Assessment and genetic improvement of aluminium tolerance in South African winter bread wheat cultivars

By

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Declaration

I Mamotlole Patricia Motupa, hereby declare that this dissertation, prepared for the degree of *Magister Scientiae Agriculturae*, which was submitted by me to the University of the Free State, is my original work and has not been submitted previously to any other University/Faculty. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

Signature ……..

Dated ……

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List of abbreviations

$AlCl_3$	Aluminium chloride
ALMT1	Aluminium Activated Malate Transporter
ARC	Agricultural Research Council
ARC–SGI	Agricultural Research Council-Small Grain Institute
Ave	Average
ºC	Degrees Celsius
$CaCl_2$	Calcium chloride
CAPS	Cleavage Amplified Polymorphic Sequence
CIMMYT	International Maize and Wheat Improvement Center
DNA	Deoxyribonucleic acid
DH	Double haploid
F_1	First generation
F_2	Second generation
h	Hour(s)
KNO_3	Potassium Nitrate
L	litre(s)
Max	Maximum
Min	Minimum
min	Minute(s)
mm	Millimetre(s)
mM	Millimolar
$MgCl_2$	Magnesium chloride
$NaIO_3$	Sodium Iodine
NIL	Near-Isogenic Lines
NH_4NO_3	Ammonium nitrate
$(N_4H)_2SO_4$	Ammonium sulphate
PEP	Phosphoenolpyruvate
PR	Primary root
pH	Power of hydrogen
QTL	Quantitative Trait Loci
RG	Root re-growth

RL	Root length
ROS	Reactive oxygen species
RTI	Root tolerance index
S	Stained portion of roots
SR	Secondary root
w/v	Weight per volume

CHAPTER 1
General introduction

The enormous economic importance of bread wheat (*Triticum aestivum* L.), the increasing human population and the increasing food demand world wide makes wheat genetic improvement necessary at many levels to ensure food security (Rodriguez Milla & Gustafson, 2001). There is an increase in acidic soil in wheat production areas worldwide, which causes a threat to crop production in these regions (Nava *et al.,* 2006; Zhou *et al.,* 2007). The major growth limiting factor for wheat production on most acid soils is aluminium toxicity (Cosic *et al.,* 1994; Baier *et al.,* 1995; Kikui *et al.,* 2007; Witcombe *et al.,* 2008; Navakode *et al.,* 2009; Ryan *et al.,* 2009).

Plant roots are always exposed to aluminium in some form, fortunately, most of this aluminium occurs as harmless oxides and aluminnosilicates (Matos *et al.,* 2005). Besides the natural occurrence of soil acidity, the extensive use of ammonia and amide-containing fertilisers causes further soil acidification and aggravates aluminium toxicity that contributes to an increase in soil acidity and enhanced aluminium solubility in acid-sensitive soils at low pH (Cosic *et al.,* 1994; Zhou *et al.,* 2007). The use of aluminium tolerant genotypes provides the most effective alternative strategy for production of economically important crops in acid soils as soil improvement by liming is not always economically feasible, especially in highly acidic subsoils (Ma *et al.,* 1997; Echart *at al.,* 2002; Navakode *et al.,* 2009).

The best approach to this abiotic problem is the improvement in the aluminium tolerance of existing crop species so that they may be successfully grown in acidic soils (Tahira & Salam, 2006; Witcombe *et al.,* 2008; Dai *et al.,* 2009). Selection for aluminium tolerance offers an avenue for increasing crop production and reducing the production cost of wheat (Ma *et al.,* 1997; Zhang *et al.,* 2007; Navakode *et al.,* 2009). Wheat genotypes vary widely for aluminium tolerance (Aniol & Gustafson, 1984; Giaveno & Miranda Fihlo, 2000; Zhou *et al.,* 2007; Navakode *et al.,* 2009).

Tolerance to aluminium toxicity is genetically controlled in many plant species (Rincón & Gonzales, 1992; Carver & Ownby, 1995). Tolerance to aluminium toxicity in wheat is controlled by multiple (Aniol & Gustafson, 1984; Rincón & Gonzales, 1992) or single dominant genes (Ryan *et al.,* 1995; Matos *et al.,* 2005).

Various methods have been employed to screen and select wheat genotypes for aluminium tolerance (Polle *et al.,* 1978; Aniol, 1984; Baier *et al.,* 1995; Ma *et al.,* 1997). Aluminium toxicity causes inhibition of root growth by preventing cell division which results in reduced root penetration in the soil and significant yield reduction due to drought stress (Rincón & Gonzales, 1992; Ryan *et al.,* 1995). Rapid, reliable and effective aluminium tolerance screening techniques are needed to discriminate between sensitive and tolerant genotypes in wheat (Polle *et al.,* 1978; Giaveno & Miranda Fihlo, 2000).

The evaluation of root elongation in nutrient solutions can be useful in developing aluminium tolerant genotypes in wheat breeding programmes in a short time as plants at seedling stage can be screened for their relative aluminium sensitivity (Kochian, 1995; Giaveno & Miranda Fihlo, 2000). Selection of aluminium tolerance can be enhanced by screening for aluminium toxicity where the stress is carefully managed and by carefully choosing parents of crosses so that the physiological traits can be pyramided. This implies a reduction in the number of crosses that are made so that larger populations can be employed, an approach that is effective in breeding for multiple gene control (Witcombe *et al.,* 2008).

In order to be able to breed and grow wheat of high quality in high aluminium soils, it is important to know and understand the tolerance levels of genotypes. Selecting genotypes based on the ability of aluminium tolerant seedlings to continue root growth under induced aluminium stress allowed for gene pyramiding in some genotypes. The root growth method uses the root re-growth and root tolerance index to evaluate aluminium tolerance. The root growth parameter indentifies genotypes with good root growth under aluminium stress, but fails to detect aluminium tolerance in genotypes with poor root vigour (Hede *et al.,* 2002). Genotypes with poor root vigour can only be identified using the root tolerance index parameter.

The objectives of this study were to:

1. Identify the most efficient screening method for aluminium tolerance in South African wheat cultivars and to screen known sources of tolerance in order to measure root re-growth and root tolerance index of wheat genotypes, in aluminium containing solutions, in order to establish good levels of aluminium tolerance in local wheat cultivars.
2. To cross selected genotypes with high and low root re-growth in the presence of aluminium, in order to enhance aluminium tolerance.
3. To determine the reciprocal effects of aluminium tolerance in wheat using three F_2 cross combinations and their reciprocals.

