

**GENETIC VARIATION AND QUANTITATIVE TRAIT LOCI
MAPPING OF RESISTANCE TO ALUMINUM TOXICITY IN
COMMON BEAN**

(Phaseolus vulgaris L.)

JUSTIN NJOBVU

**A Dissertation submitted to the University of Zambia in partial fulfilment of the
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System.**

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DECLARATION

I, Justin Njobvu, hereby declare that the work presented in this dissertation was my own and has never been submitted for a degree at this or any other university.

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APPROVAL

This dissertation of Justin Njobvu was approved as fulfilling part of the requirements of the award of degree of Master of Science in Plant Breeding and Seed System by the University of Zambia.

Examiner's Name

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Signature

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ABSTRACT

Aluminum (Al) toxicity in acidic soils is a major constraint to common bean (*Phaseolus vulgaris*) production. The genetic architecture of Al toxicity resistance in common bean is not well understood. The objective of this study was to identify the quantitative trait loci (QTL) for resistance to aluminum toxicity in a mapping population of 150 F_{4:8} recombinant inbred lines (RILs) derived from parents Solwezi and AO-1012-29-3-3A. The parent, Solwezi is resistant to Al toxicity compared to AO-1012-29-3-3A. The RILs and their parents were evaluated for resistance to Al toxicity in a hydroponic system that had two nutrient solutions with 0 μ M Al (control solution) or 15 μ M Al concentration (Al stress solution). Primary traits including root length (RL), root dry weight (RDW), and shoot dry weight (SDW) were measured. Also, secondary traits including percentage reductions in RL, RDW and SDW were calculated from their respective primary traits. The RILs were genotyped using 5393 SNP markers and QTLs identified using Composite Interval Mapping. A total of Eight QTLs for Al toxicity resistance were identified on chromosomes Pv02, Pv04, Pv05, Pv06, Pv07, Pv09 and Pv10. The R² values for these nine QTL ranged from 7.6% to 14.7%. The Al-toxicity resistance QTL RL10.1 on Pv10 explained 10% of the genetic variation in RL and the Al-resistant parent Solwezi contributed the positive allele. The QTL RL10.1 overlapped with the QTL RDW10.1 identified using RDW. Another Al-resistance QTL RL.6.1 with R² of 10% was identified on Pv06 using percentage reduction in RL. The genetic architecture of Al resistance in Solwezi and AO-1012-29-3-3A population is polygenic with additive action.

DEDICATION

To my lovely capable wife Kathleen Mwamba Katunansa - Njobvu for taking care of my family affairs whilst I was pursuing this degree and encouragement she gave me during my study period, my daughters, Tionenji, Keziah and Nkumbwizya.

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CHAPTER 1

1. INTRODUCTION

Common Bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption. It is a major source of protein, carbohydrates and micronutrients such as iron and zinc. For many households in African and Latin American countries, common bean is a major source of food security and revenue (Akibode and Maredia 2012).

Aluminum (Al) toxicity in acidic soils is a major constraint to common bean productivity. Over 50% of the world's potential arable land including land where common bean is produced has acidic soils, making Al toxicity a global challenge to crop productivity (von Uexküll and Mutert., 1995). At soil pH of less 5, Al solubilizes from the non-phytotoxic silicates and oxides into phytotoxic Al^{3+} (Kinraide 1991). The root apex, which is the root elongation zone, is the most sensitive to Al toxicity. Al toxicity inhibits cell expansion and elongation of the root apex resulting in poor root growth and damaged root system. The damaged root system hinders water and nutrient uptake by the plant resulting in poor plant growth and productivity. Additionally, Al toxicity exacerbates the effects of drought and low soil fertility on crop productivity. Soil amendments such as lime application can ameliorate Al toxicity. However, for many small-scale farmers who are the major producers of beans in Africa, lime application may not be affordable.

Growing varieties of common bean resistant to Al toxicity would be a more cost effective management option for Al toxicity. Two physiological mechanisms have been proposed for resistance to Al toxicity. First is the exclusion of the Al from the root rhizosphere, which occurs through the Al-activated exudation of organic acids such as malate, citrate and oxalic acid from the roots. These organic acids convert the phytotoxic Al^{3+} ions into non-toxic forms. Correlation between citrate exudation and genotypic differences in Al toxicity resistance has been reported.

Delhaize et al. 1995 reported that Al-resistant cultivars of snap beans secrete 10 times more citric acid from its roots than Al-susceptible varieties. In common bean, Al-resistant cultivar ICA Quimbaya was reported to exude more citrate in its rhizosphere than the susceptible variety VAX-1. The second mechanism of resistance is internal detoxification of the Al, which happens when organic acids complex Al^{3+} ions into non-phytotoxic compounds in the plant (Kochian et al 2004.)

Genetic variation for Al toxicity resistance has been reported in a few plant species. An understanding of the genetic basis of this variation is important in breeding Al-resistant bean varieties. This understanding could support development of breeding tools and strategies for Al resistance. The genetic architecture of Al resistance varies depending on the plant species. Single QTL with major effects on Al toxicity resistance have been reported for Triticeae members including wheat (*Triticum aestivum* L.), rye (Miftahudin et al., 2002), and barley (Tang et al., 2000). Multiple QTL with major and minor effects have been reported to control Al toxicity in Arabidopsis (Kobayashi and Koyama 2002; Hoekenga et al., 2003; Kobayashi et al., 2005), sorghum (Magalhaes et al. 2004), rice (Nguyen et al., 2002) and maize (Ninamango-Cardenas et al., 2003). Genetic variation in Al toxicity resistance has been reported in common bean. Blair et al (2009) evaluated a total of 36 Andean and Middle American genotypes for Al toxicity resistance under hydroponic conditions. In that study they reported several Al-resistant genotypes from both Andean and Middle American gene pool. However, the Andean genotypes exhibited more Al resistance than the Middle American genotypes. Lopez-Marin et al. (2009) reported polygenic control of Al resistance, involving both major and minor effect QTL in a population of recombinant inbred lines (RILs) derived from a cross of DOR364 (Middle American) and G19833 (Andean). In that report 12 QTL for resistance to Al toxicity were

identified using root length, root dry weight, root diameter and number of root tips measured under hydroponic system with two nutrient solutions (0 μM and 20 μM Al concentrations) (Lopez-Marin et al., 2009). The phenotypic variation explained by individual QTL ranged from 10% to 23%. To date, the report by Lopez-Marin et al. (2009) is the only one on the genetic architecture of Al toxicity resistance. More genetic studies are needed to enhance knowledge on the genetic architecture of Al resistance in common bean.

1.2 Objectives

The objective of this study was to identify the quantitative trait loci (QTL) for resistance to aluminum toxicity in a mapping population of 150 $F_{4:8}$ recombinant inbred lines (RILs) derived from parents Solwezi and AO-1012-29-3-3A (AO).

1.3 Specific Objectives

- i. Evaluate 150 $F_{4:8}$ RILs for genetic variation for resistance to aluminum toxicity.
- ii. Identify QTL controlling variation for resistance to aluminum toxicity in 150 $F_{4:8}$ RILs

CHAPTER 2

2. LITERATURE REVIEW

2.1 Origin and Spread of Common Bean

The origin of bean can be traced to Mexico and Central America (Sigh et al. 1991). This region holds the greatest diversity of the genus *Phaseolus*, hosting seventy-eighty species (Freytag et al. 2002). The genus *Phaseolus vulgaris* is the most widely spread globally with highest consumption, nutritional and commercial value (Gepts. 2010). Beans are mostly categorized into Andean and Mesoamerican gene pools (Chavez-Servia et al. 2010.) Due to wide adaptability, Bean is grown in all five continents in under various climatic conditions (Chavez-Servia et al. 2016). The various local names given to landraces in Africa indicate that bean growing in Africa began before colonial time (Allen et al. 1989). In central and southern Africa, bean spread in the sixteenth century by Arab and Swahili merchants (Wortman et al. 1994).

2.2. Common Bean Production

Worldwide, 12 million metric tonnes of bean is produced with Brazil and Mexico been leading producers and consumers contributing 24% to world production. Bean growing in Africa is a major crop. It is ranked second most important source of human dietary protein and third most important source of carbohydrate (Pachico, 1993.) Bean production and trade in Africa is concentrated in areas with high population density. East Africa contributes 25% to world bean production with consumption exceeding 50 kg per capita per year (Jaetzold et al. 1993). Bean demand is projected to keep rising in tandem with human population growth. Bean production has not increased proportionally with human population growth. In many countries bean

consumption is greater than production (Chavez-Servia et al. 2010). Zambia for instance with annual production level of 23 000 metric tonnes and consumption rate of 10 kg per capita per year, grapples with annual bean deficit of 500 metric tonnes (Sichilima et al. 2016). The deficit is meet through imports. Thus to meet the growing world demand for bean, production constraints need to be addressed for production to increase.

2.3. Common Bean Production Constraints

Bean production is faced with numerous biotic, abiotic and agronomic challenges. Constraints to bean production results in low productivity with average yields in Africa ranging from 0.6 – 0.8 metric tonnes per ha (FAO, 2014.). The nature of challenges and importance vary depending on the region. Major challenges in Africa resulting in seed yield loss of 0.4 metric tonnes per ha, include angular leaf spot, Low soil nitrogen availability, bean stem maggot, low phosphorous availability, aphids, bean common mosaic and aluminum and magnesium toxicities (Wortmann et al. 1994). Aluminum toxicity is one of the main constraints to bean production in Africa.

