregating populations, or advanced breeding lines under consideration for release. Therefore, it is important to compare the laboratory screening methods with the field screening methods.

2.9 Diagnosis of Aluminium Toxicity in Plants

Diagnosis of aluminium toxicity can be done in a number of ways some of which are laboratory, hematoxylin, molecular markers and field screening. Soil Exchangeable aluminium concentration is used as a guide to the likelihood of aluminium toxicity. Wenzl *et at*., in 2002 reported that each aluminium levels greater than 15mg/kg may be a problem and above 50mg/kg toxic, in which case the economics of liming should be considered to overcome this problem.

Matsumoto *et al.* (2001) reported that the diagnosis of aluminium toxicity from visual signs in plants is unreliable and critical plant concentrations of aluminium are ill-defined. The aluminium concentration in leaves of Lucerne is of little value in determining toxicity.

There are several screening methods for aluminium-tolerance such as solution, sand and soil cultures, root re-growth and hematoxylin staining techniques, and field screening. However, reliable ranking of tolerance in the field screening is difficult because of the temporal and spatial variation in acid soils. Moreover, screening at field level is very expensive and time-consuming when a large number of genotypes are under evaluation (Garcia et al., 1979).

CHAPTER 3

3.0 MATERIALS AND METHODS

The study comprised of field and laboratory experiments. Twenty already released varieties were used and these varieties were obtained from Seedco, Maize Research Institute (MRI) and Zambia Seed Company (ZAMSEED) private seed companies. The genotypes and their names used in the research are as in Table 1.

3.1 Laboratory Experiment

The laboratory experiment was conducted at the University of Zambia, School of Agricultural Sciences, Plant Science Laboratories. It was carried out in two phases. The first phase was the determination of the discriminatory level of aluminum and second was the screening of all the twenty genotypes for aluminium tolerance.

3.1.1 Determination of Discriminatory Level

In order to establish the injurious level of aluminium concentration in the solution which will clearly discriminate between tolerant and susceptible genotypes, five genotypes were selected randomly from the twenty genotypes and tested in six different aluminium concentrations. The aluminium concentrations were 0mg/L, 4 mg/L, 8 mg/L, 12 mg/L, 16 mg/L and 20 mg/L.

Fifteen to twenty seeds from each genotype were germinated in tightly covered petri dishes for three days. The petri dishes were lined with filter papers which were moistened with distilled water.

Seedlings of uniform root length were selected and transferred to 55 ml test tubes nutrient solutions of varying aluminium concentration I.e. 0, 4, 8, 12, 16 and 29mg/L as described by Kerridge *et al* (1971).

Table 1: List of Soyabean genotypes evaluated

S/N	Variety Name	Source
1	Saga	Seedco
2	Semeki	Seedco
3	Samba	Seedco
4	Sovereign	Seedco
5	Spike	Seedco
6	Squire	Seedco
7	Soprano	Seedco
8	Sequel	Seedco
9	S810/6/10	Seedco
10	Safari	Seedco
11	Satelite	Seedco
12	Score	Seedco
13	Sirocco	Seedco
14	Scribe	Seedco
15	Magoye	ZAMSEED
16	Lukanga	ZAMSEED
17	Hernon 147	ZAMSEED
18	Hernon 147B	ZAMSEED
19	Dina	(MRI)
20	Kaleya	ZAMSEED

ZAMSEED=Zambia Seed Company, MRI=Maize Research Institute

The solution provided in mg/L, 42.61 nitrogen, 2.0 phosphorus, 23.5 potassium, 48.1 calcium, 14.6 magnesium 0.32 boron, 0.03 chlorine, 0.06 copper, 0.02 sodium, 0.03 manganese, 0.03 molybdenum and 0.16 zinc. The pH of the solution was adjusted to 4.2 and left unadjusted thereafter. Four seedlings were selected per treatment per genotype/variety.

The Completely Randomized Design was used with three replications for each genotype. One seedling was placed in each test-tube and left to grow in the green house for ten days. The seedlings were supported over the nutrient containing test-tubes by polyethylene stoppers. The test-tubes were covered with black polyethylene bags to prevent algae from growing in the test-tubes. The test-tubes were placed on racks and then taken to the green house. The test-tubes were aerated daily. The solution was changed after five days from the time of transplanting. After ten days, taproot length (TRL) and shoot length (SL) were taken using a thirty cm rule. The plants were cut into two; the root and shoot parts, parked in small envelops and taken to the oven to dry to a constant weight. The shoot (SB) and root biomass (RB) were measured.

3.1.2 Screening of Genotypes

The twenty genotypes were tested with levels of Aluminium concentration which were 0mg/L and 20mg/L. The procedure followed was as shown in determination of discriminatory level experiment above.

3.1.3 Hematoxylin Test

This test was also used for screening of genotypes for tolerance to Aluminum toxicity. The twenty genotypes were grown in the two levels of Al; 0 mg/L and 20 mg/L as above. After two days of exposure to aluminium, the roots were stained with two to three drops of hematoxylin. Based on this screening, genotypes were grouped into three categories: tolerant (clear stains), moderate tolerant (moderate stain) and susceptible (full stain) genotypes.

3.1.4 Determination of Amount of Aluminium in the Plant Tissue

Since the screening of genotypes would only determine the tolerant and susceptible genotypes, the determination of amount of aluminium in the plant tissue was carried to determine the mechanism of aluminium tolerance which the tolerant genotypes used.

Twelve genotypes from the twenty genotypes were selected randomly. Four from each of the three groups of hematoxylin stain i.e. susceptible, moderate and tolerant groups. These genotypes were grown in grown in growth media with two levels of aluminium which were 0 mg/L and 20 mg/L. After five days, the plants were cut to separate the shoots from the roots. The cut roots were then analyzed for the amount aluminium in them. The cut shoots and roots were put in envelops and taken to the oven to dry at 80^{o}C to a constant weight.

3.1.4.1 Dry Ashing

The dried plant parts were weighed then put on clean thirty milliliters crucibles. These organic plant materials were then ashed in a furnace at 500^{o}C for two hours. After cooling, the ashes were digested in 20 mL of 0.1M nitric acid, HNO_3. The digests were then put in 100 mL containers and filled to 100 mL mark with distilled water.

3.1.4.2 Determination of Aluminium

Amount of aluminium that remained in the growing solution, that in roots and shoots solutions were determined. Ten mL from each sample solution was collected and the amount of Aluminium in it was determined by Atomic Absorption Spectrometry (AAS).

The following calculation was used to determine the amount of aluminium in the sample ;

mg of Al kg^{-1} = concentration*dilution factor/ weight of sample

3.2 Field Experiment

The field experiment was carried in order to confirm if the laboratory results will be repeatable under field conditions, and also to determine which field parameters can be used to select for aluminium-tolerance in the field. The field experiment was conducted at Liempe University of Zambia Farm and at Seedco Lusaka West Research Farm.

The twenty genotypes were planted at both sites with 100 kilograms per hectare seed rate in four replications in a Randomized Complete Block Design.

Each plot had six rows of five meters long with 45 centimeters between rows. The total experimemtal area resulting to 0.378 ha.

At both sites, 300 kilograms per hectare of soya mix was applied at time of planting. The seeds were inoculated with a Glycinmax innoculant. The parameters which were measured at both sites include;

- Days to flowering
- Days to maturity
- Days to shutter
- Number of pink root nodules
- Number of non-pink root nodules
- Disease scoring
- Plant height
- Root biomass
- Number of pods
- Grain weight
- Yield.

Days to Flowering (DF_{50})

This was taken as the number of days from the date of planting to the date when fifty percent of the plants in a plot flowered.

Days to Maturity (DM)

This was taken as the number of days from the date of planting to the date when ninety-five percent of plants in the plot had their pods dried.

Days to Pod Shattering (DS)

This was taken as the number of days from the date of maturity to the date when three of the plants in a plot started pod shattering.

Number of Root Nodules per Plant (RN)

This was taken a week after flowering date. Four plants per plot were pulled from the ground and root nodules were counted. The number was averaged and recorded.

Number of Pink Root Nodules Per Plant (PRN)

This was taken a week after flowering date. Four plants per plot were pulled from the ground and root nodules were bisected to check for those that had a pink colour. The pink root nodules were counted for each plant and the average of the four plants was taken and recorded.

Disease Score

Disease score was done per plot using a scale of one to five where one implies no disease found and five denotes the whole plot was infested with the disease.

Plant Height (PH)

This was taken as the average height, from the ground to the top most part of the plants, per plot in centimeters using a meter rule. This was taken after maturity.

Number of Pods per Plant (PP)

This was taken after maturity by counting the number of plants for four representative plants per plot and the average of the four was taken.

Root Biomass (RB)

This is the mass of dried roots. Four representative plants were selected, cut at the base and an augor was used to pull out the roots. These roots were then washed on a sieve, packed in envelops and oven dried. Weights were taken when the roots attained a constant dry weight. Then the weights were averaged.

Grain Weight (GW)

This was taken after harvest as the weight of the shelled grains per plot in kilograms.

Yield (YLD)

This was calculated by converting the GW to tones per hector.

CHAPTER 4

4.0 RESULTS

Significant differences were observed for most of the parameters which were measured and / or derived in the laboratory and field analyses of variance as shown in tables 2 and 18.

4.1 Laboratory Experiment

4.1.1 Determination of Discriminatory Level of Aluminium

For most of the parameters which were measured and/or derived in the laboratory analysis, significant differences were observed during the determination of the discriminatory level of aluminium as shown by Table 2. Significant differences were seen in genotypes and aluminium concentrations at $P \leq 0.001$. This implies the different genotypes tested in this experiment responded differently at different levels of aluminum. While genotype by aluminium concentration interaction showed no significant differences as shown by Table 2, indicating that the genotypes did not react similarly at different levels of aluminium.

4.1.1.1 Tap Root Length

Significant differences were observed in genotypes and aluminium concentrations at $P \leq 0.05$ as can be seen in Table 2.

4.1.1.2 Root Biomass

Genotype, aluminium concentrations and genotype by aluminium concentrations showed significant differences at $P \leq 0.05$ as indicated by Table 2. Sovereign had the highest root biomass of 0.31g and the lowest were Semeki and Safari with 0.08g as shown by Table 3.

4.1.1.3 Shoot Length

Aluminium concentration levels were highly significant at $P \leq 0.001$ while genotypes were significantly different at $P \leq 0.01$. Genotype by aluminium concentration levels

interaction was not significant as shown by Table 2. The highest Shoot Length was observed in Soprano, 12.90 cm, and the lowest in Spike, 10.40cm, as shown in Table 3.

4.1.1.4 Shoot Biomas

Highly significant differences at $P \leq 0.001$ were seen among genotypes and also due aluminium concentration levels while genotype by aluminium concentration levels were not significantly different. Sovereign showed the highest weight of 0.93g and the lowest was Spike with 0.73g as shown in Table 3.

4.1.1.5 Aluminium Concentration Levels' Means

Table 4 shows that there was a general tendency of reduction in most of the parameters taken such as Root Biomass, Shoot Biomass, Shoot Length and Tap Root Length. As the aluminium concentration level increased, the figures for each parameter the parameters mentioned reduced.