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CHAPTER 2
Literature review

2.1 Consumption and economical importance of wheat worldwide

Wheat is one of the most important and widely cultivated crops in the world, occupying 17% of all cultivated land (Dreisigacker & Melchinger, 2004). The global consumption of wheat, which is third after rice (*Oryza sativa* L.) and maize (*Zea mays* L.), continuously increased during the past decades. Wheat is used mainly for human consumption and supports nearly 35% of the world population (Dreisigacker & Melchinger, 2004; Raman *et al*., 2006). Its importance derives from the properties of wheat gluten, a cohesive network of endosperm proteins that stretch with the expansion of fermenting dough, yet hold together when heated to produce a "risen" loaf of bread. Only wheat, and to a lesser extent rye (*Seceale cereal* L.) and *Triticale*, has this property. Wheat is nutritious, easy to transport and to store. Wheat's diversity of uses, nutritive content, and storage qualities has made wheat a staple food for more than one third of the world's population. The demand for wheat is expected to grow faster than for any other major agricultural crop. To meet the needs of the growing world population, the forecast of demand for the year 2020 varies between 840 and 1050 million ton for human consumption (Foreign Agricultural Service, 2002; Dreisigacker & Melchinger, 2004).

2.2 Aluminium toxicity

Aluminium is the most abundant light metal that makes up 7% of the earth's crust and is the third most abundant element after oxygen and silicon (Ma *et al*., 2001). Plant roots are therefore almost always exposed to aluminium in some form. Dissolution of just a small fraction of the aluminium compounds in soil results in serious aluminium toxicity to susceptible plant species. Fortunately, not all forms of aluminium are toxic; it is the soluble forms that are implicated in the toxicity of acid soils. Trivalent cations are toxic to plants in general and Al^{3+} is considered to be the major phytotoxic form, although some studies have implicated the di- and monovalent forms of aluminium also play a role in aluminium toxicity (Tang *et al*., 2000; Ma *et al*., 2001; Delhaize, 2004).

When aluminium is in contact with water the metal undergoes hydrolyses and the Al^{3+} form dominates under acidic conditions that can reach toxic levels for the plants, while the $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ forms are prevalent at a pH level of between 5 and 7 that is not toxic for the plants (Blamey *et al*., 1992; Delhaize, 2004; Panda & Matsumoto, 2007).

As the pH increases, the solid phase aluminium $Al(OH)_3$ can form and under alkaline conditions $Al(OH)_4^{-}$ is the most prevalent form in the soil. This form is then also not accessible for the plants and thus harmless. Aluminium also has the ability to form many ligands that makes the chemistry of aluminium in soil difficult to understand. Even in solutions of known aluminium and pH composition, the effect of various forms of aluminium on roots can be difficult to analyse. Like zinc, manganese, copper and iron, the more acid the soil, the more aluminium will be dissolved into the soil solution. If the pH is allowed to drop much below 5.5, the availability of manganese and aluminium is increased to the point that they could become toxic for plants (Blamey *et al*., 1992; Delhaize, 2004; Panda & Matsumoto, 2007).

Aluminium toxicity is a major factor limiting wheat production on acid soils worldwide (Blamey *et al*., 1992; Luo & Dvořák, 1996; Drummond *et al*., 2001; Jozefaciuk & Szatanik-Kloc, 2001; Tang *et al*., 2000; 2002; 2003; Delhaize, 2004; Kochain *et al*., 2005). Acid soils occur mainly in two global belts: the northern belt, with a cold, humid climate, and the southern tropical belt, with warmer, humid conditions. Wheat producers must contend with acid soils in the USA, Australia, Canada, the Southern Cone region of Southern America, and the Carpathian basin region of Europe, Central Africa, and more recently, South Africa. Locations undergoing increasing acidification include the wheat belts of the USA, Canada, Australia, and South Africa (Carver & Ownby, 1995).

In South Africa, wheat is planted in three distinct environmental conditions. The summer rainfall region of South Africa contributes about 50% of total annual wheat production followed by the winter rainfall region in the Western Cape that contributes 30% and the central irrigation areas including the Northern Cape with 20% of the wheat production annually. These three production areas of South Africa, which are the major wheat production regions, are limited by increasing soil acidification. According to Bosch & Otto (1995) approximately 0.4 million hectares of wheat producing areas in the summer rainfall region of South Africa are considered critically acidic with a pH (KCl) lower than

pH4.5. Most of these areas are in the high rainfall regions of South Africa with good yield potential. In the winter rainfall region of South Africa, another 0.07 million hectares have critical soil acidity. This acidification of soil thus makes the expansion of wheat production in South Africa difficult (Carver & Ownby, 1995).

2.3 Genetics of aluminium tolerance in cereals

Genetic variation in response to aluminium toxicity has been found not only among plant species but also within species and among developed cultivars. These plants differ significantly in their susceptibility to aluminium toxicity in acid soils and these differences are genetically controlled. While most cultivars are sensitive to aluminium, tolerant genotypes can be found in most plant species. Genes encoding aluminium tolerance are mainly found among landraces or minor cereals (rye populations) (Aniol, 2004). When subjected to aluminium stress, the tolerant individuals would have more roots and produce greater shoot yield than the aluminium sensitive individuals (Tang *et al.,* 2001; 2003; Gustafson, 2005; Ma, 2005).

The tolerance to aluminium toxicity exhibited by certain species, and cultivars within species, depend on the prevention of aluminium uptake by roots or upon its detoxification on entering the cytosol. While the expression of aluminium tolerance in wheat appears to be a polygenic trait, e.g. in cultivar Atlas 66 (Tang *et al*., 2002), in other cultivars a large proportion of the tolerance can be attributed to a single dominant gene (Ryan *et al*., 1995).

Over 20 genes induced by aluminium stress have been isolated from a range of plant species, including wheat, rye, rice, soybean (*Glycine max* L.), tobacco (*Nicotiana tabacum*), and *Arabidopsis*. Most of the aluminium-induced genes seem to be general stress genes that are induced by a range of different plant stresses (Mossor-Pietraszewska, 2001; Fontecha *et al.,* 2007).