2.4. Acidic soils and Associated Aluminum Toxicity

Acidification of soils emanates from acidic parental material from which the soil developed and leaching of basic cations resulting from excessive rainfall exacerbates the problem (Fageria et al. 2008). Further, industrial pollution results in formation of acid rain coupled with continuous use of nitrogenous fertilizers significantly contribute to acidification of soils (Haynes et al. 2001). Industrialization and intensification of agriculture are significant factors that increase soil acidity.

Poor fertility of acidic soils is due to several factors. Toxicity resulting from hydrolyzed Al is the most limiting constraint for crop production on 67% of global acidic soil (Hede et al. 2001). It was estimated that 50% of the world's potential arable land including land where common bean is produced is characterized by acidic soils (von Uexküll and Mutert, 1995). In Eastern, Central and Southern Africa Soil acidity and associated aluminum toxicity affect 52% of bean producing

areas (Beebe et al. 2014). Al toxicity accounts for 25 – 80% yield loss for crop production on acidic soils (Singh et al. 2011). Thus, Al toxicity is a major constraint to common bean productivity.

Favorable crop growth requires suitable soil pH because it affects availability, absorption and utilization of nutrients. Favorable crop growth occurs around Neutral pH (McCauley et al. 1999). Lower pH limit recommended for bean production is 5.8 (Anderson et al. 2013). Thus bean production is greatly hampered on acidic soils. Acidic soils by definition are soils with a pH of 5.5 and below (Kochian et al. 2004). The dissolution of Al is accelerated on acidic soils (Moore, 2001). Hydrolyses of Al progresses steadily as soil acidity increases in solution such that the trivalent species, Al^{3+} dominates in more acidic condition, whereas the $\text{Al}(\text{OH})^{2+}$ (divalent) and $\text{Al}(\text{OH})_2^+$ (monovalent) species form as pH increases towards neutral pH and in alkaline condition Al exists in solid compounds such as aluminum hydroxide ($\text{Al}(\text{OH})_3$) (Delhaize et al. 1995).

Many factors accounts for poor fertility of acidic soils including deficiencies of Calcium, Magnesium, Molybdenum and Phosphorus as well as toxicities of Aluminum and Manganese (Hede et al. 2001). However, Al toxicity is the most limiting constraint for crop production on 67% of the total acidic soil by area (Hede et al. 2001). Worldwide, 30% of total arable land is characterized by acidic soils and 60% of acidic soils occur in the tropics and subtropics (Kochian et al. 2004).

2.5. Phytotoxic Effect of Al on Plant Growth

The toxic effect of aluminium severely limits root development and resulting in poor yield (Havlin, et al. 2005). In comparison with other crops, common bean is considered relatively sensitive to aluminium toxicity (Beebe, 2016). The excess exchangeable Al^{3+} ions is the major growth limiting factor on many acidic soils. Al toxicity symptoms in plants are manifested in reduced root and shoot growth as well as reduction in biomass accumulation (Poschenrieder et al. 2008). Within 2 hours of growth in soil with toxic levels of Al, inhibition of root elongation occurs ((Kochian et al. 2004). Al^{3+} ions in soil solution hinders cell division in plant roots, interferes with nodule initiation, reduces phosphorous availability forms in soils, decrease root respiration, interferes with enzymes governing the deposition of sugars in cell wall, increase cell

wall rigidity, and reduces uptake, transport and use of plant nutrients and water by plants (Havlin, et al. 2005).

2.6. Mechanism of resistance to Al toxicity in Common Bean

Excess Al in the soil solution triggers the production of organic acids in plants that are involved in detoxification of excess Al either internally in the root or externally in the rhizosphere (Mossor-Pietraszewska. 2001). Aluminum detoxification in cells involves release of citrate anions that form stable compounds with cytosolic Al^{3+} ions followed by sequestration of the compound into the vacuole (Kochian et al. 2005). External detoxification involves secretion of organic acids by the plant into the rhizosphere that binds Al^{3+} ions making it non-phytotoxic. In Snapbean, Mayasaka et al. (1991) found that Al tolerant genotype secreted 10 times more citric acid compared to the sensitive cultivar. In wheat Delhaize et al. (1993) demonstrated that Al tolerant genotypes secreted 5 – 10 times more malic acid than Al sensitive genotypes. Organic acids have a chelating effect on toxic Al^{3+} ions in plants forming stable compounds that are non-phytotoxic to plants.

2.7. Amelioration of the effect of Al toxicity

Globally, liming is a common practice employed to neutralize soil acidity. Lime application at proper dosage reduces soil acidity and subsequently ameliorates the adverse effects of Al and Mn toxicities (Fageria et al. 2008). In sustainable strategies for improving crop productivity on acidic soils, tolerant varieties are used which reduces lime requirement (Hede et al. 2001). Liming is an additional input on crop production costs and is not effective at neutralizing subsoil acidity (Kochian et al. 2004). In other localities farmers have adopted environmentally degrading practices to improve crop production on acidic soils. In Northern Zambia for instance where acidic soils are widely spread, shifting cultivation is practiced to provide ash to ameliorate effect of Al toxicity in the soil (Giller et al. 2004). Breeding and utilization of Al tolerant varieties can greatly contribute to increased productivity on acidic soils and reduce the cost associated with application of lime. Development of varieties resistant to aluminum toxicity is expected to improve bean productivity on acidic soils and extend production hectareage to marginal areas (Everson et al. 2002). To breed genotypes with improved resistance to Al toxicity, reliable and efficient screening methods must be developed and employed by the breeder (Hede et al. 2001).

Phenotypic evaluation employed with marker assisted selection would improve efficient of developing Al toxicity resistant varieties in beans.

2.8. Al toxicity tolerance evaluation in Nutrient solution

Hydroponic technique is a widely used system for evaluation of genotypes for resistance to Al toxicity. Plant nutrient solution of varying Al concentrations have being used in several studies for screening genotypes for tolerance to Al toxicity (Rodrigues et al. 2017, Rangel et al. 2005, Wu et al 2000). In a QTL study, hydroponic system with two nutrient solutions; the control containing 0 μM Al and the stress solution with 20 μM Al were used to conduct phenotypic evaluation of genotypes for resistance to al toxicity in beans (Lopez-Marin et al. 2009). Evaluation in nutrient solution is cost effective. It provides for a procedure of solely investigation the effects of Al on plants and eliminates possible effects of other mineral interactions that arise in acid soils such as those of Manganese toxicity and Phosphorous deficiency. Important caution highlighted by Singh et al. (2011), is that hydroponic screening require validation with evaluation under homogeneous target acidic soil in field condition to increase reliability of molecular tools developed.

2.9. Genetics of Al toxicity resistance

Crops and varieties of the same crop species differ widely in their susceptibility to aluminum toxicity, thus resistance to aluminum toxicity is genetically controlled (Havlin et al., 2005). Further, resistance to Al toxicity in common bean is reportedly, a quantitatively inherited trait (Araujo et al. 1992). Measurements of root traits and derived variable obtained by comparing measurements of non-stressed to stressed plants are common parameters used in several studies for detection of QTLs for Al resistance. In a study on common bean by Lopez-Marin et al. 2009, Sixteen QTLs for resistance to Al toxicity were detected on eight chromosomes. Using only the relative variable of root length reduction of non-stress compared to stress plants in rice, three QTLs for resistance to Al toxicity were identified (Wu et al. 2000). QTLs associated with resistance to Al toxicity have been identified in other crops such as Alfalfa, Maize, Wheat, Soybean and Rye (Singh et al. 2011).

Comparing biotic to abiotic constraints in common bean, little information is known about genetics of resistance to abiotic stresses such as resistance to Al toxicity (Panthania et al 2014.)

Accurate understanding of the basis of resistance to aluminum toxicity in common bean is currently lacking. QTL mapping associated with resistance to aluminum toxicity will thus, provide insight on the genomic regions that govern the trait in bean. Identification of candidate genes controlling the trait will lead to marker development for use in selection of genotypes for resistance to aluminum toxicity. Marker assisted selection (MAS) coupled with phenotypic selection improves efficiency of breeding compared to conventional approaches alone (Collard, 2005).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Plant Material

A mapping population of 150 F_{4:8} RILs derived from a cross between two Andean parents Solwezi and AO-1012-29-3-3A (AO) was used. Solwezi is resistant to Al toxicity while AO is susceptible. This mapping population was derived using single seed descent method and was used in two recent QTL mapping studies (Kamfwa et al., 2018; Kamfwa et al., 2019). Solwezi is a climber (Type III growth habit) with a red mottled seed type. It is a Zambian landrace and widely grown in Zambia. AO is a determinate dark red kidney bean variety developed and released cooperatively by Sokoine University of Agriculture, Oregon State University, USDA-ARS and the University of Puerto Rico.

3.2 Phenotypic Evaluations for Al toxicity Resistance

The parents and RILs were evaluated in the screen house at University of Zambia (Latitude-15.39°S, Longitude-28.33°E), Lusaka Zambia for Al toxicity resistance using a hydroponic system. The hydroponic system had two nutrient solutions with 0 µM Al (control solution) or 15 µM Al (Al stress solution).