The 0mg/L level had the highest root biomass of 0.25g followed by 4mg/L with 0.24. The lowest root biomass of 0.08g was seen in concentrations 16 and 20 mg/L. Shoot length was highest at 0mg/l with 15.15 cm, followed by 4mg/L with 15.20cm. The Shoot length was seen at aluminium concentration level 20mg/L. Table 4 shows that shoot biomass decreased from 1.16g to 0.54g as aluminium concentration level increased from 0 to 20 mg/L.

The reduction rate increased for root biomass, shoot length and tap root length from zero to twelve mg/L. The reduced more at sixteen mg/L after which the reduction lessened.

Table 2: Analysis of Variance for the parameters measured and/or derived from five genotypes evaluated in the Plant Science Laboratory at the University of Zambia

Sources of Variation	DF	TRL	SL	SB	RB
		M.S	M.S	M.S	M.S
Genotype (G)	4	16.81***	17.66**	0.10***	0.16*
Al conc (Al)	5	33.24***	96.61***	0.80***	0.15*
G*Al	20	1.03ns	2.45ns	0.01*	0.10*
Error	60	2.85	3.99	0.004	0.05
CV		28.80	16.6	8.00	13.90
Std Error		1.69	2.00	0.07	0.21

***=Significant at P=≤0.001, **Significant at P=≤0.01, *= Significant at≤0.05, ns=non significant, Std = Standard, CV= Coefficient of Variation, M.S=mean square

Table 3: Means for the parameters measured and/or observed from five genotypes evaluated in the Plant Science Laboratory at the University of Zambia

Genotype	RB	SB	SL	TRL
Semeki	0.08	0.87	12.55	5.09
Soprano	0.13	0.82	12.94	6.58
Sovereign	0.31	0.93	12.17	5.02
Safari	0.08	0.82	12.16	5.14
Spike	0.17	0.73	10.36	7.17
LSD	**0.83**	**0.42**	**0.15**	**0.12**

LSD=least significant difference, RB=root biomass, SB=shoot biomass, SL=shoot length, TRL=taproot length

Table 4: Aluminium concentration levels' means for the parameters measured and/or derived from five genotypes evaluated in the Plant Science Laboratory at the University of Zambia

Al Levels (mgL^{-1})	RB	SB	SL	TRL
0	0.25	1.16	15.18	7.72
4	0.24	0.99	14.20	6.92
8	0.23	0.89	12.72	6.50
12	0.11	0.79	11.88	5.73
16	0.05	0.62	9.34	4.33
20	0.05	0.54	8.89	3.93
LSD	**0.55**	**0.11**	**0.08**	**0.56**

LSD=least significant difference, RB=root biomass, SB=shoot biomass, SL=shoot length, TRL=taproot length

4.1.1.6 Effect of Aluminium Concentration on Shoot Biomass for Genotypes

Table 5 shows that Soprano had the highest percentage reduction in shoot biomass at four mg/L indicating that it was sensitive even at low levels on aluminium concentration. Safari on the other hand had the highest reduction percentage at twenty mg/L. Semeki, Sovereign and Spike had their highest reduction in shoot biomass at sixteen mg/L. Even though Semeki and Spike had their highest percentage reduction in shoot biomass at sixteen mg/L, Spike had highest percentage reduction, among the genotypes, in shoot biomass of 37.47 at 16 mg/L alumuinium while the lowest percentage of 13.33 was at 12 to 16mg/L in Semeki which was not very different from Soprano with 13.84 percent. This implies that Spike was the most affected genotype by aluminium among the genotypes and Semeki the least affected. Hence sixteen mg/L was chosen to be the most discriminatory level of aluminium. Although this was the case, it was noticed that there was still reduction in most parameters at twenty mg/L. Therefore, 20 mg/l was used in this experiment for effective discrimination.

Table 5: Percentage Decrease of Shoot Biomass from aluminium Level to another for five Genotypes

	GENOTYPES									
	SEMEKI		**SOPRANO**		**SOVEREIGN**		**SAFARI**		**SPIKE**	
AL CONC	**SHOOT(g)**	**% REDUCTION**	**SHOOT(g)**	**% REDUCTION**	**SHOOT(g)**	**% REDUCTION**	**SHOOT(g)**	**% REDUCTION**	**SHOOT(g)**	**% REDUCTION**
0	1.14		1.13		1.23		1.18		1.13	
4	1.09	4.39	0.97	**13.84**	1.12	8.68	1.00	14.69	0.88	21.62
8	0.99	9.17	0.87	10.59	1.04	6.62	0.88	12.61	0.80	9.05
12	0.90	9.09	0.80	8.05	0.94	9.96	0.73	16.70	0.70	12.52
16	0.78	**13.33**	0.73	8.75	0.65	**30.83**	0.63	13.40	0.44	**37.47**
20	0.76	2.56	0.63	13.70	0.60	8.20	0.49	**22.05**	0.42	5.08

4.1.2 Screening of Genotypes

4.1.2.1 Tap Root Length

Based on the analysis of variance shown in Table 6, highly significant differences at P ≤ 0.001 where seen among genotypes, aluminium concentration levels and Genotype by aluminium concentration interaction.

The means in Table 7, among the genotypes, at 0mg/L, Sovereign, Hernon 147B and Spike had the highest taproot lengths of 31.15, 28.83 and 26.02 cm respectively. The lowest were Squire, 2.73 cm, and Satelite with 9.35cm. At 20 mg/L Semeki, Spike and Squire had the highest taproot lengths of 22.90, 19.53 and 18.17cm respectively. While the lowest taproot lengths at this level were 5.38, 6.37 and 6.40 cm observed in Lukanga, Satelite and S810/6/10 respectively.

4.1.2.2 Lateral Root Length

Genotype, aluminium concentrations and genotype by aluminium concentration interaction were highly significantly different at P ≤ 0.001 as shown in Table 6. Lukanga and Saga had the highest lateral root lengths at 0mg/L with 1.012 and 1.00cm respectively as can be seen in Table 7. The lowest at this level were 0.05, 0.48 and 0.57 cm observed in Hernon 147B, Semeki and Sirocco in the same order. The highest taproot lengths at 20mg/L were observed in Samba and Magoye with 0.88 cm each while the lowest were observed in Squire and Hernon 147B with 0.03cm each.

4.1.2.3 Number of Lateral Roots

Table 6 shows high significant differences among genotypes, aluminium concentration levels and genotype aluminium concentration levels at P ≤ 0.001.

In comparison with other genotypes, the means in Table 7 shows that Spike, Semeki, and S810/06/10 had the highest number of lateral roots at 0mg/l of 55, 47 and 38

respectively, while the lowest at this aluminium level were Sequel, Hernon 147B and Samba with 9, 10 and 11 respectively. At 20 mg/L, the highest numbers of lateral roots were 61, 58 and 51 observed in genotypes Spike, Dina and Squire respectively.

4.1.2.4 Shoot Length

Highly significant differences were observed among genotypes, between aluminium concentration levels and genotypes by aluminium concentration levels at $P \leq 0.001$ as Table 6 shows.

Hernon 147B, S810/6/10 and Safari had the highest shoot lengths at 0 mg/L of 16.63, 13.80 and 13.48 cm respectively as shown in Table 7. The lowest shoot lengths at this aluminium level were 3.03, 5.38 and 6.20 cm observed for Score, Sequel and Spike respectively. While at 20 mg/L, the highest shoot lengths were observed in Squire, 14.03 cm, Sovereign, 11.93 cm and Hernon 143B with 11.0 cm. The lowest at this aluminium level were Satelite, Sirocco and Spike with 4.20, 4.21 and 4.73 cm respectively.

4.1.2.5 Root Biomass

Genotypes showed highly significant differences in RB at $P \leq 0.001$ while Aluminium concentration levels and genotype by Aluminium concentration levels were not significantly different as shown in analysis of variance of Table 6.

Results in Table 7 shows that Lukanga, Saga and Dina had the highest root biomass at 0mg/L with 1.12, 0.96 and 0.90 g respectively, while the lowest at this aluminium level were 0.05, 0.46 and 0.57 g observed in genotypes Hernon 147B, Semeki and Sirocco respectively. At 20 mg/L, 0.88, 0.87 and 0.84 g were the highest root biomasses observed in genotypes Samba, Satelite and Saga, while the lowest at this Al level were 0.02, 0.03 and 0.30g observed in Soprano, Hernon 147B and S810/6/10 respectively.

4.1.2.6 Shoot Biomass

Highly significant differences were seen among genotypes, aluminium concentration levels and genotype by aluminium concentration levels interaction at $P \leq 0.001$ in Table 6.

Sirocco, Dina and Hernon 147B had the highest shoot biomass at 0mg/L,as shown in Table 7, with 2.31, 1.19 and 1.10g respectively, while at this same Al level the lowest shoot biomass were 0.51, 0.52 and 0.53g observed in Spike, Sequel and S810/6/10 respectively. At 20 mg/L, sirocco, Dina and Sovereign had the highest shoot biomasses of 2.15, 1.00 and 0.96 respectively, while the lowest shoot biomasses at this aluminium level were 0.21, 0.33 and 0.35g observed in genotypes Hernon 147B, S801/6/10 and Spike respectively.

4.1.2.7 Aluminium Concentration Level Means

As aluminium concentration increased from zero mg/L to twenty mg/L, there was a reduction in all parameters taproot length, lateral root length, shoot length, root and shoot biomass. While the number of lateral roots increased with the increase in aluminium concentration from zero to twenty mg/L as shown in Table 8.

At level zero mg/L, the tap root rength, lateral root length, number of lateral roots, shoot length and shoot biomass were 18.96 cm, 2.93 cm, 27, 8.46 cm, 0.81 g and 0.78 g respectively. While, at twenty mg/L, they were 12.77 cm, 1.72 cm, 34 , 6.81 cm, 0.66 g and 0.54 g in the same order.

Table 6: Analysis of Variance for the parameters measured from eighteen (18) genotypes evaluated in the Laboratory.