A single gene controls the inheritance of aluminium tolerance in barley (*Hordeum valgare* L.). While barley cultivars exhibit a range of variation for aluminium tolerance, in many instances this appears to be due to the action of a single locus, with different alleles conferring different degrees of aluminium tolerance (Tang *et al*., 2000). Rye,

barley and sorghum (*Sorghum bicolor* L.), like wheat, have an inheritance pattern with a single locus explaining the genotypic differences (Panda & Matsumoto, 2007).

More recently, a gene that activates citrate secretion has been isolated and associated with aluminium tolerance in barley and sorghum. In addition, cysteine synthase was reported to play a key role in aluminium response in rice (Hu *et al.,* 2008). The conserved positions of the barley aluminium tolerance gene *Alp*, on the long arm of chromosome 4 (Magalhaes, 2006; Wang *et al.,* 2006a) and that of *Alt3* on the long arm of rye 4R, show that aluminium tolerance in the *Triticeae* is controlled by parallel mutations in orthologous loci. This apparent conservation appears to persist across a wider evolutionary continuum, as a major aluminium tolerance QTL on rice chromosome 3 is likely orthologous to the aluminium tolerance loci in the *Triticeae* (Magalhaes, 2006).

Genetic variability exists among the cereal species for tolerance to acidic soils (pH<5.5), where common wheat is less tolerant than rye but more tolerant than durum wheat (*T. durum* L.) (Aniol & Gustafson, 1984; Johnson Jr *et al*., 1997; Mossor-Pietraszewska, 2001).

The various hexaploid genotypes (AABBDD) have the highest degree of tolerance. The A genome species exceeded the B genome species but not the tetraploids (AABB) at a lower acidity level. The importance of the D genome for acid tolerance was demonstrated by increased sensitivity of a tetraploid derivative lacking the D genome from cultivar Canthatch, a hexaploid cultivar and restoration of tolerance in the reconstituted hexaploid by addition of the D genome from several sources. Increased tolerance is provided by the R genome from rye, either by itself or in combination with durum or hexaploid wheat genomes as hexaploid or octoploid triticale (Carver & Ownby, 1995; Stodart *et al.,* 2007; Zhou *et al.,* 2007).

The majority of the observed variability with respect to aluminium tolerance in wheat could be explained by the hypothesis of two or three gene pairs (Aniol, 1995; Gupta, 1997; Riede & Anderson, 1996). Each gene pair affecting the same character, with complete dominance of each gene pair, but either recessive homozygote, is epistatic to effects of the other gene (Aniol, 1995; Luo & Dvořák, 1996; Gupta, 1997).

In hexaploid wheat, major genes influencing tolerance to aluminium are located on the short arm of chromosome 5A and the long arm of chromosomes 2D and 4D. Major genes influencing aluminium tolerance in rye are located on chromosomes 3R, 4R and the short arm of chromosome 6R (Aniol, 1995; Ma *et al*., 2000; Mossor-Pietraszewska, 2001).

Based on co-linearity among the genomes of rice, wheat, barley, rye, and sorghum, aluminium tolerance loci corresponding to aluminium tolerance QTL on wheat 4DL were mapped in chromosome 3 in rice and 7RS in rye. A wheat gene (*ALMT1*) encoding an aluminium activated malate transporter was isolated from aluminium resistant wheat line, ET8 recently (Kikui *et al.,* 2007; Panda & Matsumoto, 2007; Ryan *et al.,* 2009). *ALMT1*-like genes have also been isolated from several other species. *ALMT1-1* expression is associated with aluminium tolerance in wheat. Cultured tobacco cells over-expressing this gene also show an increase in aluminium tolerance (Panda & Matsumoto, 2007; Stodart *et al.,* 2007).

Different genetic systems for aluminium tolerance could conceivably prevail in seedling versus adult plants, or in a laboratory versus field environment (Johnson Jr *et al*., 1997). A number of over-expressed genes under aluminium stress was reported from different plant species, including the organic acid pathway featuring citrate synthase gene, or the anti-oxidant pathway with genes for superoxide dismutase and glutathione peroxidase, pathogen defence such as genes for β-1,3-glucanase and phenylalanine ammonia, signal transduction such as cell wall-associated receptor kinase 1 (*WAK1*) gene, and the general stress-responsive pathway such as blue copper binding protein gene. Most of these genes can also be induced by other biotic and abiotic stresses. Identification of these genes was based on comparison of gene expression levels of a single genotype under aluminium stressed versus non-stressed conditions, or between two genotypes with different genetic backgrounds under aluminium stressed conditions (Guo *et al*., 2007).

Maternal and cytoplasmic inheritance does not play a role in aluminium tolerance control in hexaploid wheat. In maize, cytoplasmic inheritance is also not involved in aluminium tolerance. Dominance plays a major role in the inheritance of aluminium tolerance in barley (Gupta, 1997). The inheritance of aluminium tolerance is usually determined from

F_2 populations instead of more sophisticated mating designs needed to detect gene interactions. This was the case for the cross, Cardinal (aluminium tolerance)xGK Zombor (aluminium susceptible), in which epistatic effects at two loci were hypothesised based on root length measurements in nutrient solutions. Inheritance of root length in acidic soil was also not monogenic (Carver & Ownby, 1995). Gupta, (1997) suggested that where aluminium tolerance is heritable, both pedigree and recurrent selection methods should improve plants for these traits.

2.4 Genetic makeup of aluminium tolerance in wheat

Only two species of *Triticum* are commercially important: the hexaploid species, *T. aestivum*, also known as bread wheat; and the tetraploid species, *T. durum,* the durum wheat used in making pasta. They are products of natural hybridization of perennial wild types, none of which is cultivated on a large scale today. Wild emmer, *Triticum dicoccides* (*T. turgidum* ssp. *dicoccides*, 2n = 4x = 28), (AABB) (Valkoun, 2001; Dreisigacker & Melchinger, 2004) was identified as the donor of the A and B genomes of durum and bread wheat. Tetraploid wheat later outcrossed with goat grass (*T. tauschii,* 2n=2x=14), (DD) (Valkoun, 2001; Dreisigacker & Melchinger, 2004) resulted in bread wheat (*T. aestivum* L. em Thell., 2n = 6x = 42) with the additional D genome. The origin of wild emmer is still a matter of controversy, but there is a general conclusion that it's A genome comes from the wild diploid wheat, Einkorn (*T. monococcum* L. 2n = 2x = 14), (AA) and its B genome is related to the genome coming from a species of *Aegilops* (Valkoun, 2001; Dreisigacker & Melchinger, 2004).