The seed for parents and RILs were pre-germinated on petri dishes before transferring them to the nutrient solution. For pre-germination, seeds were sterilized in 1% sodium hypochlorite solution, rinsed in distilled water and placed on filter paper on a clean petri dish. The petri dishes with seed were placed in the incubator at 25°C. Water was lightly sprayed on seed in petri dishes at 24 hours interval during incubation. After 72 hours of incubation, seed embryo development progressed with extension of the radicle. After pre-germination, the seeds were transferred into

plant nutrient solution in the screen house. The control and Al stress nutrient solutions were prepared using a modified protocol by Kerridge and Kronstad (1968). Al was provided in the form of Aluminum Potassium Sulphate dodecahydrate $[\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$. The pH of the solution was adjusted to 4.2 using 0.1 M HCl and 0.1 M NaOH. The nutrient solution was held in 1 liter plastic container. Four uniform pre-germinated seeds for each genotype (RIL and parents) were placed on perforated cork on the lid of the nutrient solution container. The placement was carefully done to ensure the radicle completely immersed in nutrient solution but the shoot grew above the cork on the lid. The 1 litre containers were arranged in a completely randomized design with two replications. The germinated seeds were grown in the nutrient solution for seven days in the screen house at University of Zambia. During the growth period, the aquarium air pump was used to aerate the nutrient solution once per day through the hole on the lid of the container. After seven days of growth, seedlings were removed from the nutrient solution and separated into root and shoot. A measuring ruler was used to measure root length (RL) before roots were dry. The roots and shoots were then put in separate paper bags and then oven-dried at 65°C for 48 hours. After drying, shoot dry weight (SDW) and root dry weight (RDW) were measured using an electronic balance. Three secondary variables i.e., percentage reduction in RL, RDW and SDW were derived from their respective primary variables (variable under Al stress divided variable under control and then multiplied by 100%). These three secondary variables were considered as indices for Al toxicity resistance.

3.3 Phenotypic Data Analysis

Statistical analyses for phenotypic data were conducted in SAS 9.3 (SAS Institute, 2011). A t-test was conducted between the parents for both primary (Root Length, Root Dry Weight and Shoot dry Weight) and secondary traits (derived variable of percentage reduction in stress

compared to control). Analysis of variance (ANOVA) was conducted using PROC MIXED on all the traits based on the following statistical model:

$$Y_{ik} = \mu + \alpha_i + \gamma_k + \varepsilon_{ik}$$

Where: Y_{ik} was the response variable e.g., RDW for genotype i , replication k ; α_i was the fixed variable effect of the genotype (RIL) i ; γ was the random variable effect of a replication; ε was the residual associated with replication k in genotype i .

3.4 DNA Extraction and Genotyping

DNA was extracted from leaves of the 150 RILs and parents grown in the GH at MSU using a previously described protocol (Cichy et al., 2015). DNA samples were genotyped using the Illumina BARCBean6K_3 BeadChip with 5398 SNPs (Song et al., 2015) in the Soybean Genomics and Improvement USDA Laboratory (USDA-ARS, Beltsville Agricultural Research Center) in MD, USA. The SNP genotyping was conducted on the Illumina platform following the Infinium HD Assay Ultra Protocol (Illumina Inc.). SNP alleles were called using GenomeStudio Software from Illumina, Inc.

3.5 Genetic Map Construction

The 5398 SNP markers were filtered for polymorphisms. After this filtering 760 SNPs were polymorphic between Solwezi and AO. These polymorphic SNPs were used to build a genetic linkage map for the 150 RILs using the software JoinMap version 4.1 (Van Ooijen, 2011). In JoinMap additional filtering was done to remove markers with severe segregation distortion from the expected 1:1 ratio from further analyses. Additional filtering was also done to leave one marker on a mapped position if more than one mapped to the same position. In JoinMap markers

were grouped into linkage groups using a logarithm of odds (LOD) score threshold score of 5. A regression mapping procedure was used to order markers within linkage groups, and map distances between markers were estimated from recombination frequencies using the Kosambi mapping function implemented in JoinMap. Linkage maps were displayed using MapChart (Voorrips, 2002).

3.6 QTL Analysis

QTL analysis was conducted using composite interval mapping (CIM) method implemented in the software Win QTL Cartographer version 2.5-011 (Wang et al., 2012). CIM was conducted using the following control parameters: (i) model 6 (Standard model), (ii) 5 control/background markers, (iii) 10 cM window size, and (iv) forward and backward multiple regression model, (v) 1 cM walk speed (genome scan interval). A permutation test (Doerge and Churchill, 1996) for each trait was conducted in QTL Cartographer (1000 permutations) to determine a genome-wide LOD threshold at $P=0.05$ for declaring a QTL significant. The position with the highest LOD score for a given testing region was considered as the position of the QTL. The amount of phenotypic variance explained by a QTL at a given test position was determined using the coefficient of determination (R^2) from the QTL cartographer software program. The QTL were named based on guidelines provided by the Genetics Committee of Bean Improvement Cooperative (Miklas and Porch, 2010). Briefly, the letters at the beginning of the name represents the trait abbreviation, the number that immediately follows the abbreviation, but preceding the period represents the linkage group (which is also the chromosome number), the number after the period represents the number of this QTL in the order of discovery. The single published article Lopez-Marin et al. (2009) was used to order discovery of QTL for Al resistance.

CHAPTER 4

4 Results

4.1 Phenotypic Analysis

The t-test results (Table 1) revealed significant ($P<0.01$) differences between the parents Solwezi and AO in RL and RDW in the control and Al stress solutions. Root length for Solwezi was longer in both the control (10.87 cm) and Al stress solutions (7.5 cm) than AO in the control (8.75 cm) and Al stress solutions (6.12 cm). However, there were no significant differences between Solwezi and AO for percentage reduction in root RL. Solwezi had higher RDW in both control (36.0 mg) and Al stress solution (34.7 mg) than AO in control (23.0 mg) and Al stress solution (12.5 mg). Also, the percentage reduction in RDW for Solwezi (22.7%) was significantly less than that for AO (48.9%). Significant ($P>0.01$) differences in SDW between Solwezi and AO were observed in the control but not Al stress solution. The percentage reduction in SDW for AO (12.9%) was significantly ($P<0.01$) greater than that for Solwezi (0.33%).

Analysis of variance results (Table 2) show that there were significant ($P<0.01$) differences between RILs in RL, RDW and SDW in both control and Al stress solutions. Also, differences in percentage reductions in RL, RDW and SDW between RILs were significant ($P<0.01$). The mean RL for the RILs in the control was 10.1 cm compared to 7.1 cm in Al stress solution. The mean percentage reduction in RL for the population was 26.9%. The mean RDW for the population in the control was 30.97 mg compared to 22.64 mg in Al stress solution. The mean percentage reduction in RDW for the population was 27%. The histograms (Figure 1 - 9) for all

six primary and secondary traits showed a continuous distribution. Also, transgressive segregation on both sides of the spectrum was observed for all six traits.

Table. 1. Summary of t - test results for Means of root length (RL), root dry weight (RDW), shoot dry weight (SDW) and percentage reductions (in RL, RDW and SDW) for parents Solwezi and AO-1112-29-3-3A (AO) evaluated in 0 μ M Al and 15 μ M Al solutions.

Genotype	RL 0 μ M Al (cm)	RDW 0 μ M Al (mg)	SDW 0 μ M (mg)	RL 15 μ M Al (cm)	RDW 15 μ M Al (mg)	SDW 15 μ M Al (mg)	RL reduction %	RDW reduction %	SDW reduction %
Solwezi	10.87 \pm 0.4	36.0 \pm 50.3	228.5 \pm 1.3	7.5 \pm 0.2	34.7 \pm 0.4	227.5 \pm 1.3	30.9 \pm 1.0	22.7 \pm 0.8	0.33 \pm 0.01
AO	8.75 \pm 0.14	23.5 \pm 2.0	276.3 \pm 1.3	6.12 \pm 0.24	12.5 \pm 0.41	240.5 \pm 4.2	29.98 \pm 2.6	48.9 \pm 2.3	12.94 \pm 0.6
t statistic	4.72**	6.75**	28.47**	4.37**	32.91**	5.24**	0.33 ^{ns}	5.42*	20.5**

^{ns} not significant

*Significant at 0.05 probability level

**Significant at 0.01 probability level

\pm Standard Error of the mean

Table. 2. Summary of ANOVA results for Means and ranges of root length (RL), root dry weight (RDW), shoot dry weight (SDW) and percentage reductions (in RL, RDW and SDW) for 150 RILs evaluated in 0 μM Al and 15 μM Al solutions.

Genotype	RL 0 μM Al (cm)	RDW 0 μM Al (mg)	SDW 0 μM (mg)	RL 15 μM Al (cm)	RDW 15 μM Al (mg)	SDW 15 μM Al (mg)	RL reduction %	RDW reduction %	SDW reduction %
Mean									
Squares	4.8**	99.22**	7029.55**	6.22**	100.8**	6364.51**	452.39**	171.78**	389.43**
Mean	10.12 \pm 0.4	30.97 \pm 0.6	258.84 \pm 5.4	7.40 \pm 0.4	22.64 \pm 0.8	234.76 \pm 4.5	26.97 \pm 4.8	26.98 \pm 3.1	9.03 \pm 1.6
Min	6.13	14.15	123.0	1.88	9.5	114.8	2.63	1.19	0.11
Max	14.12	53	394.0	11.13	47.5	362.8	69.33	71.01	45.28
CV	5.2	3.1	3.0	7.3	4.7	2.7	25	16.4%	24.9
LSD	1.04	1.89	0.15	1.07	2.13	12.59	13.3	8.72	4.44

^{ns} not significant

*Significant at 0.05 probability level

**Significant at 0.01 probability level

\pm Standard Error of the mean

Reduction % is derived variable calculated by finding the difference of a means in 0 μM Al and 15 μM Al solutions expressed as a % of the mean in 0 μM Al.