Sources of Variation	DF	TRL	LRL	# LR	SL	SB	RB
Genotype (G)	17	151.45***	19.88***	929.86***	47.611***	1.07***	0.38***
Al conc (Al)	1	1035.00***	36.45***	1341.58***	73.23***	0.62ns	1.59***
G*Al	14	55.41***	21.56***	187.08***	36.23***	0.09ns	0.16***
Error	64	4.92	1.19	12.64	1.23	0.32	0.05
CV		4.0	6.4	11.8	4.5	1.7	2.2
Std Error		2.22	1.09	3.56	1.11	0.92	0.21

*** = Significant at P ≤0.001, ** = Significant at P≤0.01, * = Significant at≤0.05, DF=degrees of freedom, CV= coefficience of variance, G*AL=genotype by aluminium interaction, ns = non significant, Std = Standard error, RB=root biomass, SB=shoot biomass, SL=shoot length, TRL=taproot length, LRL=lateral root length, #LR=number of lateral roots

Table 7: Means of parameters measured and/or derived from eighteen soyabean genotypes in the Laboratory

Genotype	TRL		LRL		# LR		SL		RB		SB	
Al **Level mg/L**	**0**	**20**	**0**	**20**	**0**	**20**	**0**	**20**	**0**	**20**	**0**	**20**
Satelite	9.35	6.37	0.91	0.87	34	24	7.20	4.20	0.93	0.87	0.59	0.40
S810/10/6	14.02	6.40	0.90	0.30	38	36	13.80	4.83	0.90	0.30	0.53	0.33
Saga	10.05	8.28	1.00	0.84	31	32	11.30	8.25	0.96	0.84	0.56	0.46
Safari	16.85	10.85	0.87	0.76	23	27	13.48	8.57	0.87	0.76	0.67	0.54
Semeki	25.95	22.90	0.48	0.46	47	32	6.98	6.39	0.46	0.46	0.77	0.89
Soprano	20.90	10.35	0.70	0.02	35	44	6.82	7.42	0.70	0.02	0.54	0.55
Sovereign	31.15	16.87	0.76	0.56	16	24	9.22	11.93	0.76	0.12	1.03	0.96
Sirocco	24.07	17.73	0.57	0.33	13	19	5.70	4.21	0.57	0.33	2.31	2.15
Spike	26.02	19.53	0.86	0.62	55	61	6.20	4.73	0.86	0.62	0.51	0.35
Score	19.27	11.93	0.79	0.84	12	16	3.03	8.55	0.78	0.84	0.94	0.75
Scribe	17.15	10.84	0.90	0.65	30	36	6.23	4.74	0.89	0.65	0.57	0.41
Squire	2.73	18.17	0.86	0.03	37	51	6.18	14.03	0.86	0.03	0.76	0.93
Samba	17.52	15.60	0.89	0.88	11	31	7.40	7.03	0.89	0.88	0.57	0.54
Sequel	22.70	12.60	0.85	0.84	9	14	5.38	7.83	0.85	0.84	0.52	0.39
Hernon 147B	28.83	8.58	0.05	0.03	10	31	16.63	11.0	0.05	0.03	1.10	0.21
Magoye	17.22	13.87	0.75	0.88	25	46	7.77	7.93	0.75	0.88	0.73	0.47
Lukanga	11.60	5.38	1.12	0.83	21	26	10.08	9.55	1.12	0.83	0.70	0.50
Dina	15.87	13.55	0.90	0.55	35	58	8.87	4.85	0.90	0.52	1.19	1.00
LSD	**0.56**		**0.26**		**0.41**		**0.28**		**0.25**		**0.33**	

LSD=least significant difference, RB=root biomass, SB=shoot biomass, SL=shoot length, TRL=taproot length, LRL=lateral root length, #LR=number of lateral roots

Table 8: Means of parameters measured and/or derived from nineteen soyabean genotypes in the Laboratory

Al levels (mgL^{-1})	TRL	LRL	# LR	SL	RB	SB
0	18.96	2.93	27	8.46	0.81	0.78
20	12.77	1.76	34	6.81	0.66	0.54
LSD (5%)	**0.85**	**0.42**	**0.37**	**0.42**	**0.08**	**0.11**

LSD=least significant difference, RB=root biomass, SB=shoot biomass, SL=shoot length, TRL=taproot length, LRL=lateral root length, #LR=number of lateral roots

4.1.2.7 Percentage Reduction in Shoot Biomas for Genotypes at Two Levels (0 and 20 Mg/L) of Aluminium Concentration

The highest percentage decrease in shoot biomass was 75.51 by Hernon 147B which appeared not very different from Spike with 74.42 followed by S810/6/10. The lowest percentage decrease in shoot biomass was seen in Semeki with 5.77 which was not so different from Samba with 6.74 followed by Saga with 12.50 which also was not different from Safari with 12.64 percent as shown in Table 9.

The results from the screening of genotypes in the laboratory showed that Semeki and Samba were tolerant, while Hernon 147B, S810/6/10 and Spike were susceptible to high levels of aluminium.

Table 9 : Percentage decrease in SB for genotypes Screened in the Laboratory under 0mg/L and 20mg/L

Genotype	0mg/L	20mg/L	% Decrease in SB
Satelite	0.91	0.72	20.88
S810/6/10	0.9	0.3	66.67
Saga	0.96	0.84	12.50
Safari	0.87	0.76	12.64
Semeki	0.52	0.49	5.77
Soprano	0.7	0.58	17.14
Sovereign	0.76	0.42	44.74
Sirocco	0.58	0.49	15.52
Spike	0.86	0.22	74.42
Score	0.79	0.61	22.78
Scribe	0.89	0.69	22.47
Squire	0.86	0.73	15.12
Samba	0.89	0.83	6.74
Sequel	0.85	0.64	24.71
Hernon 147B	0.49	0.12	75.51
Magoye	0.75	0.58	22.67
Lukanga	1.12	0.83	25.89
Dina	0.9	0.52	42.22
LSD		**0.33**	

LSD=least significant difference, SB=shoot biomass

4.1.3 Hematoxylin Test for Selection of Varieties Tolerant to High Levels of Aluminium

The hematoxylin Test was carried out as another way of screening the genotypes for tolerance to high levels of aluminium.

Different genotypes stained differently with Hematoxylin because they have different levels of tolerance to aluminium as can be seen in Figure 4.

Semeki, Samba, Scribe, Squire, Sirocco, Score, Kaleya and Saga showed a clear stain. Sovereign, Statelite, Safari, Sequel, Soprano, Lukanga, Dina and Magoye showed a moderate stain while S810/6/10, Spike an d Hernon 147 were fully stained as indicated by Table 10.

These results from the Hematoxylin test shows that Semeki , Samba, Squire, Kaleya and Saga were tolerant to high levels of auminium while Spike, Hernon 147 B and S810/6/10 were susceptible. This was in line with the results obtained under the screening of genotypes in the laboratory.

Clear Stain **Moderate Stain** **Full Stain**

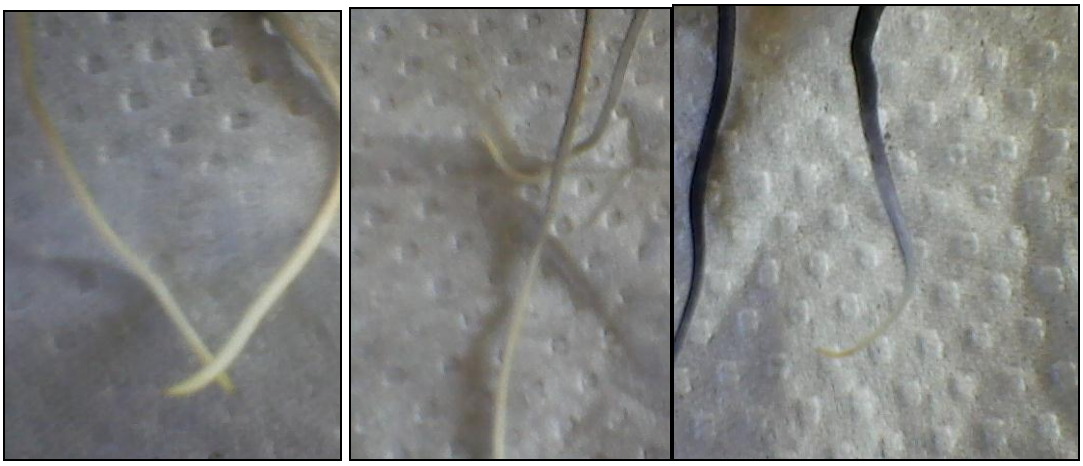


Figure 4: Picture showing Full, Moderate and Clearly Stained Root after Staining with Hematoxylin

Table 10 : Genotypes Screened in the Laboratory grouped according to Hematoxylin Stain Intensity

Genotypes	Stain Intensity		
	Full	**Moderate**	**Clear**
Samba			✓
Semeki			✓
Scribe			✓
Squire			✓
Sirocco			✓
Score			✓
Kaleya			✓
Saga			✓
Sovereign		✓	
Satelite		✓	
Safari		✓	
Sequel		✓	
Soprano		✓	
Lukanga		✓	
Dina		✓	
Magoye		✓	
S810/6/10	✓		
Spike	✓		
Hernon 147	✓		

4.1.4 Mechanism of Tolerance Determined by Determining Amount of Al in the Plant Tissue through Plant Tissue Analysis

Since the screening of genotypes in laboratory and the hematoxylin test only showed the genotypes tolerant and susceptible, plant tissue analysis was done to determine whether the mechanism of tolerance is Inclusion or Exclusion of aluminium.

Highly significant differences at $P \leq 0.001$ were observed in genotypes for mg of aluminium per kg of sample for root and shoot as shown in the analysis of variance Table 11.

Table 12 shows the amounts of aluminium in mg per kg of sample measured for root and shoot for each genotype. Hernon 147 had the highest amount of 549.22 mgAl /kg for root sample followed by Spike with 543.38 and S810/6/10 with 508.21 mg Al/ kg. While Samba had the lowest amount with 97.33 mg Al/kg of root sample followed by Semeki with 108.61 and Lukanga with 116.47 mg Al/ kg. The rest were intermediaries which ranged from 116.47 to 508.21 mg Al/kg.

Hernon 147 maintained the highest even for amount of Al / kg in shoot with 31.31 mgAl/ kg followed by Spike with 251.35 and S810/6/10 with 231.77 mg Al/kg. On the other hand, Samba also maintained the lowest with 28.08, followed by Sirocco with 38.10 and Semeki with 42.78 mg Al/kg for shoot sample.

The genotypes which were tolerant according to laboratory screening and Hematoxylin test, Samba and Semeki, had the lowest amount of Al both in the root and shoot. While those that were susceptible by the two experiments, Spike, S1810/6/10 and Hernon 147B, had the highest amount of Al in the shoots and roots.

The ratio of root to shoot, as indicated in Table 12, was highest in Sirocco with 3.52 followed by Magoye with 3.13. The lowest ratio was observed with Hernon 147 with 1.76 which was not significantly different from Soprano with 2.00.

Generally the ratio was more than one in all the genotypes, implying that the Al partitioning was high in the roots as Table 12 shows.

Table 11: Analysis of Variance for the mg of Aluminium per sample for twelve genotypes which underwent Plant tissue analysis

Sources of Variance	DF	ROOT	SHOOT
Genotype	12	88999.00***	26464.30***
Error	23	2852.00	689.40
CV		22.90	25.7
Std Error		53.40	22.26
LSD		9.20	4.44

***=Significant at P=≤0.001, **Significant at P=≤0.01, *= Significant at≤0.05, Std = Standard, CV=coefficient of variation, LSD=least significant difference, DF-degree of freedom

Table 12: Average amounts of Aluminium mg per Kilogram of the root and shoot samples for each genotype tested

GENOTYPE	ROOT	SHOOT	ROOT:SHOOT RATIO
SEMEKI	108.61	42.78	2.54
SATELITE	147.18	58.43	2.52
SPIKE	543.38	251.35	2.16
SOVEREIGN	151.83	60.12	2.53
SIROCCO	136.74	38.10	3.59
MAGOYE	197.36	63.04	3.13
HERNON 147	549.22	311.31	1.76
SAMBA	97.33	28.08	3.47
KALEYA	124.16	40.79	3.04
SOPRANO	133.56	66.65	2.00
LUKANGA	116.47	42.73	2.73
S810/6/10	508.21	231.77	2.19
LSD	9.20	4.44	

LSD=least significant difference

4.2 Field Experiment

The results from soil sample analyses from four different portions of Liempe and Lusaka West Farms show that Liempe Farm had higher soil aluminium of 0.44 meq/100g and a lower pH of 3.5 than Lusaka West farm with soil aluminium content of 0.11meq/100g and pH of 6.5as shown in table 13.