Genes introduced through the D genome control the intrinsic baking qualities that set *T. aestivum* apart from other species of *Triticum*. Each of the different bread wheat genomes contributes seven chromosomes and shows similar physical characteristic across the genomes, also defined as homologous groups (Dreisigacker & Melchinger, 2004). Genes from homologous groups can compensate for each other, which makes wheat highly tolerant to genetic changes e.g., mutations or losses of individual chromosomal segments (Valkoun, 2001; Dreisigacker & Melchinger, 2004).

Homologous groups allow breeders to accumulate favourable alleles (up to six per locus) for the enhancement of desired traits. Recombination between homologous chromosomes is suppressed, leading to a pairing pattern similar to that of diploid crops. Because of its allopolyploid nature, the genomes of bread wheat show a high homology with those of several diploid and tetraploid wild species. Consequently, genes from wild wheat species can be introgressed into cultivated wheat through recombination of the homoeologous chromosomes, and undesirable gene linkages can often be broken, using repeated backcrossing to cultivated wheat (Valkoun, 2001). Moreover, chromosome recombination allows a simultaneous gene transfer from different chromosomes, as well as introgression of polygenic traits, in which the genes are dispersed on different chromosome segments (Valkoun, 2001; Dreisigacker & Melchinger, 2004).

The genetics of aluminium tolerance in wheat has been examined extensively and aluminium tolerance in some wheat cultivars is polygenic and is controlled by a single major gene in other cultivars (Aniol & Gustafson, 1984; Tang *et al*., 2002; Matos *et al.,* 2005; Ryan *et al.,* 2009). There is also evidence to suggest that more than one aluminium tolerance gene may exist in certain wheat cultivars e.g. in cultivar Atlas 66 (Tang *et al*., 2002; Raman *et al.,* 2008). A differential response of wheat to aluminium has been reported, and several attempts have been made to determine the inheritance of this character. Major genes, controlling tolerance to aluminium were located on chromosomes of the A and D genomes of hexaploid wheat, but the physiological processes controlled by these genes are still unknown (Aniol, 1995).

Wheat crosses between aluminium tolerant and sensitive varieties showed that aluminium tolerance segregates as a single, dominant locus. However, the segregation patterns of other crosses suggested that two loci are responsible for tolerance. One aluminium tolerance locus, called *Alt*$_{BH}$ or *Alt2*, was mapped to the long arm of chromosome 4D (Gustafson, 2005). Aluminium tolerance in the tolerant wheat cultivar BH 1146 is conditioned by a single major locus that controls nearly 85% of the phenotypic variation in a cross with the aluminium sensitive cultivar Anahuac. The locus designated *Alt*$_{BH}$, was genetically mapped to the long arm of chromosome 4D (Magalhaes, 2006).

A major QTL on chromosome 4DL was identified in wheat cultivars BH 1146, Atlas 66 and Chinese Spring (Luo & Dvořák, 1996; Riede & Anderson, 1996; Ma *et al.,* 2005; Zhou *et al.,* 2007; Cai *et al.,* 2008; Navakode *et al.,* 2009) and three additional QTLs located on 5AS, 2DL and 7AS were identified to contribute to aluminium tolerance in wheat cultivar Chinese Spring (Luo & Dvořák, 1996; Fontecha *et al.,* 2007; Guo *et al.,* 2007; Panda & Matsumoto, 2007). The Chinese Spring cultivar chromosome arms 6AL, 7AS, 3DL, 4DL, 4BL and 7D were also found to have genes controlling aluminium tolerance located on them (Panda & Matsumoto, 2007; Ryan *et al.,* 2009).

Tang *et al.* (2002) determined the aluminium tolerance of near isogenic lines (NILs) of the cultivars Century and Chisholm (Century –T and Chisholm –T). The cultivar Atlas 66 aluminium tolerance gene present in each NIL acted by increasing aluminium inducible malate release from root tips, but conferred only a portion of the aluminium tolerance of cultivar Atlas 66 in both instances. Tang *et al*. (2002) concluded that differences in aluminium tolerance between the NILs and cultivar Atlas 66 can be attributed to malate release differences, and not differential phosphate release. It was also indicated that genetic variation at more than one locus underlies the malate mediated aluminium tolerance differences in cultivar Atlas 66, when compared with cultivars Century and Chisholm. Aluminium inducible malate released from root apices was significantly higher in the NILs compared with the recurrent parents, but less than that observed in cultivar Atlas 66. In contrast, root phosphate release was significantly lower than previously reported in cultivar Atlas 66, with no major differences observed among cultivars (Tang *et al*., 2002).

Management options complimentary to the use of lime are required to address soil acidity and aluminium toxicity. One option is to exploit the genetic variability in crop germplasm to breed and select plant genotypes with greater tolerance to aluminium toxicity, phosphorus, calcium, magnesium and molybdenum deficiencies (Gupta, 1997; Tang *et al.,* 2001).

Breeding for aluminium tolerance in wheat accounts for an increase in seed yield of 3.2% per year on acid soils, estimated over a period of 10 years (Raman *et al.,* 2006), as well as to minimise the inputs required, such as lime (Miyasaka *et al.,* 1989). Selection

and breeding for aluminium tolerance are important approaches for increasing grain yield in acid soils (Giaveno & Miranda Filho, 2000). Growing aluminium tolerant cultivars is one of the best strategies for improving wheat productivity in acidic soils (Pei-guo *et al.,* 2007).

Fortunately, genetic variation in aluminium resistance exists in wheat, and the adoption of aluminium resistant cultivars may provide an additional strategy to combat subsurface soil acidity (Tang *et al.,* 2001; Raman *et al.,* 2002; Zhou *et al.,* 2007). Landraces, the ancestral genotypes of cultivated wheat, can be examined for novel variations in aluminium tolerance, which may not have been characterised or have been lost during the development of modern wheat cultivars (Stodart *et al.,* 2007).