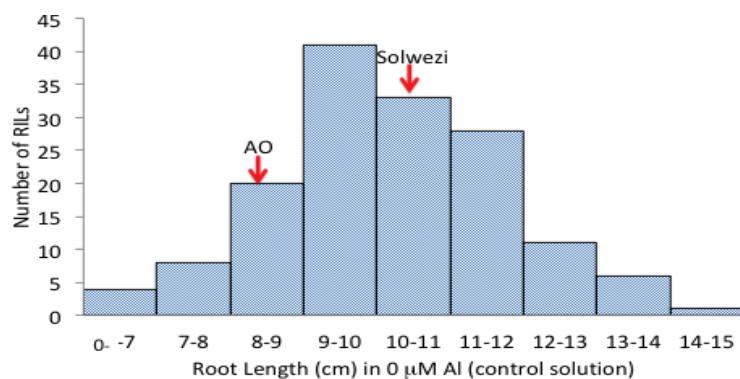


Figure 1.1. Histogram of 150 RILs and the parents (Solwezi and AO) grown in non stressed (0 μ M Al) solution for root length.

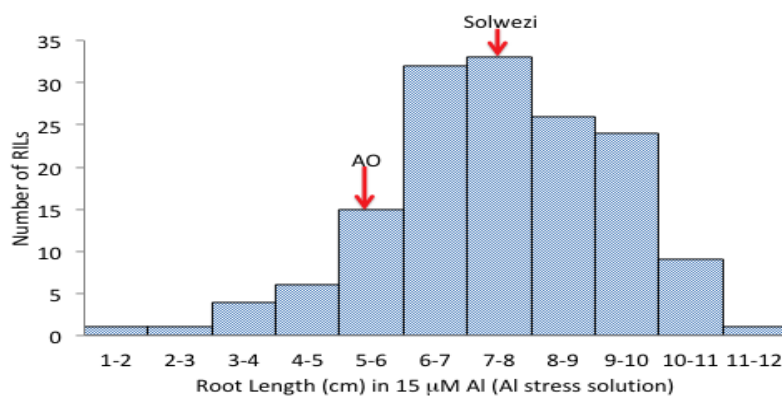


Figure 1.2. Histogram of 150 RILs and the parents (Solwezi and AO) grown in stressed (15 μ M Al) solution for root length.

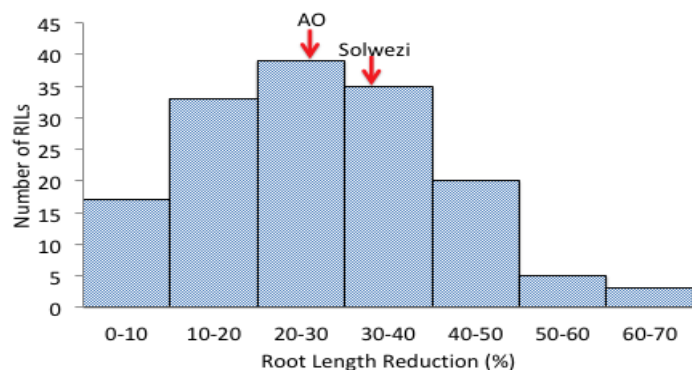


Figure 1.3. Histogram of 150 RILs and the parents (Solwezi and AO) grown in non stressed (0 μ M Al) and stressed (15 μ M Al) solution for root length reduction %.

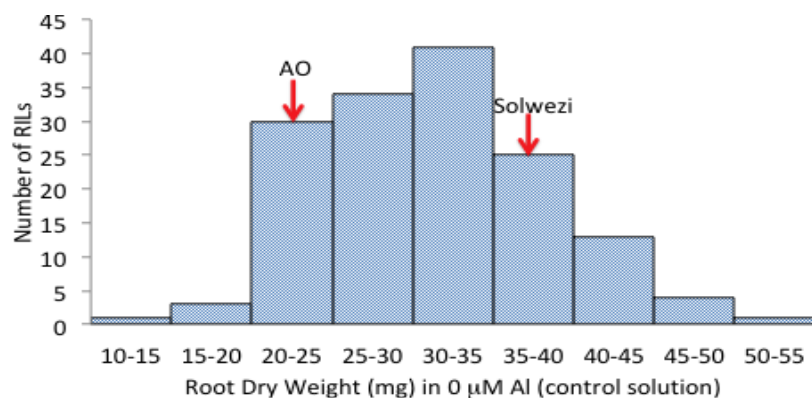


Figure 1.4. Histogram of 150 RILs and the parents (Slowezi and AO) grown in non stressed ($0 \mu\text{M Al}$) solution for root dry weight.

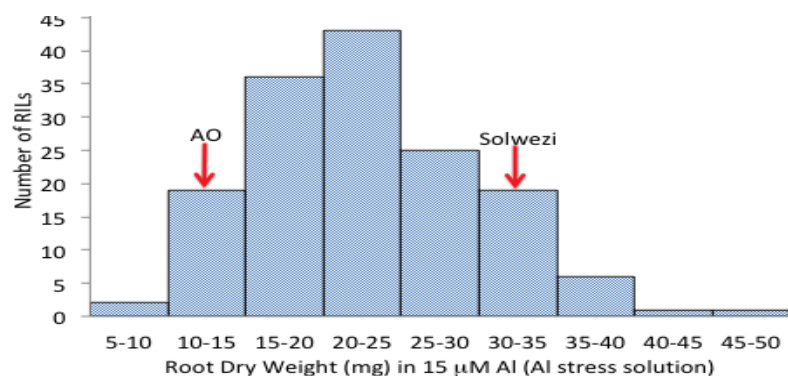


Figure 1.5. Histogram of 150 RILs and the parents (Slowezi and AO) grown in non stressed stressed ($15 \mu\text{M Al}$) solution for root dry weight.

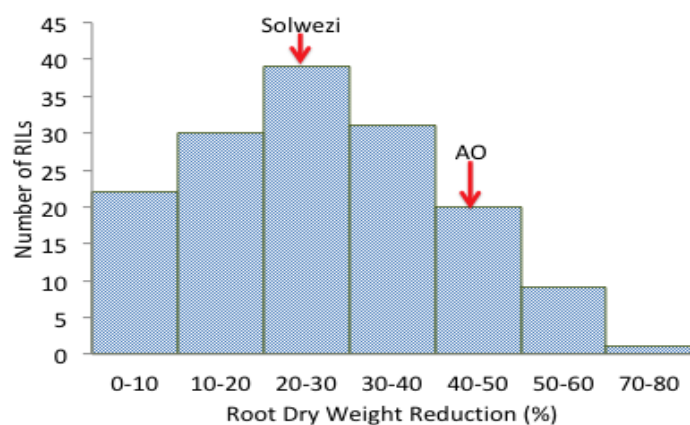


Figure 1.6. Histogram of 150 RILs and the parents (Slowezi and AO) grown in non stressed ($0 \mu\text{M Al}$) and stressed ($15 \mu\text{M Al}$) solution for root dry weight reduction %.

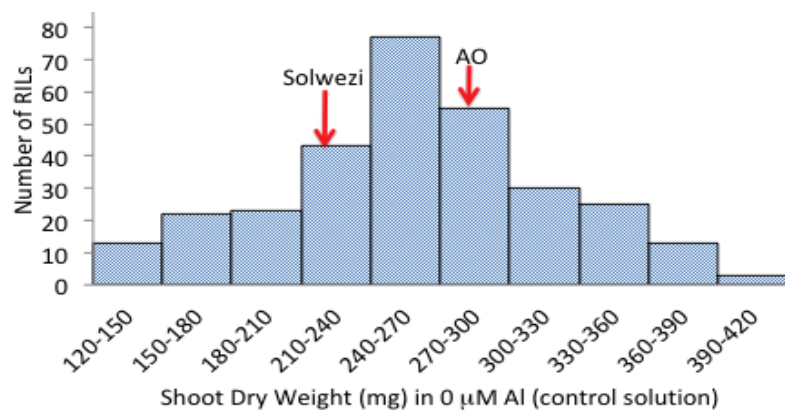


Figure 1.7. Histogram of 150 RILs and the parents (Solwezi and AO) grown in non stressed (0 μ M Al) solution for shoot dry weight.

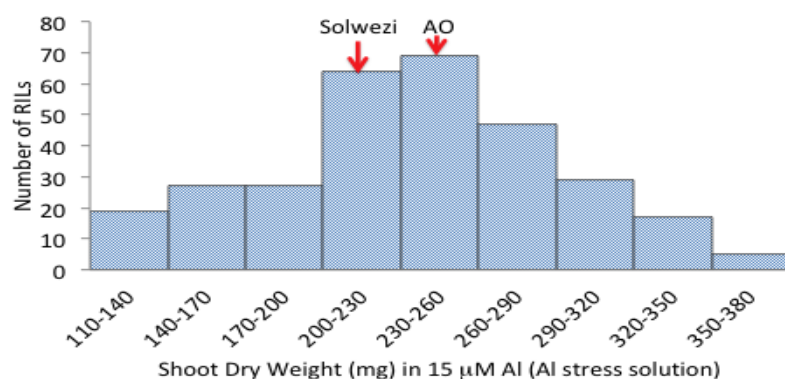


Figure 1.8. Histogram of 150 RILs and the parents (Solwezi and AO) grown in stressed (15 μ M Al) solution for shoot dry weight.