Table 13: Aluminium and pH levels for the Lusaka West and Liempe Farms

Site	Al^{3+} meq/100g	pH
Liempe	0.44	4.3
Lusaka West	0.11	6.5

The site specific analyses of variance for both Liempe and Lusaka West Farm as shown in tables 13 and 14 respectively, indicate that significant differences at $P \leq 0.01$ for parameters measured and/or derived in the field.

4.2.1 Grain Yield

The general observation shown by tables16 and 17 is that most varieties had high higher yields at Lusaka West Farm than Liempe Farm. The mean yield for Lusaka West Farm was 2.94 t/ha while Liempe Farm was 1.70t/ha.

Table 17 shows the top five genotypes with highest yields at Lusaka West Farm were Squire, Semeki, Safari, Satelite and Spike with 4.08, 3.94, 3.83, 3.62 and 3.60t/ha respectively. The lowest yields were observed in genotypes Scribe, Dina, Score, Magoye and Sequel with 2.33, 1.97, 1.87, 1.82 and 1.75t/ha respectively. The rest were intermediaries.

Highest yields at Liempe Farm were observed in genotypes Safari, Semeki, Samba, Squire and Kaleya with, 3.17, 3.16, 2.75, 2.68 and 2.31 t/ha respectively, which implied that these genotypes were resistant to high levels of soil aluminium . The lowest yields were 0.91, 0.85, 0.84, 0.47 and 0.44t/ha observed in Magoye, Soprano, S810/6/10, Spike and Hernon 147B respectively as Table 16 shows, indicating that these genotypes were susceptible to high levels of soil aluminium.

The percentage decrease in yield between the two locations was done to determine which genotype was highly affected by high soil Al levels. Table 21 shows the highest percentage reduction in yield in Hernon 147 with 83.3 percent followed by Hernon 147 B with 82.61 percent. The lowest in decrease was observed in Kaleya with 16.67 percent followed by 17.65 percent in Semeki which was not significantly different from Sirocco with 17.95 percent.

4.2.2 Weight of 100 Grains

There was a reduction in the weight of 100 grains from 16.24kg at Lusaka West Farm to 14.39kg at Liempe Farm as can be observed in tables 16 and 17.

Table 16 shows the highest 100 grains' weights of 19.50, 18.00, 17.5, 16.5 and 16.25kg which were observed in genotypes in the order of Semeki, Satelite, Lukanga, Scribe and Score evaluated at Liempe Farm. The lowest weight was observed in S810/6/10 with 9.50kg.

While 23.25, 22.00, 18.25, and 17.25 kg were the highest weights from Lusaka West Farm observed in genotypes Squire, Satelite, Hernon 147B and Hernon 147, and Score data from Table 17 indicates. The lowest weights were observed in genotypes Magoye and Dina with 10.00 and 11.50 kg respectively.

4.2.3 Number of Pods Per Plant

Lusaka West had higher number of pods per plant with the mean of 81.27 than Liempe with the mean of 40.54 as illustrated in tables 16 and 17.

The highest number of pods at Lusaka Liempe Farm was 69.00, 68.52 and 66.50 observed in genotypes Safari, Samba and Semeki respectively. While Lusaka West had

the highest number of pods in genotypes Magoye and Soprano with 149.2 and 132.7 Pods per Plant as shown in Table 17.

4.2.4 Number of Pink Root Nodules Per Plant and Number of Root Nodules Per Plant

Tables 16 and 17 show that Liempe had the lowest number of pink nodules with the mean of 2.38 as well as number of root nodules per plant of 4.17 than Lusaka West which had 7.16 number of pink nodules and 10.18 number of root nodules per plant implying that there was more nodulation at Lusaka West Farm than at Liempe Farm.

Squire, Semeki and Safari had the highest number of pink nodules per plant of 3.50, 3.25 and 3.25 respectively at Liempe Farm. Spike and Hernon 147B had the lowest with 1.25 each as shown by Table 16.

Meanwhile, at Lusaka West Farm, Table 17 shows that the highest pink nodules were observed in Score with 9.75 and lowest in Samba with 4.5.

The ratios from Table 18 shows that Spike, Hernon 147B and S810/6/10 had the lowest PN:RN ratio of 33 each which implies that the fixation efficiency was low. While the highest fixation efficiency was observed in genotypes Samba and Semeki with 83 and 80 PN: RN ratios respectively.

4.2.5 Root Biomass

Tables16 and 17 show that most of the genotypes had higher root biomass at Lusaka West Farm than at Liempe Farm. Lusaka West had the highest root biomass with the average of 6.08 grams and Liempe had the lowest with the mean of 3.53 grams.

Semeki and Squire had the highest root biomass at Liempe Farm of 5.53 and 5.18 grams respectively while the lowest root biomass was observed in Soprano, Spike and Hernon 147B with 1.92, 1.23 and 1.16 in the same order as shown by Table 16.

Meanwhile Table 17 shows that Spike had the highest Root Biomass of 9.00 grams which indicates that the roots of Spike were affected by high levels of soil Aluminium at Liempe. The lowest Root Biomass at Lusaka West was 4.00 grams observed in Scribe.

4.2.6 Plant Height

Most genotypes had lower plant heights at Liempe Farm than at Lusaka West Farm as can be seen from tables 16 and 17 with the means of 47.15 and 74.47 cm respectively.

The highest plant heights at Liempe Farm, as shown by Table 16, were 65.00 and 60.75cm observed in genotypes Dina and S810/6/10 respectively. The lowest at this site were Hernnon 147B and Magoye with 38.25 and 37.75 in that order.

The plant height means at Lusaka West Farm, in Table 16, show Hernon 147B and Sovereign had the highest with 89.25and 89.50 cm respectively, while 48.25cm observed in Sequel was the lowest.

4.2.7 Days to Fifty Percent Flowering and Maturity

All the genotypes evaluated flowered and matured earlier at Liempe Farm than at Lusaka West Farm as shown in both tables 16 and 17. The means for flowered fifty percent flowering and maturity at Liempe were 40.84 and 109.50 days respectively. . The means for flowered fifty percent flowering and maturity for Lusaka were 46.01 and 116.2 in that order.

The latest genotype to flower at Liempe Farm as can be seen in Table 16 was Dina with the mean of 52.75 days followed by Score and Samba with the mean of 44.00 days. The earliest was Soprano with 31.50 days. At the same site, Score and Dina were the latest to mature at 124.20 and 122.00 days, and the earliest were Squire and Spike at 87.00 and 98.00 days.

Meanwhile, Spike and Satelite were as the earliest to flower at 41.50 and 41.75 days as can be seen in means in Table 17. The latest to flower was Dina at 58.00 days. The earliest to mature at this site were Hernon 147 and Satelite at 109.5 and 109.8 days respectively.

4.2.8 Days to Shatter

The general indication shown in tables 16 and 17 was that the genotypes shuttered early at Liempe Farm than at Lusaka West Farm. This was evident in the means with Liempe at 19.59 and Lusaka West at 24.16 days.

Among the genotypes, the earliest to shatter at Liempe Farm was S810/6/10 at 11.00 days and the latest Satelite at 24.50 days as shown in Table 16.

Table 17 shows that the earliest pods to shatter at Lusaka West were Magoye followed by Sequel at 15.75 and 17.75 days respectively. The latest were Lukanga and Hernon at 26.25 and 26.00 days respectively.

4.2.9 Genotype by Location Interaction for Field Experiment

Table 19 shows that at $P \leq 0.001$, significant differences, in the parameters measured, between locations, genotypes and the interaction of genotype by location. This shows that the genotypes responded differently at Liempe and Lusaka West implying that

there was a diversified genetic base for Aluminium tolerance, for a breeder to select from.

In line with the results of the analysis of variance in Table 18, Figure 5 also shows that there is high interaction between genotypes and location. Spike and S810/6/10 had higher yield at Lusaka West and were found to have the lowest yield at Liempe farm. Samba and Semeki which had yields lower than Spike and S810/6/10 happened to have higher yields than the three genotypes (Spike, Hernon 147B and S810/6/10) at Liempe Farm.

There was a general tendency in all the genotypes evaluated in the field in the way they responded to different parameters. In all the parameters taken, there was a reduction in the genotypes' performance from Lusaka West farm to Liempe. Generally the performance of genotypes considering all the parameters was low at Liempe compared to Lusaka West Farm. This is shown by Table 20. The yield parameter was used to further show there was a reduction in the genotypes performance, which is shown by Table 21. The data in this table shows that the highest percentage reduction in Hernon 147 with 83.3 percent followed by Hernon 147 B with 82.61 percent. The lowest in percentage decrease was observed in Kaleya with 16.67 percent followed by 17.65 percent in Semeki which appeared to be not different from Sirocco with 17.95 percent.

The varieties Samba and Semeki which were chosen to be tolerant in the both the Laboratory screening and Hematoxylin test also showed tolerant in field screening.

4.3 Association of the Field Measured Parameters to Yield

A stepwise multiple regression analysis was carried out where yield was regressed on the other parameters measured and/or derived at Liempe Farm to select which of the independent variables has the greatest cause and effect relationship on yield.

The total variation from the study was 68.4 percent as shown in Table 22. The greatest contribution to yield was pink root nodules with 51.3 percent. Others were plant height with 15.5 percent and 100 grains' weight with 3 percent. The contributions of the rest of the parameters were not significant.

4.4 Comparison of Laboratory Results to Field Results

An Orthogonal Contrast, whose results are shown in Tables 14 was done to compare the tolerant (Samba and Semeki) and susceptible Spike, Hernon 147B and S810/6/10) varieties selected in the Laboratory Screening and Hematoxylin on their performance with regards to field parameters number of pink root nodules and yield. Number of pink nodules was used because it had the highest cause and effect on yield.

The F.values in Table 14 shows that, at five percent level of significance, the comparison between tolerant and susceptible varieties was significant. This implies that there were differences in number of pink nodules and yields at Liempe Farm between the two sets of varieties. Therefore, the laboratory screening can be used in selecting genotypes tolerant to high levels of soil aluminium.

Non significant differences were observed in the comparisons between tolerant and tolerant varieties, and also between susceptible and susceptible varieties as shown by the smaller F. values than the tabulated.

Table 13: Analysis of Variance for the parameters measured and/or derived from nineteen genotypes evaluated at Liempe Farm during 2012/2013 growing season in Zambia

Sources of Variation	DF	YLD	100GW	PP	PRN	RN	RB	PH	DF_{50}	DM	DS
Replications	3	0.27	2.56	17.66	0.75	1.73	0.54	131.00	3.40	117.86	0.79
Genotypes	18	3.13***	27.60***	177.42**	1.84***	1.53***	7.15***	234.54**	79.48***	272.55***	67.09***
Orthogonal Comparison											
Samba vs Rest	1	1.87		1.88							
Samba & Semeki vs Spike, Hernon 147B & S810/6/10	1	193.10***		10.63***							
Samba vs Semeki	1	0.42		0.13							
Spike vs Hernon 147B & S810/6/10	1	0.33		0.17							
Error	30	0.16	5.75	65.10	0.96	0.71	0.21	11.17	0.76	123.55	0.80
LSD (5%)		0.46	3.02	9.56	3.45	5.23	3.21	10.2	0.49	16.55	1.34
C.V		19.07	14.81	16.64	12.4	3.51	1.23	18.5	0.83	10.46	4.74

DF=degree of freedom, LSD=least significant difference, C.V-coefficient of variation, YLD=yield in tones per hectare, 100GW=weight of 100 grains, PP=number of pods per plant, PRN=number of pink root nodules, RN=root nodules, RB=root biomass in grams, PH=plant height in centimeters, DF_{50}=days from planting to when 50% of the plants in a plot flowers, DM=days from planting to the time 50% of plants in a plot matures, DS=days from maturity to the time the first pods in a plot shatters.