2.5 Tolerance mechanisms

2.5.1 Physiological mechanisms of aluminium tolerance

Due to the fact that aluminium can interact with a number of extracellular and intracellular structures, different mechanisms to manage aluminium toxicity exist. The exclusion mechanism enhances plant tolerance to aluminium stress by preventing excess uptake of aluminium ions from entering the root apex cells. Central to the exclusion mechanism is the root tips that secrete organic acids such as malate and citrate or oxalate to chelate aluminium in the rhizosphere that change the pH of the rhizosphere. If aluminium does cross the plasmalemma, the ATPase pump located in the plasmalemma excludes the metal (Kochian, 1995). The internal mechanism reduces aluminium toxicity by immobilisation, compartmentalisation or detoxification of the aluminium ions that have penetrated the plant cells (Drummond *et al*., 2001; Mossor-Pietraszewska, 2001; Ma, 2005; Wang *et al.,* 2006b, Guo *et al.,* 2007).

The internal mechanism is characterized by the production of specific proteins capable of forming complexes with the toxic aluminium components (Giaveno & Miranda Filho, 2000). The basic difference between the two mechanisms is the site of detoxification. The exclusion mechanism prevents aluminium from crossing the plasma membrane to accumulate inside the plant cells (symplasts), while the possible mechanism for internal resistance are the chelatization of aluminium in the cytosol and compartmentation of aluminium in the vacuole or to detoxify this metal when it penetrates the cells by the

evolution of aluminium tolerance enzymes that elevated tolerance of the enzymatic activity in the cells (Kochian, 1995; Drummond *et al*., 2001; Mossor-Pietraszewska, 2001; Ma, 2005; Wang *et al.,* 2006b; Kikui *et al*., 2007).

Several possibilities have been proposed for each type of mechanism and organic acids play an important role in the detoxifying plants from aluminium both internally and externally. Some organic acids can form stable complexes with aluminium, thereby preventing the binding of aluminium to cellular components, resulting in the detoxification of aluminium in plant species (Ma, 2005; Raman *et al*., 2006; Wang *et al.,* 2006b).

2.5.2 Exclusion mechanism for aluminium tolerance

For organic acids to detoxify aluminium in the rhizosphere, organic acids must be transported from the cytosol to the apoplasm. At the near-neutral pH of the cytoplasm, organic acids are almost entirely dissociated from their protons and exist as organic anions. It is these organic acid anions that are probably transported out of the root cell. Although many types of organic acids are found in root cells, only one or two specific organic acids are secreted in response to high aluminium levels (Ma *et al*., 2001). Increasing pH in the rhizosphere reduce the aluminium solubility and its potential toxicity, which favour the formation of less-toxic aluminium forms such as aluminium hydroxides and aluminium phosphates, and would also help the exudation of organic acids from roots (Wang *et al*., 2006b). Aluminium activated efflux of organic acid anions from the roots is a well established mechanism that was proposed to be used by a range of aluminium tolerant plants (Ma, 2005).

Many aluminium tolerant plant species are known to secrete organic acids from their roots in response to aluminium treatment. Citrate, oxalate, and malate are some of the commonly released organic acid anions that can form sufficiently strong complexes with Al^{3+} to protect plant roots. Malate is released from the roots of aluminium tolerant cultivars of wheat; citrate from aluminium tolerant cultivars of snapbean (*Phaseouls vulgaris*), maize, *Cassia tora* and soybean; and oxalate from buckwheat (*Fagopyrum esculentum*) and taro (*Colocasia esculenta*). Some plant species, such as aluminium tolerant triticale (x *Triticosecale Wittmack*), rapeseed (*Brassica napus*), oats (*Avena*

sativa), radish (*Raphanus sativus*) and rye release both malate and citrate (Gustafson, 2005; Ma, 2005; Kikui *et al*., 2007).

A high correlation between organic acid anion secretion and aluminium tolerance has been established in some species such as wheat and barley (Gustafson, 2005; Ma, 2005). These organic anions are able to chelate aluminium ions and exclude them from root apices (Gustafson, 2005; Ma, 2005; Kikui *et al*., 2007). In some of these species, the increased secretion of organic acids by these plants is localised to the root apex and depends upon the presence of Al^{3+} in the external solution. In several of these examples the efflux of organic acids occurs primarily from the root apices and this makes good sense since this is the part of the root system most susceptible to aluminium toxicity (Kochian, 1995; Ma *et al*., 2001; Delhaize, 2004). It is neither possible for all the Al^{3+} in the soil to be detoxified by root exudates nor is it necessary. The root apex is particularly sensitive to Al^{3+}, therefore only the cations that immediately surround the apical root cells need to be detoxified. Secretion needs to continue as the root apex moves through an acid soil to replace the organic acids that diffuse away from the root or are broken down by micro-organisms (Ma *et al*., 2001).

There are two temporal patterns of organic acid release, on the basis of the timing of secretion.

Pattern I

No discernible delay is observed between the addition of aluminium and the onset of organic acid release. For example, in wheat and buckwheat (*Fagopyrum esculentum*), the secretion of malate or oxalate was detectable within 15 to 30 min after exposure to aluminium (Delhaize *et al*., 1993; Zheng *et al*., 1998; Ma *et al*., 2001; Delhaize, 2004; Ma, 2005).

Pattern II

Organic acid secretion is delayed for several hours after exposure to Al^{3+}. For example, in *C. tora*, maximal efflux of citrate occurs after 4 h exposure to aluminium (Ma *et al*., 1997) and in rye, citrate and malate efflux increases steadily during a 10 h period (Li *et al*., 2000), which suggests that gene induction is required. Some inducible proteins

could be involved in organic acid metabolism or in the transport of organic acid anions (Ma *et al*., 2001; Delhaize, 2004; Ma, 2005).

In maize, it appears that aluminium might trigger both a rapid efflux of citrate as well as a delayed release, which increases during a 48 h period (Pellet *et al*., 1995; Piñeros & Kochian, 2001). The rapidity of the Pattern I response suggests that aluminium activates a pre-existing transport mechanism and that the induction of novel proteins is not required (Ma, 2000; Delhaize, 2004). Aluminium might simply activate a transporter on the plasma membrane to initiate organic anion efflux. By contrast, the delay observed in Pattern II-type secretion might indicate that protein induction is required. These induction proteins could be involved in organic acid metabolism or in the transport of organic acid anions out of the root cells and/or in the synthesis of organic acids (Ma *et al*., 2001; Delhaize, 2004; Ma, 2005). In addition to pattern I and II, another pattern was found in aluminium tolerant cultivars of barley, which responds to aluminium stress by secretion of citrate from the roots. Secretion of citrate is very rapid but affected by low temperature (Ma, 2005).