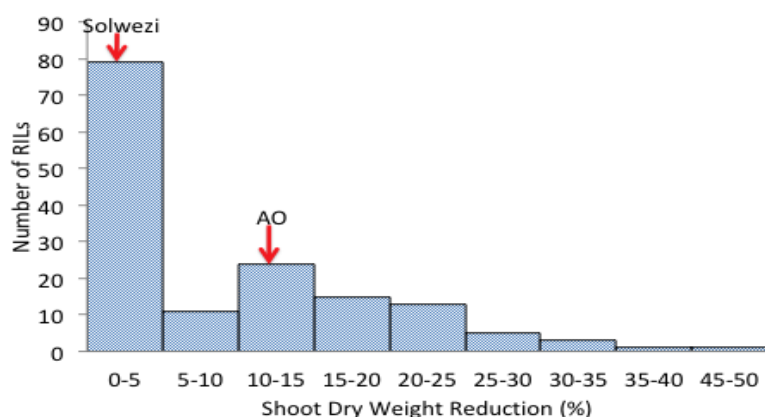


Figure 1.9. Histogram of 150 RILs and the parents (Solwezi and AO) grown in non stressed (0 μ M Al) and stressed (15 μ M Al) solution for shoot dry weight reduction %

4.2 Map Construction

A total of 11 linkage groups (representing 11 chromosomes) with a total genetic distance of 613.6 cM were constructed from 518 SNPs markers. The number of SNPs per linkage group ranged from 16 on Pv01 to 84 on Pv04.

4.3 QTL Analysis

A QTL was considered to be associated with resistance to Al toxicity based on two criteria used by Lopez-Marin et al. (2005): (i) if the QTL was identified in Al stress solution but not in the control, (ii) if the QTL was for percentage reduction in RL, RDW or SDW. Based on these criteria, a total of eight QTLs for Al toxicity resistance were identified (Table 3). Four QTLs for RDW and SDW were identified in the control solution (Table 3), and were not considered to be for Al toxicity resistance.

4.3.1 Total Root Length

Four QTL R12.1, R16.1, R17.1 and R110.1 for RL were identified on chromosomes Pv02, Pv06, Pv07 and Pv10. The QTL R12.1, R16.1 and R17.1 with R^2 values 7.7%, 9.7%, 8.1% and 9.5%, respectively, were for percentage reduction in RL. The parent Solwezi contributed positive allele at the QTL R12.1 while parent AO contributed the positive allele at QTL R16.1 and R17.1. The QTL R110.1 for RL was identified in Al stress solution but not in the control, thus it was considered to be for resistance to Al toxicity. R110.1 explained 9.5% variation in RL and the parent Solwezi contributed the positive allele.

4.3.2 Root Dry Weight

A total of three QTL Rdw4.1, Rdw7.1 and Rdw10.1 for RDW were identified on Pv04, Pv07 and Pv10. The QTL Rdw4.1 ($R^2=7.6\%$) and Rdw7.1 ($R^2=7.6\%$) were identified in the control solution, and were considered not to be associated with Al toxicity resistance. The QTL Rdw10.1 ($R^2=10.3\%$) for RDW was identified in the Al stress solution but not in the control. This QTL was considered to be for Al toxicity resistance. The Al-tolerant parent Solwezi contributed the positive allele at the QTL Rdw10.1. The QTL Rdw10.1 (for RDW) and Rl10.1 (for RL) co-localized on Pv10.

4.3.3 Shoot Dry Weight

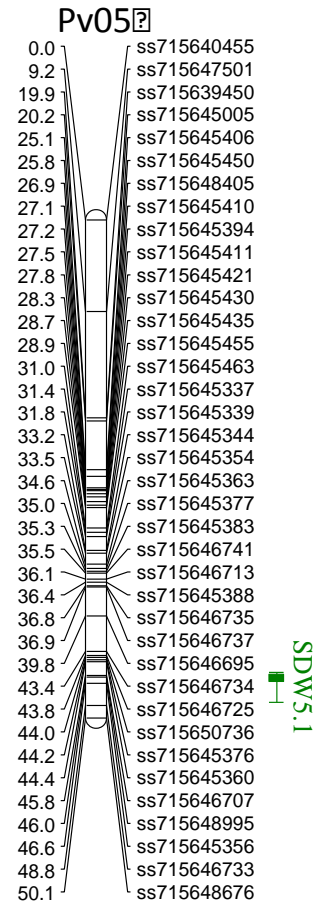
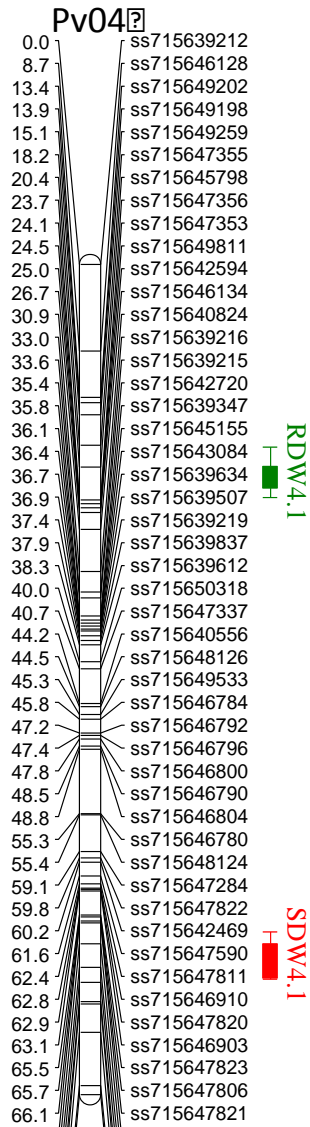
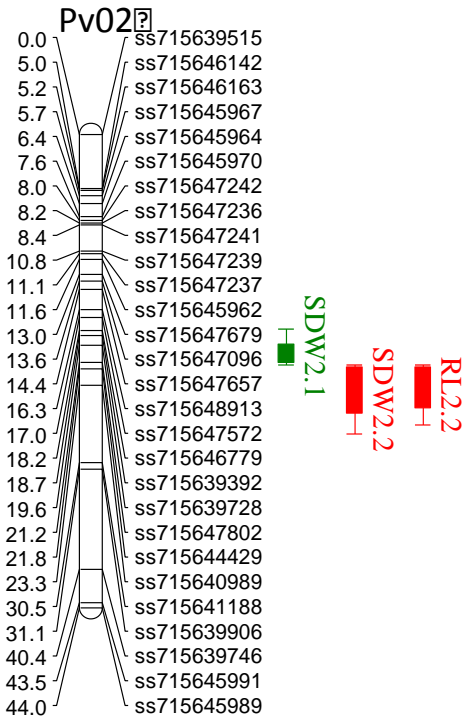
A total of three QTL for SDW were identified on Pv02 (Sdw2.2), Pv04 (Sdw5.1) and Pv09 (Sdw9.1). The QTL Sdw2.2, Sdw4.1 and Sdw9.1 were identified in Al stress solution but not in the control, therefore they were considered to be for Al toxicity resistance. The R^2 values for Sdw2.2, Sdw4.1 and Sdw9.1 were 8.5%, 10.2% and 14.7%, respectively, and the parent Solwezi contributed the positive alleles at all three QTL.

Two QTL (Sdw2.1 and Sdw5.1) were identified in the control solution. These two QTL were considered not to be associated with Al toxicity resistance.

Table 2. Quantitative trait loci for root length (RL), root dry weight (RDW), shoot dry weight (SDW) and percentage reductions (in RL, RDW and SDW) identified in a population of 150 recombinant inbred lines (RILs) derived from a Solwezi x AO-1012-29-3-3A.

Trait	QTL Name	CHR	Treatment	Nearest Marker (Position in Mb)	QTL Interval (Mb)	LOD	R ² %	Eff	Source
Root length	RL10.1 ^{SA}	Pv10	15 µM Al	ss715646970 (37.9)	40.0 - 37.9	3.4	9.5	0.6	SZ
Root length	RL2.1 ^{SA}	Pv02	% Reduction	ss715640989 (17.5)	17.5-18.5	3.0	7.7	4.3	SZ
Root length	RL6.1 ^{SA}	Pv06	% Reduction	ss715649019 (17.9)	3.1 – 17.9	3.5	9.7	4.6	AO
Root length	RL7.1 ^{SA}	Pv07	% Reduction	ss715648692(2.3)	1.8 – 2.3	3.1	8.1	4.1	AO
Root Weight	RDW4.1 ^{SA}	Pv04	0 µM Al	ss715645798 (43.8)	43.8 - 45.3	3.8	7.6	2.0	SZ
Root Weight	RDW7.1 ^{SA}	Pv07	0 µM Al	ss715645208 (50.6)	50.1- 51.0	3.9	7.6	3.0	SZ
Root Weight	RDW10.1 ^{SA}	Pv10	15 µM Al	ss715646970 (38.0)	37.9- 40.0	4.0	8.8	3.0	SZ
Shoot Weight	SDW2.1 ^{SA}	Pv02	0 µM Al	ss715639728 (41.7)	41.0 -42.1	3.8	7.7	20	SZ
Shoot Weight	SDW5.1 ^{SA}	Pv05	0 µM Al	ss715646707 (40.0)	40.0-40.4	4.4	9.6	31	AO
Shoot Weight	SDW4.1 ^{SA}	Pv04	15 µM Al	ss715649433 (0.5)	0.5 -1.7	3.7	10.2	21	SZ
Shoot Weight	SDW9.1 ^{SA}	Pv09	15 µM Al	ss715647170 (34.2)	33.0- 34.2	5.6	14.7	24	SZ
Shoot Weight	SDW2.2 ^{SA}	Pv02	% Reduction	ss715640989 (17.5)	17.0 - 18.5	3.3	8.5	5.6	SZ

QTL = quantitative trait loci; SA=Solwezi x AO-1012-29-3-3A population; CHR = Chromosome; Mb = Million base pair; LOD = Logarithm of odds; R² = Percentage of phenotypic variance explained by the QTL; SZ = Solwezi; AO= AO-1012-29-3-3A; Effe = allelic effect; ss=prefix for SNP name