Table 145: Analysis of Variance for the parameters measured and/or derived from nineteen genotypes evaluated at Lusaka West Farm during 2012/2013 growing season in Zambia

Sources of Variation	DF	YLD	100GW	PP	PRN	RN	RB	PH	DF_{50}	DM	DS
Replications	3	0.21	0.53	742.19	2.10	1.73	0.46	24.25	0.22	4.43	5.30
Genotypes	18	2.34***	36.22***	2387.14**	7.71***	6.33***	5.51**	356.22***	52.54***	93.37***	34.87***
Error	30	0.16	1.53	179.58	0.40	1.89	1.97	44.24	1.81	4.89	2.15
LSD (5%)		0.63	1.152	9.56	1.10	2.03	1.64	8.67	1.50	3.25	1.34
C.V		14.86	6.47	16.64	10.59	13.80	19.4	8.21	2.30	1.94	4.74

***=Significant at P=≤0.001, **Significant at P=≤0.01, *= Significant at≤0.05, DF=degree of freedom, LSD=least significant difference, C.V-coefficient of variation, YLD=yield in tones per hectare, 100GW=weight of 100 grains, PP=number of pods per plant, PRN=number of pink root nodules, RN=root nodules, RB=root biomass in grams, PH=plant height in centimeters, DF_{50}=days from planting to when 50% of the plants in a plot flowers, DM=days from planting to the time 50% of plants in a plot matures, DS=days from maturity to the time the first pods in a plot shatters.

Table 156: Means of Parameters measured and/or derived from nineteen soyabean genotypes at Liempe during the 2012/2013 growing season in Zambia

Genotype	YL	100GW	PP	PN	RN	RBM	PLHT	DF	DM	DS
Safari	3.17	15.00	24.00	3.25	4.50	4.84	48.75	42.00	112.5	22.75
Semeki	3.16	19.50	53.50	3.25	4.25	5.53	46.75	40.75	112.00	21.00
Samba	2.75	15.50	52.25	2.75	3.50	4.25	52.50	44.00	112.50	23.25
Squire	2.68	15.50	28.00	3.50	4.75	5.18	46.75	42.50	87.00	22.75
Kaleya	2.31	12.25	35.75	2.00	4.75	2.55	45.00	41.00	103.00	25.00
Sovereign	2.31	15.00	26.00	2.25	4.00	4.75	41.25	37.75	112.00	20.00
Scribe	2.22	16.50	59.25	2.50	4.00	4.88	55.50	43.25	112.00	22.75
Lukanga	2.09	17.50	26.00	1.75	3.00	3.75	40.75	37.75	103.80	22.00
Sirocco	1.90	13.25	27.75	3.00	4.25	3.35	41.00	43.00	110.80	22.00
Satelite	1.68	18.00	66.75	2.50	5.00	2.22	42.75	38.00	105.20	24.50
Hernon 147	1.41	15.25	46.00	2.50	4.25	2.80	42.75	43.50	113.50	18.25
Score	1.20	16.25	68.25	2.50	3.75	4.97	48.75	44.00	124.20	21.25
Dina	1.00	11.50	45.00	2.50	3.50	3.84	65.00	52.75	122.00	20.50
Sequel	1.00	14.62	33.25	2.75	5.25	4.45	41.00	41.00	115.80	13.50
Magoye	0.91	11.00	18.50	1.50	3.75	1.92	37.75	35.00	107.00	16.75
Soprano	0.85	12.75	17.50	1.50	5.25	2.32	54.50	31.50	112.20	16.50
S810/6/10	0.84	9.50	69.00	2.75	4.00	3.87	60.75	43.00	111.20	11.00
Spike	0.47	11.25	27.00	1.25	3.75	1.23	53.00	38.00	98.00	14.75
Hernon 147B	0.44	13.25	46.50	1.25	3.75	1.16	38.25	37.25	106.20	13.75
Means	**1.703**	**14.39**	**40.54**	**2.38**	**4.17**	**3.53**	**47.51**	**40.84**	**109.50**	**19.59**
5% LSD	**0.46**	**3.02**	**9.56**	**3.45**	**5.23**	**3.21**	**10.20**	**0.49**	**16.55**	**1.34**
C.V.	**19.07**	**14.81**	**16.64**	**12.40**	**3.51**	**1.23**	**18.50**	**0.83**	**10.46**	**4.74**

DF=degree of freedom, LSD=least significant difference, C.V-coefficient of variation, YLD=yield in tones per hectare, 100GW=weight of 100 grains, PP=number of pods per plant, PRN=number of pink root nodules, RN=root nodules, RB=root biomass in grams, PH=plant height in centimeters, DF_{50}=days from planting to when 50% of the plants in a plot flowers, DM=days from planting to the time 50% of plants in a plot matures, DS=days from maturity to the time the first pods in a plot shatters.

Table 167: Means of Parameters measured and / or derived from nineteen soyabean genotypes at Lusaka West Farm during the 2013/2013 growing season

Genotype	YL	100GW	PP	PN	RN	RBM	PLHT	DF	DM	DS
Squire	4.08	23.25	76.25	6.50	10.25	6.63	75.25	43.50	115.00	24.00
Semeki	3.94	16.50	76.25	6.500	10.00	6.400	79.25	45.25	114.8	25.50
Safari	3.828	16.25	56.33	6.000	10.00	6.000	78.25	45.50	111.0	28.25
Satelite	3.62	22.00	68.75	6.500	9.750	5.000	68.50	41.75	109.8	27.50
Spike	3.60	17.00	69.75	7.750	10.00	9.000	76.25	41.50	118.0	23.75
S810/6/10	3.56	16.00	63.25	8.500	11.25	5.575	74.50	45.50	118.8	24.50
Samba	3.44	17.00	76.00	4.500	7.750	5.250	73.75	46.00	114.2	26.25
Sircco	3.27	16.75	70.67	5.333	10.25	6.500	73.25	45.00	113.8	23.50
Hernon 147B	3.26	18.25	66.00	8.000	11.00	5.375	89.25	45.50	113.5	26.00
Hernon 147	2.96	15.75	71.50	7.500	9.750	6.750	80.25	44.50	109.5	24.75
Lukanga	2.96	14.75	47.00	7.000	10.00	6.175	72.25	42.75	110.0	26.25
Soprano	2.75	15.50	132.7	8.250	10.25	4.500	71.50	48.75	117.8	24.75
Sovereign	2.50	16.00	85.67	6.500	10.50	7.375	89.50	43.25	122.8	23.50
Kaleya	2.37	13.75	81.75	10.25	12.75	5.000	60.50	48.00	116.0	24.00
Scribe	2.33	18.25	84.75	7.250	9.000	4.000	66.50	46.50	115.2	24.00
Dina	1.97	11.50	98.50	6.500	9.250	6.750	80.00	58.00	126.8	25.25
Score	1.87	17.25	94.75	9.750	13.25	7.250	76.25	46.75	124.2	23.75
Magoye	1.82	10.00	149.2	6.750	9.000	5.250	81.75	46.75	117.2	15.75
Sequel	1.75	16.25	75.00	6.750	9.500	6.750	48.25	49.50	120.0	17.75
Means	**2.94**	**16.42**	**81.27**	**7.162**	**10.18**	**6.080**	**74.47**	**46.01**	**116.2**	**24.16**
LSD (5%)	**0.63**	**1.152**	**9.56**	**1.10**	**2.03**	**1.64**	**8.67**	**1.50**	**3.25**	**1.34**
C.V	**14.86**	**6.47**	**16.64**	**10.59**	**13.80**	**19.4**	**8.21**	**2.30**	**1.94**	**4.74**

DF=degree of freedom, LSD=least significant difference, C.V-coefficient of variation, YLD=yield in tones per hectare, 100GW=weight of 100 grains, PP=number of pods per plant, PRN=number of pink root nodules, RN=root nodules, RB=root biomass in grams, PH=plant height in centimeters, DF_{50}=days from planting to when 50% of the plants in a plot flowers, DM=days from planting to the time 50% of plants in a plot matures, DS=days from maturity to the time the first pods in a plot shatters.

Table 178: Ratios of Pink Root Nodules: Root Nodules to Determine the fixation Efficiency at Liempe Farm

Name	PRN	RN	% RATIO OF PN:RN
SAFARI	3	5	60
SAMBA	5	6	83
SATELITE	3	6	50
SEQUEL	3	6	50
SEMEKI	4	5	80
SCRIBE	2	4	50
SCORE	3	4	75
SOVEREIGN	2	4	50
SOPRANO	3	5	60
SPIKE	2	6	33
SIROCCO	3	5	60
S810/10/6	1	3	33
SQUIRE	3	5	60
LUKANGA	1	2	50
DINA	3	4	75
MAGOYE	2	4	50
HERNON 147	2	4	50
KALEYA	3	4	75
HERNON 147B	1	3	33

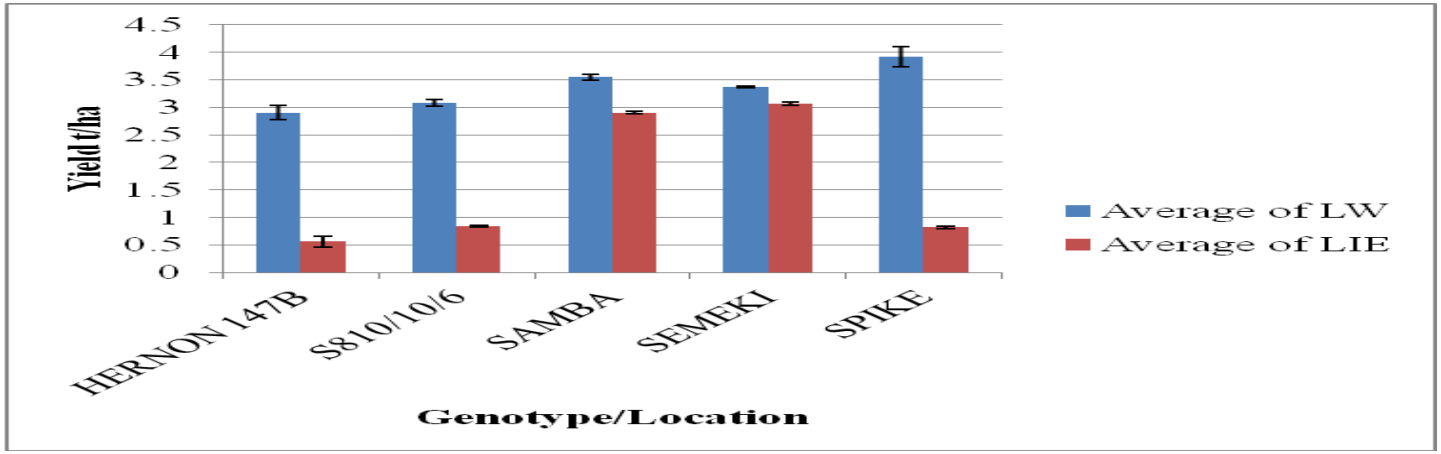
PRN=pink root nodules, RN=root nodules

Table 189: Analysis of Variance for the parameters measured and/or derived from nineteen genotypes evaluated at two locations (Liempe and Lusaka West Farms) during 2012/2013 growing season in Zambia