Although root apices of aluminium tolerant seedlings synthesise more malate than those of sensitive seedlings in response to aluminium, root apices of both genotypes show similar activities of phosphoenolpyruvate (PEP) carboxylase and malate dehydrogenase, two enzymes important in malate synthesis. Since the root apices of aluminium sensitive and aluminium tolerant genotypes have the same capacity to synthesise malate, the differences in efflux probably lie in their relative ability to transport malate across the plasma membrane in response to aluminium. Therefore the *Alt1* locus could code for a malate-permeable channel responsive to aluminium or for a component of the pathway that regulates the activity of the putative channel (Delhaize & Ryan, 1995).

2.5.3 Internal tolerance mechanisms

Aluminium is detoxified *in vivo* by aluminium accumulating plants. The internal mechanism are those which operate within the symplasm and are mediated at the cellular level either by detoxification or immobilisation of aluminium ions that have penetrated into plant cells (Delhaize, 2004; Ma, 2005; Wang *et al.,* 2006b). Some plant species, mostly woody species have the remarkable ability of accumulating aluminium in

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

The organic acids are possibly secreted to the outside via ion channels, which are the ion transporters. Anion channels activated by aluminium have been identified in patch-clamp studies with aluminium tolerant wheat root tip protoplasts and in maize, suggesting that these anion channels are involved in aluminium tolerance (Panda & Matsumoto, 2007). Buckwheat is a species that also exudes oxalate in response to aluminium and its high level of aluminium tolerance may be a result of both external and internal detoxification mechanisms (Delhaize, 2004).

From the analysis of root tips, membrane patches and whole cells, a putative mechanism has emerged by which aluminium may activate a plasma membrane bound anion channel. Aluminium might directly bind and then activate a membrane protein or an associated receptor, or might indirectly activate the channel via cytosolic components. The two most important families of channel proteins are the chloride channel family and a subset of the ATP-binding cassette (ABC) protein super-family.

In yeast (*Saccharomyces cerevisiae*), Pdr12, an ABC protein, assists the carboxylate efflux (Panda & Matsumoto, 2007). Some circumstantial evidence suggests that the carboxylate transporter involved in aluminium tolerance may be an ABC transporter. Guard cell plasma membrane containing slow anion channels seem to have several similarities with anion channels in aluminium tolerant wheat and maize, and both are inhibited by the ABC transporter antagonist diphenylamine-2-carboxylic acid (Panda & Matsumoto, 2007).

2.5.4 Other mechanisms

There is considerable evidence associating organic acids in the aluminium tolerance mechanisms of many species. Other species apparently use mechanisms that do not rely on organic acids. *Brachiaria decumbans*, an extremely aluminium tolerant species, does not secrete organic acids in response to aluminium and so must possess different

ways of dealing with toxic levels of aluminium in the soil solution (Delhaize, 2004). Since the phytotoxic form of aluminium is largely dependent on pH, a mechanism based on increasing the pH around root apices should provide a degree of protection from aluminium. Support of such a mechanism comes from a study of an aluminium tolerant *Arabidopsis* mutant (*alr1*). This mutant was found to exhibit an aluminium induced increase of pH in the solution immediately surrounding the root apex and this resulted in a decrease in Al^{3+} activity (Delhaize, 2004). Rhizosphere is a dynamic micro-environment, in which many new substances are released constantly and more secondary compounds will be produced under environmental stress. The rhizosphere can influence plant growth and crop productivity (Wang *et al*., 2006b). The rhizobia of some legume species are more sensitive to aluminium than their host plants. The symbiotic N_2 fixation process itself is apparently less sensitive to aluminium than the process of nodule formation. Aluminium toxicity and low pH are more important than manganese toxicity and calcium deficiency in limiting the activities of rhizobia on cowpea (*Vigna sinensis* L.) and soybean roots (Foy, 1984).

The presence of more than one gene and more than one mechanism of aluminium tolerance in cultivar Atlas 66 raise the possibility that different aluminium tolerance genes may encode distinctly different aluminium tolerance mechanisms, specifically either aluminium inducible malate or constitutive phosphate exclusion from root tips (Tang *et al*., 2002). Though in many cases organic acid efflux and aluminium resistance are correlated, no such correlation was observed in rye, suggesting that in some plants other intracellular mechanisms operate to induce aluminium tolerance (Panda & Matsumoto, 2007).

2.6 Beneficial effects of aluminium on gene expression

The physiological functions of aluminium in plants is not clear, but low levels of aluminium can have a beneficial effect on plant growth, especially in aluminium tolerant plant species (Foy, 1984; Ritchie, 1989). A number of plants that have shown positive growth response to aluminium include rice, tropical legumes, *eucalyptus*, tea (*Camellia sinensis*), peach (*Prunus persice*), sugar beet (*Beta vulgaris*), maize inbred and wheat. Beneficial effects of added aluminium in rice cultivars and the growth stimulus was greater in aluminium tolerant cultivars than in aluminium sensitive cultivars.

A wide range of proteins is induced by aluminium stress in wheat and other plant species. Aluminium induced proteins include membrane-bound, cytosolic, cytoskeletal and exudate proteins and many of these have been implicated as general stress-response proteins, others have been associated with oxidative and other stresses (Foy, 1984; Hamilton *et al*., 2001).

2.7 Physiological and biochemical effects of aluminium

Foy (1984) and Mossor-Pietraszewska (2001) reported that excess aluminium interferes with cell division in root tips, formation of lateral roots, increased cell wall rigidity by cross linking pectins and reduced deoxyribonucleic acid (DNA) replication by increasing the rigidity of the DNA double helix. Excess aluminium also fixes phosphorus in a less available form in the soil and on plant root surfaces that decreases root respiration. The metal also interferes with a number of enzymes governing sugar phosphorylation and the deposition of cell wall polysaccharides. The excess aluminium available modifies the structure and function of the plasma membrane and interferes with the uptake, transport and use of several essential nutrient elements, including calcium, magnesium, potassium, phosphorus and iron that is essential for normal plant development (Foy, 1984; Mossor-Pietraszewska, 2001).

2.8 Uptake and distribution of aluminium

The plasma membrane represents the primary target of aluminium toxicity. The primary sites of aluminium uptake are the peripheral cells of the root cap and mucilagenous secretions around the roots and only small amounts penetrate through the leaves. Phosphorous accumulates in the roots when toxic levels of aluminium occur, possibly as an aluminium phosphate precipitate (Mossor-Pietraszewska, 2001; Ma, 2005).