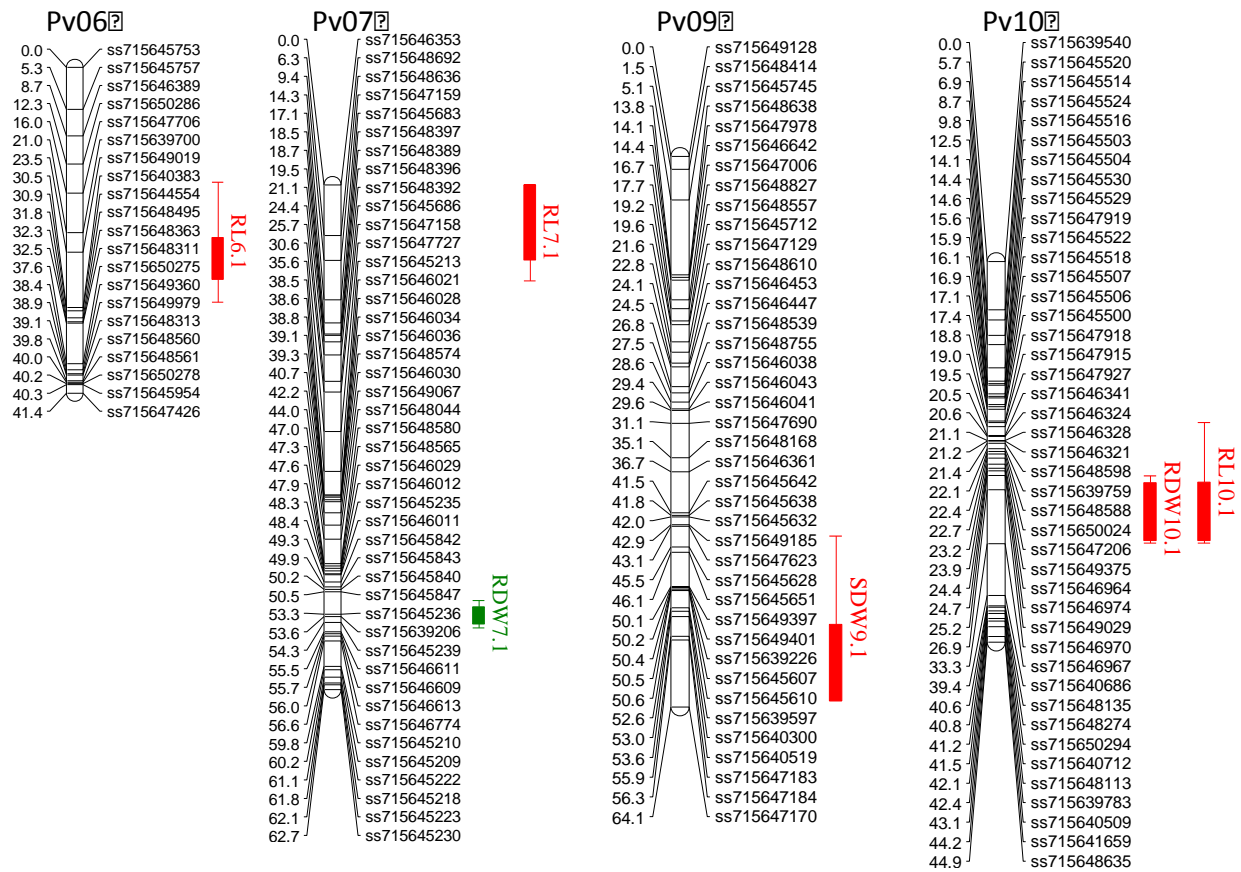


Figure 2. Linkage maps with quantitative trait loci for root length (RL), root dry weight (RDW) and shoot dry weight (SDW) identified in a mapping population of 150 RILs evaluated in 0 μM Al and 15 μM Al solutions in a hydroponic system in the screen house at University of Zambia, Lusaka, Zambia. The QTL in green are for resistance to Al while those in green were not for Al resistance.

CHAPTER 5

5. Discussion

Root development and growth is highly affected by Al toxicity and root traits such as root length and root weight are used in determining Al toxicity resistance. The t-test results from the current study revealed that the parents Solwezi and AO were significantly different in RL, RDW and SDW in both the control and Al stress solutions. These t-test results suggest that Solwezi and AO have different allele combinations at the genes involved in Al toxicity resistance. The parent Solwezi had significantly longer RL and larger RDW than AO parent in both the control and Al stress solutions. Further, the percentage reduction in RDW for Solwezi was smaller than for AO. These RL, RDW and percentage reduction results confirmed that Solwezi was more resistant to Al toxicity than AO. The difference in Al toxicity resistance between Solwezi and AO could be due to differences in their adaptation to Al toxicity. Solwezi is a Zambian landrace that is well adapted and widely grown in the northern part of Zambia where Al toxicity is prevalent. The genotype AO was developed in Puerto Rico, is an introduction to Zambia and may lack adaptation to Al toxicity.

The histograms for all six traits in the current study showed a continuous distribution suggesting that Al toxicity resistance in the mapping population is a quantitatively inherited trait. This result was supported by the multiple QTL identified for Al toxicity resistance. A total of eight QTLs were identified for RL, RDW and SDW, and their respective relative variables on Pv02, Pv04, Pv06, Pv07, Pv09 and Pv10. These eight QTL were considered to be the genetic basis of Al resistance variation observed in the mapping population. The R^2 values for these QTLs ranged from 7.6% to 14.7% with most QTL having R^2 less than or equal to 10%. This suggests that Al

resistance in the mapping population is polygenic involving mainly QTL with $R^2 \leq 10\%$ and additive action. The genetic complexity of Al resistance reported in the current study is consistent with previous reports of polygenic control in common bean (Lopez-Marin et al., 2005), arabidopsis (Kobayashi and Koyama 2002; Hoekenga et al. 2003; Kobayashi et al. 2005), sorghum (Magalhaes et al. 2004), rice (Nguyen et al., 2002) and maize (Ninamango-Cardenas et al., 2003). Both parents Solwezi and AO contributed positive alleles at the identified QTLs for Al toxicity resistance. This result was supported by the transgressive segregation that was observed for all six traits in the current study. The transgressive segregants with higher resistance than the tolerant parent Solwezi could be used in breeding for enhanced Al toxicity resistance.

Two QTL RL2.1 and SDW2.2 for Al resistance identified in this study using RL and SDW, respectively, co-localized on Pv02. These QTL mapped to the genomic region near the previously reported QTL Srl2.1 for Al resistance (Lopez-Marin et al. 2009). Possibly, the QTL RL2.1 and SDW2.1 identified in this study overlaps with Srl2.1 for Al resistance (Lopez-Marin et al. 2009) and may be the same. This overlap is possible because of the extensive linkage disequilibrium in populations of RILs and large confidence interval for the previously identified QTL Srl2.1 (Lopez-Marin et al. 2009). In the current study, a QTL RL7.1 for Al resistance was identified in the distal region of Pv07. Lopez-Marin et al. (2009) also reported two QTLs for Al resistance, Ard7.1 and Srl7.1 on the distal region of Pv07. The QTL RL7.1 identified in the current study most likely overlaps with either one of these two previously identified QTLs on Pv07. The possible overlap of QTLs on Pv02 and Pv07 reported in the current study and by Lopez-Marin et al. (2009) may suggest that these QTLs underlie genes involved in Al resistance with stable expression in different genetic backgrounds.

Two QTL (Rdw4.1 and Rdw7.1) for RDW and two QTL (Sdw2.1 and Sdw5.1) for SDW were identified in the control solution. These four QTLs for RDW and SDW were considered not to be for AI resistance but could be associated with constitutive expression for root biomass or shoot biomass.

CHAPTER 6

6. CONCLUSION AND RECOMMENDATIONS

This study sought to determine genetic variation for resistance to aluminum toxicity in a mapping population of 150 $F_{4:8}$ recombinant inbred lines (RILs) derived from parents Solwezi and AO-1012-29-3-3A (AO) and identify QTL controlling variation for resistance to aluminum toxicity.

The study revealed significant genetic variation with additive gene action for resistance to Al toxicity in Root length, Root dry weight, Shoot dry weight and % Reduction for these traits between the Parents and among the RILs.

The current study has identified five novel QTLs for resistance to Al toxicity on chromosomes Pv04, Pv06, Pv09 and Pv10 providing further insights into its genetic architecture in common bean.

Two major QTLs identified in this study were SDW4.1^{SA}, SDW9.1^{SA} with R^2 values 10.2 and 14.7 respectively. These QTLs with $R^2 \geq 10\%$ should be validated in population with different genetic backgrounds because they have potential to be used for marker development for utilization in marker-assisted selection to improve the trait of resistance to Al toxicity in common bean.

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APPENDICES

Appendix 1. List of Variables and how to measure

No.	Trait	Abbreviation	Unit	Description
1	Total root length	RL	cm	Each plant removed from at 7 days of growth in nutrient solution, cut to separate roots and shoots. RL and SL measured using a 30 cm ruler.
3	Root Dry Weight	RDW	mg	Cut root and shoot parts each put into separate envelopes, oven dried at 65°C for 48 hours and weighed using analytical scale.
4	Shoot Dry Weight	SDW	mg	
5	Reduction%			Derived by finding the difference between means of measured parameter in 0 μM Al and 15 μM Al solution for RL, RDW and SDW and expressing it as a percentage of the mean in 0 μM Al.