Sources Of Variation	DF	DF_{50}	DM	DS	PRN	RN	PP	RB	PH	YLD
Replication	6	2.16	57.68	3.44	1.67	1.4	504.50	0.84	30.32	0.21
Location (L)	1	1019.76***	1654.80*	794.15***	864.34**	1366.15***	62393.60***	240.17***	27140.49***	58.76***
Genotypes (G)	18	115.37***	301.86***	88.08***	4.07***	4.12***	2580.60***	9.37***	443.17***	4.72***
L*G	18	16.94***	71.15*	13.49***	5.58***	3.99***	1038.1***	3.63***	138.01***	0.80***
Error	105	1.513	69.77	1.26	0.43	1.25	171.7	0.77	50.54	0.16
CV %		2.8	7.4	5.1	19.2	15.5	21.5	18.10	11.60	17.0
LSD		11.72	1.62	1.31	1.61	18.68	1.21	3.35	0.54	2.32

*** = Significant at P≤0.001, ** = Significant at P≤0.01, * = Significant at≤0.05, ns = not significant, CV= Coefficient of variance, LSD=Least significant difference, YLD=yield in tones per hectare, 100GW=weight of 100 grains, PP=number of pods per plant, PRN=number of pink root nodules, RN=root nodules, RB=root biomass in grams, PH=plant height in centimeters, DF_{50}=days from planting to when 50% of the plants in a plot flowers, DM=days from planting to the time 50% of plants in a plot matures, DS=days from maturity to the time the first pods in a plot shatters.

Figure 5: Genotype by Location Interaction



LW=Lusaka west farm, LIE=liempe farm

Table 20: Means of Parameters measured and/or derived from nineteen soyabean genotypes at two locations during the 2012/2013 growing season

Locations	DF	DM	DS	PRN	RN	PP	RB	PC	PH	YLD
Liempe	41	110	20	2	4	41	3.6	40.7	47.8	1.7
LW	46	116	24	7	10	81	9.1	81.3	74.5	2.9
LSD (5%)	**0.66**	**4.38**	**0.82**	**0.61**	**0.31**	**8.57**	**0.32**	**5.21**	**5.92**	**0.10**

LW=Lusaka west, LSD=Least significant difference, YLD=yield in tones per hectare, 100GW=weight of 100 grains, PP=number of pods per plant, PRN=number of pink root nodules, RN=root nodules, RB=root biomass in grams, PH=plant height in centimeters, DF_{50}=days from planting to when 50% of the plants in a plot flowers, DM=days from planting to the time 50% of plants in a plot matures, DS=days from maturity to the time the first pods in a plot shatters.

Table 191 : Grain Yield means (ton/ha) for nineteen genotypes evaluated at Liempe and Seedco Lusaka West Farm

Genotype	Liempe	LW	Across location Means	% Decrease in yield between the two locations
Safari	2.3	3.8	3.1	39.47
Samba	2.7	3.3	3.0	18.18
Satelite	1.8	3.6	2.7	50.00
Sequel	0.9	1.8	1.3	50.00
Semeki	2.8	3.4	3.1	17.65
Scribe	2.2	3.3	2.7	33.33
Score	1.2	1.9	1.6	36.84
Sovereign	2.3	3.6	3.0	36.11
Soprano	1.4	2.8	2.1	50.00
Spike	0.9	4.2	2.5	79.86
Sirocco	3.2	3.9	3.6	17.95
S810/10/6	0.9	3.9	2.4	76.95
Squire	3.2	4.1	3.6	21.95
Lukanga	2.1	3.0	2.5	30.00
Dina	1.0	2.0	1.4	50.00
Magoye	0.8	1.8	1.3	55.56
Hernon 147	0.5	3.0	1.7	83.33
Kaleya	2.0	2.4	2.1	16.67
Hernon 147B	0.4	2.3	1.4	82.61
LSD (5%)	**0.1**	**0.4**		

LSD=least significant difference, LW=Lusaka west farm, LIE=liempe farm

Table 202: Relationship between field measured parameters and yield

Parameter	Partial Square	R-Model Square	R-F Value	Pr>F
PRN	0.513	0.513	73.6	0.00
PH	0.155	0.657	32.0	0.00
PP	0.03	0.648	6.72	0.00

PRN=pink root nodules, PH=plant height, PP-pods per plant

CHAPTER 5

5 DISCUSSION

Highly significant differences were observed among genotypes for all the parameters measured and/or derived from the field and laboratory as shown in the analysis of variance in results for both experiments.

5.1 Performance of Genotypes Evaluated in the Laboratory Under Zero and Twenty Milligrams Per Litre

Genotypes, aluminium concentration levels and the interaction of the genotype by aluminium concentration were significantly different in most of the parameters taken in the laboratory. The genotypes responded differently to the parameters taken in the laboratory because these genotypes have different genetic combinations. Furthermore, the genotype by aluminium level of concentration interaction was highly significant, implying that there was a large genetic base for a breeder to select from.

Table 7 shows that tap root length, shoot length, shoot and shoot biomass were higher at 0 mg/L Al concentration than at 20 mg/L. Ryan *et al*, 1993 reported that the primary effect of aluminium concentration is to inhibit root growth and development with subsequent effect on nutrient and water uptake. Cell division and root elongation is affected within hours.

However, number of lateral roots was higher in 20 mg/L than in 0 mg/L of aluminium. Soyabean tends to develop lateral roots when exposed to high aluminium concentrations as a defence mechanism. This coincides with the results that Munyinda (1986) obtained for wheat where the wheat varieties showed an increase in the number of lateral roots with an increase in aluminium

concentration. This response by soyabean was to compensate for the loss in root length as aluminium concentration increased.

There was a differential effect on root biomass between 0 and 20 mg/L. As taproot length reduced due to aluminium concentration increase, the shoot biomass and shoot length also reduced. The shoot length and biomass were inhibited due to limiting supply of water and nutrients resulting from reduction of root length. However, other genotypes such as Semeki and Samba had a very small percentage decrease in shoot biomass compared to Spike, Hernon 147B and S810/6/10. This is because Semeki and Samba increased the number of lateral roots at 20mg/L compared to Spike, Hernon 147B and S810/6/10, therefore, there was still a relative amount of water and nutrients taken up to the plants and so maintained the shoot length and biomass to a certain point.

5.2 Relationship Between Hematoxylin Test Results And Laboratory Measured Parameters as Indicators of Aluminium Tolerance

The Hematoxylin test was carried out to help select the genotypes tolerant to high levels of aluminium and also acted as a check on the results obtained from the Laboratory screening.

The Hematoxylin results in Table 10 show that Samba, Semeki, Scribe, Squire, Sirocco, Score, Kaleya and Saga stained clear with Hematoxylin while S810/6/10 implying that these genotypes had no free moving aluminium to react with hematoxylin in the roots. Such kinds of genotypes are tolerant to high levels of aluminium in the soil. While Spike and Hernon 147 had free moving aluminium in the roots which reacted with hematoxylin giving a dark stained implying that these genotypes are susceptible high levels of soil aluminium. Others genotypes were the intermediates.

These same genotypes were among the genotypes that had a lower percentage decrease in shoot biomass between 0 and 20mg/L (Table 9). There are similarities in results obtained from the two experiments. This is consistent with the findings done by Polle *et al*., 1978, who showed that hematoxylin stain has also proved to be useful in determining the Aluminium tolerance of plants.

Hematoxylin reacts with the free moving Al in the root. The fact that some roots were clear (tolerant) and others had a full stain (susceptible) after staining with Hematoxylin does not clearly show the mechanism of tolerance. The genotypes which had clear stains might either mean that the aluminium was not taken up by the plant or that the aluminium was bound to an extent that it cannot freely move in the plant system and have negative effects. Hence, based on the genotypes that were chosen to be tolerant and susceptible by both the Hematoxylin and Laboratory tests, a plant tissue analysis was done.

5.3 Plant Tissue Analysis to Determine the Mechanism of Tolerance and The Aluminium Partitioning in The Plant

Root aluminium contents were generally lower for the tolerant genotypes (Samba, Lukanga and Semeki) than for the sensitive genotypes (Spike, S810/6/10 and Hernon). It is, therefore, reasonable to assume that the Al-tolerance in these soyabean genotypes involved aluminium exclusion (Delhaize and Ryan, 1995; Kochian, 1995) from the root. Although shoot aluminium content was also considerably lower for the tolerant genotypes than for the sensitive genotypes, no indication of internal detoxification (Ma et al., 2001) was observed.

Though root aluminium contents of tolerant genotypes were lower than those of sensitive genotypes (Table 12), root aluminium content (mg/kg) was greater than that of the shoot in both

cases. The root to shoot Aluminium content ratios for the genotypes were in the range of 1.76 to 3.59.

5.4 Field Performance of Genotypes Grown in Location with High pH (Liempe Farm) And Medium pH (Lusaka West Farm)

Significant differential responses were seen in genotypes, locations and genotypes by location for most of the variables measured and/or derived from the field experiments.

Some of such variables include number of pink root nodules per plant, number of root nodules per plant, root biomass, plant height, number of pods per plant, 100 seeds weight, days to flower, days to pod shattering, days to mature and yield.

The differences in genotypes' responses to these variables, in one location, could have been due to the fact that these genotypes are from three different seed companies representing a wide range of genetic diversity among them. This is in line with what Kumar (2011) stated that the occurrence of significant differences among pigeon pea genotypes for tolerance to Aluminium toxicity indicated the scope of genetic improvement for aluminium tolerance in pigeon pea. He further reportedd that the variation in response was most likely due to difference in genetic potential of pigeon pea genotypes.

Across location differences amongst the genotypes was mainly due to their different responses to high levels of aluminium in the soil. Different genotypes yielded differently in one location to the other location. It was noticed that some genotypes which yielded high at Lusaka West had lower yields in Liempe for example genotypes Spike, S810/6/10 and Hernon 147B which were amongst the high yielding varieties at Lusaka West with 3.6, 3.56 and 3.26t/ha had 3.4.2t/ha and 3.9t/ha at Lusaka West, reduced their yields to 0.47, 0.84 and 0.44t/ha at Liempe farm

respectively. The reduction in yield, which was clearly shown in Tables 16 and 17, was as a result of the effect of high soil aluminium. Such genotypes, despite being higher yielding in some areas, are susceptible to high soil aluminium. On the other hand, some genotypes had relatively good yields at Lusaka West and performed relatively well at Liempe farm in terms of yields resulting in small percentage decrease in yields. Examples of such genotypes were Kaleya, Semeki and Sirocco with 16.67, 17.65 and 17.95 percents respectively. This implies that despite the high soil aluminium, the genotypes could still give a good yield though reduced. Such genotypes are able to tolerate such soils.