2.9 Plant symptoms to aluminium toxicity

Aluminium toxicity is associated with gross changes in root morphology (Kochian *et al*., 2005). One hypothesis is that the sequence of toxicity starts with perception of aluminium by root cap cells, followed by signal transduction and a physiological response within the root meristem (Panda & Matsumoto, 2007). The primary and earliest

symptom of aluminium toxicity is a rapid inhibition of root growth (Ryan *et al*., 1995; Kochian *et al*., 2005). Within the root, the root apex, and more specifically the distal part of the transition zone within the apex, is the primary target of aluminium toxicity (Kochian *et al*., 2005). The primary effects of aluminium on root membrane permeability may appear only after a few minutes or even hours after exposure to aluminium (Mossor-Pietraszewska, 2001).

Within meristematic and root cap cells, aluminium toxicity is associated with increased vacuolation and turnover of starch grains, as well as disruption of dictyosomes and their secretory function (Carver & Ownby, 1995; Wang *et al.,* 2006b). In the root cap cells, Golgi bodies are sensitive to aluminium and structural modifications after exposure to aluminium include a lower frequency of Golgi bodies in the cells, resulting in a decrease in mucilage secretion (Panda & Matsumoto, 2007).

Division and elongation of root cells result in root elongation. Aluminium is known to induce a decrease in mitotic activity in many plants, and the aluminium induced reduction in the number of proliferating cells is accompanied by the shortening of the region of cell division in maize (Panda & Matsumoto, 2007). Aluminium toxicity results in inhibited root elongation, which yields swollen root apices and poor or no root-hair development. Root tips and lateral roots become thickened and may turn brown. The root system as a whole appears coralloid (Foy, 1984; Mossor-Pietraszewska, 2001; Tang *et al*., 2003; Kochian *et al*., 2005). This extensive root damage results in a reduced root system and limited water and mineral nutrient uptake. Young seedlings are generally more susceptible to aluminium toxicity than older plants (Foy, 1984; Mossor-Pietraszewska, 2001; Tang *et al*., 2003). The rapid inhibition of root growth is caused by a number of different mechanisms, including aluminium interactions within the cell wall and the plasma membrane (Kochian, 1995). The production and development of root border cells also vary with genotype. Aluminium seriously inhibits the production and release of root border cells, resulting in clumping of border cells in cultivar Scout 66 and to a lesser extent in the cells of cultivar Atlas 66. Both of these cultivars are aluminium tolerant wheat cultivars (Panda & Matsumoto, 2007).

Because aluminium is so reactive, there are so many potential sites for injury, including: the cell wall, the plasma membrane, signal-transduction pathways, the root cytoskeleton and (DNA) or nuclei. X-ray micro-analysis and secondary ion mass spectro-analysis have indicated that a significant fraction of aluminium in roots is associated with apoplastic binding sites, predominantly in walls of cells of the root periphery (Kochian *et al*., 2005; Panda & Matsumoto, 2007).

Among the many components of the cell wall network, pectins have been proposed to be a critical site for aluminium-cell-wall interactions. Aluminium interactions lead to the displacement of other cations fundamental for cell-wall stability. Consequently, the strong and rapid binding of aluminium can alter cell-wall structural and mechanical properties, making it more rigid, leading to a decrease in the mechanical extensibility of the cell wall required for normal cell expansion (Mossor-Pietraszewska, 2001; Kochian *et al*., 2005).

Early in the season, wheat plants affected by acidity show symptoms of nitrogen deficiency, the leaves become pale; particularly the oldest leaves which turn yellow and die early. Later in the season, these plants show symptoms of drought stress well before wheat plants growing on less acidic soil (Scott & Fisher, 1989). Aluminium toxicity does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Mossor-Pietraszewska, 2001).

In some plants the foliar symptoms resemble those of phosphorus deficiency, manifested by overall stunting; small, dark green leaves, late maturity; purpling of stems, leaves, and leaf veins and yellowing and death of leaf tips. In other plants, aluminium toxicity appears also as an induced calcium deficiency or as reduced Ca^{2+} transport within plants, causing curling or rolling of young leaves, inhibited growth of lateral branches, or a collapse of growing points or petioles (Foy, 1984; Carver & Ownby, 1995; Wang *et al.,* 2006b). Aluminium stress decreases total chlorophyll concentration and photosynthetic rate, but the decline in transpiration rate is most severe (Wang *et al.,* 2006b). Aluminium also induces iron deficiency symptoms in rice and sorghum (Foy, 1984).

The common responses of shoots to aluminium toxicity include; cellular and ultrastructural changes in leaves, increased rates of diffusion resistance, reduction of stomatal aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot biomass (Mossor-Pietraszewska, 2001). Wang *et al.* (2006b) reported that there are two responses to aluminium: an initial acute inhibition of growth that is followed by later chronic aluminium effect on growth.

Aluminium toxicity decreases drought tolerance and the use of subsoil nutrient (Wang *et al.,* 2006b). Deleterious effects of subsurface soil acidity on crop growth will thus be influenced by the extent to which a plant depends on the surface soil for supply of water and nutrients, especially when the topsoil dries out (Jozefaciuk & Szatanik-Kloc, 2001; Tang *et al*., 2001; Stodart *et al*., 2007).

Genetic improvement of crops for acid soil tolerance has been accelerated by the availability of screening criteria for detecting aluminium tolerance. Laboratory and greenhouse based techniques are widely employed which are usually non destructive and can be applied in early developmental stages from seedlings only a few days old to flowering stage of the plants. Field based screening techniques are more laborious, time consuming and expensive (Carver & Ownby, 1995; Wang *et al.,* 2006b).

Aluminium toxicity is first apparent on root growth, and the use of nutrient solution culture with defined concentrations of aluminium is the most common screening medium for aluminium tolerance. The method has proven to be a reliable measure of aluminium tolerance for a number of species, as it provides easy access to root systems, tight control over nutrient availability and pH, and non-destructive measurement of tolerance (Carver & Ownby, 1995; Delhaize, 2004; Wang *et al.,* 2006b).