Appendix 2. T - Test Analysis for Parents

	Root Length in 0 μ M Al		Root Length in 15 μ M Al		Root Length reduction %	
	Solwezi	AO	Solwezi	AO	Solwezi	AO
Mean	10.87	8.75	7.50	6.12	30.93	29.98
Standard Deviation	0.85	0.29	0.41	0.48	2.08	5.35
Standard Error	0.43	0.14	0.20	0.24	1.04	2.67
Observations	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0	
df	3		3		3	
t Stat	4.72		4.37		0.33	
P(T<=t) two-tail	0.0092		0.0047		0.76	
t critical two-tail	3.18		3.18		3.18	

	Root Dry Weight 0 μ M Al		Root Dry Weight 15 μ M Al		Root dry weight reduction %	
	Solwezi	AO	Solwezi	AO	Solwezi	AO
Mean	36.50	23.50	34.7	12.50	22.70	48.90
Standard Deviation	0.58	4.03	0.82	0.82	1.56	5.32
Standard Error	0.29	2.02	0.40	0.41	0.78	2.36
Observations	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0	
df	3		3		3	
t Stat	6.75		32.91		5.48	
P(T<=t) two-tail	0.0066		5.24E-08		0.011	
t critical two-tail	3.18		3.18		3.18	

	Shoot dry weight in 0 μ M Al		Shoot dry weight in 15 μ M Al		Shoot dry weight reduction %	
	Solwezi	AO	Solwezi	AO	Solwezi	AO
Mean	228.50	276.25	227.5	240.5	0.33	12.94
Standard Deviation	2.65	2.06	2.65	4.20	0.01	1.22
Standard Error	1.32	1.031	1.32	2.10	0.003	0.61
Observations	4	4	4	4	4	4
Hypothesized Mean Difference	0		0		0	
df	3		3		3	
t Stat	28.47		5.24		20.50	
P(T<=t) two-tail	1.24E-07		0.003368		0.000254	
t Critical two-tail	3.18		3.18		3.18	

Appendix 3. Analysis of Variance Table for SA Population

Variate: Root Length (0 μM Al)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	1.521	1.52	5.45	
Genotype	151	724.24	4.80	17.2	<.001
Residual	151	42.10	0.28		
Total	303	767.87			

Variate: Root Length (15 μM Al)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	1.22	1.22	4.13	
Genotype	151	938.81	6.22	21.07	<.001
Residual	151	44.55	0.30		
Total	303	984.57			

Variate: Root Dry Weight (0 μM Al)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	6.37	6.37	6.96	
Genotype	151	14982.6	99.22	108.4	<.00
		7	3	7	1
Residual	151	138.13	0.914		
Total	303	15127.1			

Variate: Root Dry Weight (15 μM Al)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	0.021	0.021	0.02	
Genotype	151	15227.6	100.8	87.2	<.00
		8	4	1	1
Residual	151	174.604	1.16		
Total	303	15402.3			

Variate: Shoot Dry Weight (0 μM Al)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	15.21	15.21	0.26	
Genotype	151	1061462	7029.55	119.51	<.001
Residual	151	8882.04	58.82		
Total	303	1070359			

Variate: Shoot Dry Weight (15 μM Al)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	7.27	7.27	0.18	
Genotype	151	961041.1	6364.51	156.67	<.001
Residual	151	6134.23	40.62		
Total	303	967182.6			

Variate: Root Dry Weight Reduction %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	19.79	19.79	1.01	
Genotype	151	68310.78	452.39	23.2	<.001
Residual	151	2944.12	19.5		
Total	303	71274.69			

Variate: Shoot Dry Weight Reduction %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	1	2.926	2.926	0.58	
Genotype	151	25938.48	171.778	34.01	<.001
Residual	151	762.596	5.05		
Total	303	26704			

Variate: Root Length Reduction %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.33	0.33	0.01	
Genotype	151	58803.79	389.43	8.58	<.001
Residual	151	6854.26	45.39		
Total	303	65658.39			

Appendix 4. Genotypic Means for Evaluation of SA population

Genotype	RL 0 μ M Al (cm)	RDW 0 μ M Al (mg)	SDW0 μ M (mg)	RL15 μ M Al (cm)	RDW15 μ M Al (mg)	SDW 15 μ M Al (mg)	RL reduction %	RDW reduction %	SDW reduction %
172	9.50	32.5	253.0	9.25	30.3	246.0	2.63	6.92	2.76
12	9.62	30.8	221.0	9.25	13.3	215.5	3.85	56.90	2.49
148	9.88	53.0	290.2	9.50	42.5	210.0	3.85	19.81	27.64
105	9.38	28.0	173.8	9.00	26.8	169.2	4.17	4.46	2.58
189	8.62	30.3	291.2	8.25	22.0	276.0	4.33	27.27	5.24
184	10.50	23.0	191.2	10.00	22.3	190.5	4.76	3.26	0.39
205	8.00	30.0	224.2	7.50	26.3	197.0	4.83	12.41	12.13
166	11.75	25.3	258.8	11.13	20.5	206.5	5.21	18.80	20.14
85	9.38	26.8	370.8	8.75	23.5	307.0	6.65	12.08	17.20
27	9.12	48.5	352.8	8.50	36.3	340.8	6.92	25.27	3.39
149	9.75	31.5	278.2	9.00	18.8	152.2	7.69	40.48	45.28
45	9.75	32.8	225.8	8.88	29.3	221.8	8.13	10.66	1.75
168	10.12	31.0	263.5	9.25	25.5	231.5	8.14	17.75	12.07
25	11.25	34.0	332.5	10.25	21.3	288.8	8.70	37.50	13.16
43	11.25	26.0	325.8	10.25	19.3	306.5	8.89	25.96	5.91
145	9.38	27.3	263.5	8.50	24.3	258.2	9.32	10.97	1.92
76	11.25	48.3	311.2	10.13	47.5	305.2	9.98	1.55	1.92
34	8.62	30.3	280.2	7.75	29.0	270.0	10.17	4.13	3.66
71	9.62	36.5	273.0	8.63	32.8	268.2	10.43	10.25	1.73
210	9.25	29.5	204.5	8.25	23.5	182.5	10.82	20.32	10.75
158	10.25	27.3	252.2	9.13	26.8	220.0	10.83	1.68	12.75
18	9.12	35.0	291.5	8.13	25.5	290.0	10.92	27.14	0.52
104	10.75	32.3	280.8	9.50	24.0	231.5	11.63	24.27	17.55
37	8.12	29.0	206.5	7.13	22.3	202.5	12.26	23.28	1.94
97	11.12	38.0	257.5	9.75	35.5	250.0	12.46	6.38	2.90
208	10.50	39.3	394.0	9.13	24.8	345.0	13.30	36.94	12.44
68	11.12	40.5	289.5	9.63	35.3	287.2	13.35	12.96	0.79
87	12.00	33.0	224.2	10.38	23.8	168.5	13.57	28.03	24.81
202	9.12	44.3	280.0	7.88	30.0	279.0	13.63	32.19	0.36
57	10.75	25.5	253.2	9.25	21.0	215.5	13.96	17.65	14.89
77	8.75	30.8	246.8	7.50	26.8	236.0	14.22	13.01	4.34
214	11.12	38.0	265.0	9.50	37.0	262.2	14.60	2.63	1.04
42	12.00	37.8	340.2	10.25	32.0	245.8	14.63	14.99	27.73
30	11.12	39.5	335.8	9.50	30.5	331.0	14.65	22.78	1.41
130	8.25	29.0	296.5	7.00	15.8	287.8	15.07	45.65	2.93
209	9.62	40.0	214.2	8.13	28.0	169.0	15.10	29.91	21.08
180	11.50	39.0	343.0	9.75	26.3	233.8	15.22	32.69	31.80
212	10.38	31.3	237.2	8.63	29.3	177.0	16.35	6.39	25.40
140	9.75	23.8	213.8	8.13	19.0	184.5	16.58	19.99	13.69
81	10.38	39.8	306.8	8.63	27.5	261.0	16.98	30.81	14.78
173	9.00	27.3	306.8	6.38	16.8	306.2	29.15	38.52	0.16
183	10.75	22.3	164.2	8.88	11.8	131.0	17.42	47.15	20.19
206	11.00	46.8	280.2	9.00	39.5	278.2	17.50	15.50	0.71
54	11.38	26.5	297.8	9.38	24.0	267.5	17.56	9.37	10.15
28	11.88	29.3	248.2	9.75	25.8	239.2	17.88	11.92	3.54
94	10.00	35.3	266.2	8.13	33.5	260.2	18.67	4.99	2.25
201	12.38	31.8	235.0	10.00	30.8	192.5	19.06	3.15	18.09
125	9.62	26.8	299.0	7.75	19.5	254.0	19.46	27.03	15.05