Number of root nodules was high in Lusaka west with an average of ten as compared to Liempe farm with an average seven. This was due to the reason that aluminium has a negative effect on nodulation. Helyar, (1996) reported that Low soil pH and high soil aluminium affect nodulation and nitrogen fixation. Low pH and high aluminium restrict the survival of rhizobium in the soil. Low soil pH and high Al levels inhibit the infection process, and hence the establishment of nodulated plants. Generally individual genotypes had higher number of root nodules in Lusaka West than at Liempe farm. Further observations were seen in the reduction of number of pink root nodules from an average of four in Lusaka West Farm to two at Liempe Farm. The fixation efficiency was determined by the Pink Root Nodules: Root Nodules ratios from Table 18. The ratios for the susceptible genotypes (Spike, Hernon 147B and S810/6/10), as determined by the hematoxylin and laboratory tests, and as can be seen in the field yield data, were generally low, 33, which implies that the fixation efficiency was low. While the highest fixation efficiency was observed in tolerant genotypes (Samba and Semeki) with 83 and 80 PN: RN ratios respectively. K.R. Heiyar, in 1996, further reported that low pH and high Al., can reduce the rate of nitrogen fixation by established nodules hence it has been observed that a plant can have a high number of

root nodules, but only a few of those are pink. Implying that even though nodules could be formed on the roots, only a few are able to fix nitrogen. Some tolerant genotypes are able to nodulate more than the susceptible ones in soils with high Aluminium levels. Both the rhizobium and the plant pink root nodules can be used to select for tolerance to low soil pH and associated factors, (Helyar, 1996).

Root biomass decreased from an average of 6.08 g at Lusaka West Farm to 3.53 g at Liempe Farm as shown in tables 16 and 17 because high levels of Al in the soil impairs root growth and development. Haynes & Mokolobate, (2001) reported that the most obvious symptom of soil toxicity was inhibition of root growth. Earlier, Foy (1996) discovered that relative shoot and root dry weights in tolerant cultivars were two-fold and three-fold respectively compared to susceptible cultivars.

Significant differences were seen in days to Flower, maturity and pod shattering in the two locations. Stresses such as high soil aluminium tends to force the plants to flower, mature and shutter early because the plants are denied access to most nutrients such as phosphorus, and resort to quicken the developmental processes. Under field conditions affected plants are very susceptible to moisture stress and die easily (Helyar, 1996).

The fact that there was a general decline in plant height for most genotypes at Liempe Farm compared to Lusaka West Farm, there was a relatively reduction in number pods. Since the roots were impaired, not enough plant nutrients were taken up by the plants resulting in plant height being shorter than their normal size and hence reducing the number of pods. The shorter the plant the less pods it has and so is vice-versa.

For the same reason that not enough nutrients are taken up by the plant, the weight of 100 seeds reduces as well.

5.5 Relationship between Field Parameters and Yield as Indicators of Al Tolerance

The stepwise regression analysis results in Table 22 points out that number Pink Root Nodules and Plant Height had the highest cause and effect on yield.

The number of root nodules and number of pink root nodules also affected the yield. Helyar (1996), reported that low soil pH and high soil Al affect nodulation and nitrogen fixation in several ways. Low pH and high aluminium restrict the survival of rhizobium in the soil. Low pH soil inhibit the infection process, and hence the establishment of nodulated plants and can negatively nitrogen fixation by established nodules. Therefore, even if the rhizobium is present in the soil which is reflected by the formation of root nodules on the plant, not all of the formed root nodules can do fixation. That is why we see the number of pink nodules is less than half of the root nodules formed on the plant in some varieties such as Spike, Hernon 147 B and S810/6/10. While varieties like Semeki, Samba, Scribe, Soprano and Dina had number of pink root nodules almost half or above half of the number of root nodules formed on the plant. Helyar (1996), further reported that the nodule bacteria receive their food requirements from the host plant. In return, the bacteria use some of the food energy to convert gaseous nitrogen from the soil air, to the ammonia form of nitrogen. The plant, in turn, uses the ammonia in the production of plant proteins, and thus can be independent of soil nitrogen. The yield was affected because there was a limitation on the amount of nitrogen being fixed and hence being available for plant growth.

Plant height affects the number of pods per plant which translates to yield. Both the plant height and 100 seeds' weight are inhibited by a low supply of water and nutrients to the plant.The number of days to shatter affected yield the least, followed by pod clearance.

5.6 Comparison of Genotypes which were Considered Tolerance and Susceptible in the Laboratory on The Basis of Yield Field Parameter using Orthogonal Comparison

The results from the laboratory screening and hematoxylin tests shows that Samba and Semeki were tolerant, while Hernon 147B, S810/6/10 and Spike were susceptible. The results from the stepwise regression analysis for yield regressed on the field parameters taken at Liempe Farm showed that number of pink nodules had the highest cause and effect on the yield. Hence, an Orthogonal Contrast was conducted to compare between the performance of tolerant and susceptible varieties (according to the Laboratory screening and Hematoxylin tests) with regards to number of pink nodules and yield at Liempe Farm.

The orthogonal Contrast results in Table 14 highlighted that there were significant differences at five percent level of confidence in the comparison between the tolerant and susceptible genotypes. The comparisons between tolerant to tolerant, and susceptible to susceptible varieties showed no significant differences. This shows that the results from the laboratory screening and Hematoxylin tests agreed with the field determinants parameters of tolerance which are number of pink root nodules and yield which implies that laboratory data can be used to determine yield performance in the field. Yan & Wallace (1995), proposed that the shoot biomass is one of the three major physiological-genetic components for crop yield accumulation others being harvest index, and days to grain maturity. Furthermore, it indicates the relative importance of the vegetative biomass for the potential yield capacity and the proportion of this biomass that is subsequently remobilized to the reproductive organs (Yan & Wallace, 1995). In Table 9 Semeki

and Samba showed the lowest percentage decrease in shoot biomass of 5.77 and 6.74 respectively, while in Table 21, they were among the genotypes with the lowest percentage in yield of 17.65 and 18.18 respectively. Genotypes such as Spike and Hernon 147 had higher percentage decrease in shoot biomass of 74.42 and 75.51 as indicated in Table 9 and were among the genotypes with the highest percentage decrease in yield of 79.86 and 83.33 respectively as Table 21 indicates.

CHAPTER 6

6 CONCLUSION

The hypothesis that selection for tolerance to high levels of soil Al increases the productivity of soyabeans in areas with high levels of soil aluminium was validated in this study. There is sufficient variation in the genotypes response to high aluminium levels both in laboratory and field which showed a wide genetic base in the Zambian released soyabean genotypes. This showed that we had wide genetic base from which high yielding varieties in areas with high soil aluminium levels can be produced. However, this study has answered all the objectives of the study.

We have observed, from this study, that aluminium accumulation was higher in the root compared to the shoot and also that sensitive genotypes had high root and shoot aluminium accumulation than tolerant genotypes. Therefore, we can conclude and state that soyabean uses the exclusion type of tolerance mechanism. The aluminium partitioning in the plant is higher in the roots than the shoots.

The stepwise regression analysis of yield regressed on the independent parameters taken from Liempe Farm showed that number of pink root nodules, plant height and number of pods per plant had a greater cause and effect on yield and can therefore be used in selecting for tolerance to high levels of soil aluminium.

Hematoxylin test also conforms to the laboratory and field selection, and so this method can be used as quick way of selecting genotypes for tolerance to high levels of soil Aluminium. The 5 varieties which were among the top 5 tolerant in the laboratory and Hematoxylin tests were in the top 5 tolerant varieties in the field. This was supported by the results from the Orthogonal

Comparison which showed that significant differences were observed in the comparison between tolerant and susceptible varieties in the laboratory and Hematoxylin tests with regards to the number of pink root nodules and yield parameters obtained from Liempe Farm. Therefore, laboratory screening can be used for screening for genotypes tolerant to Aluminium toxicity.

Genotypes that were identified and selected to be tolerant to high levels of soil aluminium include Samba, Semeki, Kaleya, Squire and Sirocco.

A recommendation would be made that a study to determine what type of organic compounds are produced in external tolerance mechanism are produced by soyabean plants should be done to be sure of how soyabean tolerates high aluminium levels. The use of markers to select for tolerance in aluminium stresses should also be done to accelerate the selection process.

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APPENDICES

Appendix 1: Analysis of Variance for the determination of Discriminatory Level of Aluminium

Sources of Variation	D.F	S.S	M.S	F.PR
REP	2	0.002	0.014	
AL. Concentration	5	4.009	0.802	0.001
Genotypes	4	0.388	0.097	0.001
REP*AL. Concentration		0.057	0.006	
AL. Concentration *Genotype		0.157	0.008	0.02
Residual	48	0.181	0.004	
Total	89	4.82		

Appendix 2: Analysis of Variance for Shoot Length in Laboratory Screening of Genotypes

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Entry	17	809.381	47.611	38.70	<.001
Aluminium	1	73.255	73.255	59.55	<.001
Entry*Aluminium	14 (3)	507.217	36.230	29.45	<.001
Residual	64 (8)	78.732	1.230		
Total	96 (11)	1360.755			

Apppendix 3: Analysis of Variance for Taproot Length in Laboratory Screening of Genotypes

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Entry	17		2574.609	151.448	30.76	<.001
Aluminium	1		1035.000	1035.000	210.23	<.001
Entry*Aluminium	14	(3)	775.700	55.407	11.25	<.001
Residual	64	(8)	315.083	4.923		
Total	96	(11)	4436.367			

Appendix 4: Analysis of Variance for Shoot Biomass in Laboratory Screening of Genotypes

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Entry	17		18.1842	1.0697	3.38	<.001
Aluminium	1		0.6230	0.6230	1.97	0.165
Entry*Aluminium	14	(3)	1.2130	0.0866	0.27	0.995
Residual	64	(8)	20.2280	0.3161		
Total	96	(11)	33.3644			

Appendix 5: Analysis of Variance for Root Biomass in Laboratory Screening of Genotypes

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Entry	17		6.48207	0.38130	8.45	<.001
Aluminium	1		1.59208	1.59208	35.30	<.001
Entry	14	(3)	2.17869	0.15562	3.45	<.001
Residual	64	(8)	2.88677	0.04511		
Total	96	(11)	12.67876			

Appendix 6: Analysis of Variance for Lateral Root Length in Laboratory Screening of Genotypes

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Entry	17		338.036	19.884	16.78	<.001
Aluminium	1		36.448	36.448	30.77	<.001
Entry*Aluminium	14	(3)	301.819	21.558	18.20	<.001
Residual	64	(8)	75.821	1.185		
Total	96	(11)	743.379			

Appendix 7: Analysis of Variance for Number of Lateral roots in Laboratory Screening of Genotypes

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Entry	17		15807.55	929.86	73.55	<.001
Aluminium	1		1341.58	1341.58	106.12	<.001
Entry*Aluminium	14	(3)	2619.08	187.08	14.80	<.001
Residual	64	(8)	809.10	12.64		
Total	96	(11)	16646.91			

Appendix 8: Analysis of variance for Root in Plant Tissue Analysis

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Genotytpe	12	1067984.	88999.	31.21	<.001
Residual	23	65592.	2852.		
Total	35	1133576.			

Appendix 9: Analysis of variance for Soott in Plant Tissue Analysis

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	12	317571.7	26464.3	38.39	<.001
Residual	23	15856.5	689.4		
Total	35	333428.2			

Appendix 10: Analysis of Variance for Yield measured at Liempe Farm

Source of Variation	D.F.	S.S.	M.S.
Replications	3	0.810473	0.270158
Treatments	18	56.3366	3.12981
Error	30	4.92324	0.164108
Total	75	62.1108	

Appendix 11: Analysis of Variance for 100 grain weight measured at Liempe Farm

Source of Variation	D.F.	S.S.	M.S.
Replications	3	7.66776	2.55592
Treatments	18	496.862	27.6034
Error	30	172.426	5.74753
Total	75	749.799	

Appendix 12: Analysis of Variance for Number of Pods per Plant measured at Liempe Farm

Source of Variation	D.F.	S.S.	M.S.
Replications	3	52.9868	17.6623
Treatments	18	21193.6	1177.42
Error	30	1952.89	65.0963
Total	75	23702.9	

Appendix 13: Analysis of Variance for Number of PINK Root Nodules per Plant measured at Liempe Farm

Sources of Variance	D.F.	S.S.	M.S.
Replications	3	2.25000	0.750000
Treatments	18	33.1842	1.84357
Error	30	2.87500	0.958333
Total	75	53.9342	

Appendix 14: Analysis of Variance for Number of Plant Root Nodules per Plant measured at Liempe Farm

Source of Variation	D.F.	S.S.	M.S
Replications	3	5.19737	1.73246
Treatments	18	27.5263	1.52924
Error	30	21.3224	0.710748
Total	75	60.7763	

Appendix 15: Analysis of Variance for Root Biomass measured at Liempe Farm

Source of Variation	D.F.	S.S.	M.S.
Replications	3	1.60762	0.535873
Treatments	18	128.754	7.15299
Error	30	6.20112	0.206704
Total	75	139.433	

Appendix 16: Analysis of Variance for Plant Height measured at Liempe Farm

Source of Variation	D.F.	S.S.	M.S.
Replications	3	392.987	130.996
Treatments	18	4221.74	234.541
Error	30	335.112	11.1704
Total	75	8012.99	

Appendix 17: Analysis of Variance for Days to Flower measured at Liempe Farm

Sources Variation	D.F.	S.S.	M.S.
Replication	3	10.2105	3.40351
Treatments	18	1430.61	79.4781
Error	30	2.28947	0.763158
Total	75	1540.11	

Appendix 18: Analysis of Variance for Days to Mature measured at Liempe Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	353.579	117.860
Treatments	18	4905.95	272.553
Error	30	3706.42	123.547
Total	75	12324.9	

Appendix 19: Analysis of Variance for Days to Pod Shttering measured at Liempe Farm

Sources of Variance	D.F.	S.S.	M.S.
Replications	3	2.35526	0.785088
Treatments	18	1207.61	67.0892
Error	30	24.0197	0.800658
Total	75	1256.36	

Appendix 20: Analysis of Variance for Yield measured at Lusaka West Farm

Source of Variation	D.F.	S.S.	M.S.
Replications	3	0.617230	0.205743
Treatments	18	42.1888	2.34382
Errorr	30	4.88341	0.162780
Total	75	53.5532	

Appendix 21: Analysis of Variance for 100 Grains' Weight measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	1.57895	0.526316
Treatments	18	652.026	36.2237
Error	30	45.9211	1.53070
Total	75	714.526	

Appendix 22: Analysis of Variance for Number of Pods per Plant measured at Lusaka West Farm

Sources of Variance	D.F.	S.S.	M.S.
Replications	3	2226.57	742.189
Treatments	18	42968.6	2387.14
Error	30	5387.53	179.584
Total	75	60960.0	

Appendix 23: Analysis of Variance for Number of Pink Root Nodules measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	6.28509	2.09503
Treatments	18	138.860	7.71443
Error	30	11.9233	0.397442
Total	75	216.776	

Appendix 24: Analysis of Variance for Number of Root Nodules measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replication	3	5.21053	1.73684
Treatments	18	113.921	6.32895
Error	30	56.7895	1.89298
Total	75	225.421	

Appendix 25: Analysis of Variance for Root Biomass measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	1.39724	0.465746
Treatments	18	99.1829	5.51016
Error	30	59.0865	1.96955
Total	75	172.940	

Appendix 26: Analysis of Variance for Plant Height measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	72.7368	24.2456
Treatments	18	6411.95	356.219
Error	30	1327.26	44.2421
Total	75	8502.95	

Appendix 27: Analysis of Variance for Days to Flower measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	0.671053	0.223684
Treatments	18	945.737	52.5409
Error	30	54.2039	1.80680
Total	75	1006.99	

Appendix 28: Analysis of Variance for Days to Mature measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	13.3026	4.43421
Treatments	18	1680.95	93.3860
Error	30	146.572	4.88575
Total	75	1967.20	

Appendix 29: Analysis of Variance for Days to Shutter measured at Lusaka West Farm

Sources of Variation	D.F.	S.S.	M.S.
Replications	3	15.8947	5.29825
Treatments	18	627.605	34.8670
Error	30	64.3947	2.14649
Total	75	730.105	

Appendix 30: Combined Sites Analysis of Variance for 100 Grains' Weight

Source of variation	D.F.	S.S.	M.S.	V.R.	F pr.
Replications stratum	3	6.945	2.315	2.79	
Replication*Site stratum					
Site	1	158.217	158.217	190.93	<.001
Residual	3	2.486	0.829	0.28	
Replication*Site*Entry stratum					
Entry	18	1003.471	55.748	19.14	<.001
Site*Entry	18	146.617	8.145	2.80	<.001
Residual	105 (3)	305.832	2.913		
Total	148 (3)	1620.027			

Appendix 31: Combined Sites Analysis of Variance for Days to Flower

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Rep stratum	3		6.465	2.155	1.32	
Replication*Site stratum						
Site	1		1019.758	1019.758	624.84	<.001
Residual	3		4.896	1.632	1.08	
Replicatio*Site*Entry stratum						
Entry	18		2076.559	115.364	76.25	<.001
Site*Entry	18		304.975	16.943	11.20	<.001
Residual	105	(3)	158.854	1.513		
Total	148	(3)	3554.872			

Appendix 32: Combined Sites Analysis of Variance for Days to Mature

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Replication stratum	3		173.03	57.68	0.80	
Replicatio*Site stratum						
Site	1		1654.80	1654.80	22.95	0.017
Residual	3		216.36	72.12	1.03	
Replicatio*Site*Entry stratum						
Entry	18		5433.44	301.86	4.33	<.001
Site*Entry	18		1280.67	71.15	1.02	0.445
Residual	105	(3)	7325.96	69.77		
Total	148	(3)	15833.30			

Appendix 33: Combined Sites Analysis of Variance Days to Shutter

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Rep stratum	3		10.342	3.447	1.37	
Replication*Site stratum						
Site	1		794.150	794.150	315.53	<.001
Residual	3		7.551	2.517	2.00	
Replication*Site*Entry stratum						
Entry	18		1585.495	88.083	69.97	<.001
Site*Entry	18		242.765	13.487	10.71	<.001
Residual	105	(3)	132.178	1.259		
Total	148	(3)	2635.973			

Appendix 34: Combined Sites Analysis of Variance for Number of Pods per Plant

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Rep stratum	3		1513.6	504.5	1.83	
Replicatio*Site stratum						
Site	1		62393.6	62393.6	226.17	<.001
Residual	3		827.6	275.9	1.61	
Replication*Site*Entry stratum						
Entry	18		46450.0	2580.6	15.03	<.001
Site*Entry	18		18685.8	1038.1	6.05	<.001
Residual	105	(3)	18025.7	171.7		
Total	148	(3)	146216.8			

Appendix 35: Combined Sites Analysis of Variance for Number of Pink Nodules per Plant

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Replications stratum	3		4.9424	1.6475	1.17	
Replication*Site stratum						
Site	1		864.3551	864.3551	616.07	<.001
Residual	3		4.2090	1.4030	1.66	
Replication*Site*Entry stratum						
Entry	18		73.2905	4.0717	4.83	<.001
Site*Entry	18		100.4135	5.5785	6.62	<.001
Residual	105	(3)	88.5361	0.8432		
Total	148	(3)	1124.9739			

Appendix 36: Combined Sites Analysis of Variance for Root Biomass

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Replication stratum	3		2.5126	0.8375	2.24	
Replication*Site stratum						
Site	1		240.1724	240.1724	641.01	<.001
Residual	3		1.1240	0.3747	0.49	
Replication*Site*Entry stratum						
Entry	18		168.6364	9.3687	12.25	<.001
Site*Entry	18		65.3459	3.6303	4.75	<.001
Residual	105	(3)	80.3273	0.7650		
Total	148	(3)	557.6334			

Appendix 37: Combined Sites Analysis of Variance for Number of Plant Root Nodules

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Rep stratum	3		4.434	1.478	0.62	
Replication*Site stratum						
Site	1		1366.151	1366.151	574.25	<.001
Residual	3		7.137	2.379	1.91	
Replication*Site*Entry stratum						
Entry	18		74.151	4.120	3.30	<.001
Site*Entry	18		71.874	3.993	3.20	<.001
Residual	105	(3)	131.017	1.248		
Total	148	(3)	1638.242			

Appendix 38: Combined Sites Analysis of Variance for Yield

Source of variation	D.F.		S.S.	M.S.	V.R.	F pr.
Replication stratum	3		0.6296	0.2099	5.47	
Replication*Site stratum						
Site	1		58.7611	58.7611	1532.34	<.001
Residual	3		0.1150	0.0383	0.25	
Replication*Site*Entry stratum						
Entry	18		84.8935	4.7163	30.33	<.001
Site*Entry	18		14.2259	0.7903	5.08	<.001
Residual	105	(3)	16.3255	0.1555		
Total	148	(3)	169.1031			

Appendix 39: Orthogonal Comparison for Laboratory Results to Number of Pink Nodules at Liempe Farm

Treatment	Samba	Semeki	Spike	Hernon 147	S810/6/10				
Treatment Totals	5	4	2	1	1	Q	r∑ci²	SS=Q²/r∑ci²	F
Comparisons									
1. Samba vs Rest	4	-1	-1	-1	-1	12	80	1.80	
2. Samba, Semeki vs Spike, HernoN 147B and S810/6/10	3	3	-2	-2	-2	35	120	10.21	10.63368
3. Samba vs Semeki	-1	1	0	0	0	-1	8	0.13	0.130208
4. Spike vs Hernon 147B and S810/6/10	0	0	2	-1	-1	2	24	0.17	0.173611

Appendix 40: Orthogonal Comparison for Laboratory Results to Number of Yield at Liempe Farm

Treatment	Samba	Semeki	Spike	Hernon 147	S810/6/10				
Treatment Totals	7.59	8.32	2.57	1.2	2.81	Q	r∑ci²	SS=Q²/r∑ci²	F-value
Comparisons									
1. Samba vs Rest	4	-1	-1	-1	-1	15.46	80	2.987645	18.67278
2. Samba, Semeki vs Spike, HernoN 147B and S810/6/10	3	3	-2	-2	-2	60.89	120	30.8966008	193.1038
3. Samba vs Semeki	-1	1	0	0	0	0.73	8	0.0666125	0.416328
4. Spike vs Hernon 147B and S810/6/10	0	0	2	-1	-1	1.13	24	0.05320417	0.332526