150	9.62	38.8	266.5	7.75	22.8	261.0	19.57	41.29	2.05
66	11.50	34.3	302.2	9.13	18.5	298.0	19.95	46.13	1.41
120	7.50	18.8	329.5	6.00	9.8	318.8	20.00	47.94	3.25
124	8.50	30.0	291.8	6.75	26.0	285.8	20.31	13.33	2.04
200	7.25	21.5	331.2	5.75	16.5	317.5	20.36	23.26	4.14
17	11.00	29.8	282.0	8.75	25.3	225.2	20.45	15.11	20.11
157	10.38	24.8	318.0	8.25	16.3	262.0	20.47	34.31	17.58
143	10.25	23.3	127.5	8.13	14.3	126.8	20.77	38.71	0.59
163	13.12	33.3	243.0	10.38	30.8	233.0	20.95	7.49	4.11
136	8.25	21.3	257.5	6.50	16.3	253.8	21.05	23.53	1.45
82	11.75	38.0	248.0	9.25	35.5	216.2	21.20	6.58	12.82
127	11.12	25.3	211.8	9.88	23.5	194.5	11.24	6.90	8.15
9	9.88	27.8	215.5	7.75	18.3	209.0	21.54	34.22	3.01
5	9.75	28.5	123.8	7.63	16.0	123.0	21.84	43.86	0.61
174	7.50	24.8	243.0	5.88	18.0	211.0	21.88	27.27	13.16
3	9.00	30.0	338.8	7.00	20.5	293.8	22.22	31.65	13.28
47	9.75	32.0	275.8	7.50	20.8	265.2	23.03	35.16	3.81
15	11.75	28.8	270.2	9.00	17.8	266.2	23.37	38.26	1.48
100	8.50	30.3	255.8	6.50	19.8	202.8	23.53	34.71	20.72
41	9.00	24.3	246.5	6.88	15.8	245.8	23.61	35.03	0.30
109	10.00	28.8	278.2	7.63	23.8	244.8	23.75	17.31	12.04
138	12.00	37.8	177.5	9.13	31.5	119.8	23.78	15.59	32.54
192	12.62	25.5	196.2	9.50	24.3	153.5	24.25	4.90	21.50
190	10.25	32.5	241.5	7.75	16.3	238.0	24.39	50.00	1.45
36	9.12	22.8	260.2	6.88	19.8	205.8	24.74	13.16	20.94
169	10.88	38.3	255.5	8.13	28.0	252.8	25.08	26.72	1.06
197	12.12	33.0	254.0	9.00	30.8	253.5	25.24	6.82	0.20
99	7.88	22.5	268.2	5.88	19.8	263.2	25.45	12.20	1.86
187	11.25	29.5	285.5	8.38	23.5	182.2	25.49	20.29	36.16
142	13.62	44.0	266.2	10.13	34.3	224.5	25.64	22.16	15.65
186	9.12	27.8	163.2	6.75	25.0	160.0	26.02	9.90	1.99
207	12.00	27.8	322.2	8.88	25.3	279.2	26.04	9.01	13.34
75	8.50	25.8	296.5	6.25	25.3	227.0	26.49	1.94	23.43
108	8.88	29.3	224.8	6.38	18.0	223.0	27.88	38.33	0.77
74	8.38	35.0	255.5	6.00	21.3	250.0	28.18	39.28	2.14
107	10.62	22.5	158.0	7.50	20.0	134.5	29.37	10.97	14.87
AO3A	8.50	23.5	276.2	6.00	12.0	240.2	29.41	48.91	13.03
7	10.25	20.8	187.5	7.00	20.5	187.0	29.47	1.19	0.27
69	9.75	41.8	324.5	6.88	23.8	285.5	29.49	43.12	11.98
165	9.25	24.8	161.8	6.50	19.0	153.0	29.61	23.24	5.38
110	11.38	33.3	266.0	7.88	15.3	240.5	29.93	54.14	9.55
111	8.62	22.8	263.5	6.00	12.0	225.2	30.42	47.25	14.52
171	8.62	21.8	288.8	6.00	17.8	227.5	30.42	18.37	21.21
162	9.75	37.0	330.0	6.75	20.8	327.8	30.56	43.93	0.66
19	9.75	39.3	296.2	6.75	21.0	236.8	30.77	46.49	20.09
78	10.88	41.0	260.8	7.50	33.3	236.8	30.87	18.84	9.21
53	10.50	29.5	218.0	7.25	18.3	179.0	30.95	38.13	17.89
Solwezi	10.88	35.5	228.5	7.50	34.3	227.8	31.03	3.52	0.33
213	11.25	41.3	351.5	7.75	30.3	277.5	31.11	26.67	21.04
91	11.88	30.0	210.2	8.13	23.5	204.8	31.22	21.52	2.61
33	10.75	41.0	366.0	7.38	30.0	327.5	31.40	26.83	10.53
156	9.38	35.0	208.5	6.38	17.0	205.2	31.86	51.43	1.56
59	9.38	31.8	307.5	6.38	20.5	217.8	32.01	35.43	29.19

4	10.50	19.3	164.8	7.13	10.3	164.2	32.05	46.76	0.30
112	9.88	30.3	297.5	6.63	21.5	296.5	32.27	28.92	0.34
141	10.75	34.8	244.2	7.25	26.8	211.0	32.58	23.02	13.62
176	11.50	31.5	168.0	7.75	23.8	137.2	32.61	24.50	18.23
159	11.25	25.3	260.8	7.50	24.5	260.0	33.33	2.98	0.29
61	10.00	22.5	123.0	6.63	17.0	121.0	33.75	24.44	1.63
115	7.63	20.3	347.2	5.00	13.5	341.0	33.94	33.33	1.68
114	9.50	35.8	372.2	6.25	29.3	362.8	34.21	18.18	2.55
161	13.25	39.0	312.2	8.63	32.5	303.5	34.90	16.64	2.79
89	10.50	32.8	278.0	6.75	24.8	262.2	35.62	24.42	5.41
153	6.63	24.3	153.8	4.25	16.0	130.2	35.86	34.01	15.24
199	12.50	29.8	149.0	8.00	19.3	146.8	35.93	35.28	1.52
101	12.50	26.8	141.2	8.00	22.8	114.8	35.97	14.95	18.75
48	13.50	40.0	246.2	8.63	24.5	242.8	35.99	38.53	1.44
147	11.75	41.0	223.0	7.50	29.3	214.5	36.05	28.66	3.80
1	8.88	32.0	194.8	5.63	13.3	184.0	36.83	58.59	5.52
117	6.75	14.5	258.2	4.25	9.5	255.2	37.24	34.48	1.16
16	11.00	30.3	337.8	6.88	22.0	327.8	37.50	27.25	2.95
123	10.25	24.5	248.5	6.38	18.5	247.0	37.92	24.49	0.61
181	9.75	38.8	360.2	6.00	28.3	308.2	38.49	27.08	14.43
95	9.88	21.3	252.0	6.00	12.8	250.2	39.23	39.92	0.69
144	10.12	31.5	227.2	6.13	23.0	222.5	39.35	26.98	2.09
8	9.12	27.0	383.8	5.50	21.5	279.0	39.79	20.37	27.22
79	7.50	28.3	298.0	4.50	14.3	273.0	40.00	49.56	8.25
29	9.62	37.0	254.8	5.75	19.0	249.8	40.07	48.65	1.94
182	9.12	32.0	231.5	5.45	21.5	223.2	40.27	32.81	3.57
83	11.88	43.0	331.8	7.00	33.3	264.0	41.00	22.67	20.34
24	10.00	26.5	189.8	5.88	21.5	157.0	41.25	18.87	17.26
55	10.75	32.0	305.2	6.25	18.5	250.8	41.88	42.19	17.83
167	12.00	36.0	255.2	7.00	21.0	240.2	41.91	41.66	5.87
178	10.25	21.8	235.8	5.88	15.8	235.0	42.74	27.21	0.32
179	13.00	41.5	309.8	7.38	30.3	214.2	43.23	27.11	30.74
21	9.75	30.5	355.0	5.50	21.5	351.2	43.44	29.70	1.09
164	12.75	24.5	189.5	7.00	19.3	157.8	45.10	21.43	16.74
32	11.25	27.0	257.5	6.00	17.8	254.8	46.67	34.26	1.06
119	11.62	22.5	144.0	6.13	15.3	141.2	47.22	32.21	1.91
31	9.75	23.0	126.2	5.13	14.3	120.3	47.37	38.04	4.75
58	10.62	32.0	193.5	5.63	27.3	156.8	47.38	14.84	18.99
84	6.50	29.3	192.2	3.38	13.3	192.0	47.62	54.70	0.13
131	10.75	28.5	248.5	5.63	19.3	237.5	47.66	32.78	4.42
98	14.12	45.0	255.0	7.25	30.0	214.8	48.59	33.30	15.78
62	13.00	31.0	222.0	6.63	25.8	218.0	48.85	16.94	1.80
102	8.75	34.5	309.0	4.45	10.0	304.0	49.14	71.01	1.60
132	11.38	39.3	240.5	5.63	18.3	230.0	50.56	53.51	4.36
90	8.62	31.5	291.8	4.13	14.0	256.0	52.10	55.56	12.28
67	8.50	23.8	337.2	4.00	12.0	333.2	52.78	49.47	1.19
113	7.25	18.5	364.5	3.38	12.5	361.5	53.21	31.50	0.82
146	9.12	40.0	243.2	3.88	21.8	216.8	57.51	45.61	10.90
65	8.00	20.3	155.2	3.13	11.0	153.2	60.94	45.67	1.29
116	7.75	24.3	235.2	2.75	14.8	235.0	64.58	39.18	0.11
40	6.13	20.3	188.5	1.88	10.0	175.8	69.33	50.61	6.76
Genotype	RL 0 μM Al (cm)	RDW 0 μM Al	SDW0 μM	RL15 μM Al	RDW15 μM Al	SDW 15 μM Al	RL reduction %	RDW reduction %	SDW reduction %

		(mg)	(mg)	(cm)	(mg)	(mg)			
Mean	10.12	30.97	258.84	7.40	22.64	234.76	26.97	26.98	9.03
Min	6.13	14.15	123.0	1.88	9.5	114.8	2.63	1.19	0.11
Max	14.12	53	394.0	11.13	47.5	362.8	69.33	71.01	45.28
CV	5.2	3.1	3.0	7.3	4.7	2.7	25	16.4%	24.9
LSD	1.04	1.89	0.15	1.07	2.13	12.59	13.3	8.72	4.44
SE	0.37	0.676	5.4	0.38	0.76	4.51	4.76	3.12	1.59
Prob	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001