Variation in temperature in the growth chamber and minor fluctuation of pH of the nutrient solution can reduce repeatability of the results. Wang *et al.* (2006b) reported effective screening of wheat cultivars using very low aluminium levels in solution which minimised aluminium precipitation and more closely represented actual environment stresses compared to traditional short-term exposure with higher aluminium concentrations. Longer exposure makes solution culture technically more difficult,

requiring constant adjustment of pH, water loss and nutrient loss (Wang *et al.,* 2006b). Other variables to consider in solution based screening are nutrient composition and standards for measuring tolerance. Changes in nutrient composition can change the intensity of aluminium stress at a given concentration (Scott & Fisher, 1989; Wang *et al.,* 2006b). Higher concentration of phosphorus may lead to aluminium phosphate precipitation in aluminium solution and protect plants against aluminium toxicity. Hence phosphorus is often avoided in nutrient solution; particularly in short term aluminium exposures when phosphorus needs are satisfied by seed reserves (Wang *et al.,* 2006b).

There are two major criteria for evaluation of aluminium tolerance in nutrient solution culture. First, root length measurement is the most suitable approach for genetic and molecular studies in which a precise quantitative response for stress is needed. It is also suitable for identifying genotypes with superior alleles for aluminium tolerance. Second, root staining is quicker and more efficient. It is suitable for screening a large segregating population derived from improved germplasm (Polle *et al.,* 1978; Baier *et al.,* 1995; Wang *et al.,* 2006b).

2.10 The hematoxylin staining method

Hematoxylin stain has proved to be useful in determining the aluminium tolerance of plants (Polle *et al.,* 1978). Hematoxylin is a compound that binds aluminium *in vitro* to form a coloured complex and the absence of colour in root tips of aluminium tolerant genotypes indicates that these genotypes either exclude the aluminium or bind the aluminium in complexes that are unavailable to hematoxylin (Delhaize, 2004). When seedling roots of wheat are treated with solutions containing Al^{3+} and then stained in an aqueous solution of hematoxylin, the roots develop a pattern of staining that correlates remarkably well with their aluminium tolerance level, as estimated by root elongation methods (Polle *et al*., 1978).

The hematoxylin staining method provides a qualitative measure, while root growth measurements are quantitative and therefore can be used in conjunction with staining to evaluate relative levels of aluminium tolerance (Polle *et al*., 1978). Although hematoxylin staining of roots apices shows a semi-quantitative expression for aluminium resistance, it has been proven to be an easy, rapid, reliable, and non-destructive method for

discerning among aluminium sensitive and resistant genotypes. Hematoxylin turns dark purple when it forms a complex with aluminium so that the penetration and retention of aluminium ions in the roots can be assessed and the reaction between hematoxylin and aluminium is specific (Polle *et al*., 1978; Delhaize, 2004; Zhou *et al*., 2007).

2.11 The modified pulse method

The modified pulse method (Aniol, 1984) uses the same principle of hematoxylin staining. After germination the roots are stained with hematoxylin solution prior to transferring them into the recovery solution containing aluminium. The root staining marks the position from which root re-growth will occur during the recovery period. Aluminium tolerance is evaluated based on the ability of tolerant seedlings to show root re-growth, by measuring root growth based on the ability to present root re-growth.

This method varies from the hematoxylin method as seedlings are again submitted to a nutrient solution after been exposed to toxic aluminium levels. Genotypes differ in their ability to show re-growth of the roots (Aniol, 1984).

2.12 Root re-growth method

Two aluminium tolerance parameters are considered in this method, root re-growth and relative root growth. The root re-growth parameter is measured as root growth under aluminum stress while relative root growth is root growth compared with and without aluminium stress. After exposure to aluminium and hematoxylin, seedlings are placed in a nutrient solution to allow root re-growth (Baier *et al.,* 1995).

Quantitative measuring of stressed and non-stressed roots is done when using the root growth method. With this method as a control, a similar experiment but with aluminium, is done simultaneously. The longest two roots of each seedling are measured, averaged and the data within each genotype combined, with the removal of the seedlings from the tray. The root re-growth is calculated as the mean root growth of seedlings after being in a solution containing aluminium. Dividing root growth in the presence of aluminum by root growth in control plants over the growth time period and multiplying by 100 calculates relative root growth.

Baier *et al*. (1995) concluded that since relative root growth is the relative growth of the genotype in aluminium solution compared to its potential growth without aluminium, this parameter is a measure of aluminium tolerance alone.

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CHAPTER 3
Evaluation of screening methodology for aluminium tolerance in wheat

3.5 Introduction

The use of nutrient solution culture with defined concentrations of aluminium has proven to be a reliable measure of aluminium tolerance for a number of species, as aluminium toxicity is first apparent on root growth (Polle *et al.,* 1978; Carver & Ownby, 1995; Ma *et al.,* 1997; Delhaize, 2004; Wang *et al.,* 2006a; b). The nutrient solution culture technique is the most commonly used for screening aluminium tolerance, as it provides easy access to the root system. Effective control over environmental conditions including light, nutrient availability and pH is possible and it is also a non-destructive method for aluminium tolerance evaluation (Carver & Ownby, 1995; Delhaize, 2004; Wang *et al.,* 2006a). The nutrient solution culture methods can be used in early developmental stages from seedlings that are only a few days old to flowering stage of the plants (Baier *et al.,* 1995; Carver & Ownby, 1995; Johnson Jr *et al.,* 1997; Ma *et al.,* 1997; Karsai & Bedö, 1998; Hede *et al.,* 2002; Wang *et al.,* 2006a).

Not much work has been done in South Africa on aluminium tolerance in crops, especially in wheat, although soils in South African wheat producing areas are becoming more acidic (Bosch & Otto, 1995). In previous studies, aluminium tolerance evaluations were done in the field. However, field evaluations are time consuming and expensive (Carver & Ownby, 1995; Ma *et al.,* 1997; Aniol, 2004).

Three widely used parameters for evaluation of aluminium tolerance in breeding programmes were investigated in this study. Root re-growth (Aniol & Gustafson, 1984; Hede *et al.,* 2002; Aniol, 2004; Raman *et al.,* 2004; Zhang *et al.,* 2007), the root tolerance index (Baier *et al.,* 1995; Nava *et al.,* 2006) and root length (Cosic *et al.,* 1994; Bunta, 1999; Zhang *et al.,* 2007) were investigated in the root system. The fourth parameter that was added is the measurement of the stained portion of the root tip. This portion of the root indicates the extent to which the root tips were damaged by the

aluminium. The length of roots is very important, as longer roots will generally be able to better absorb nutrients in comparison with shorter and damaged roots.

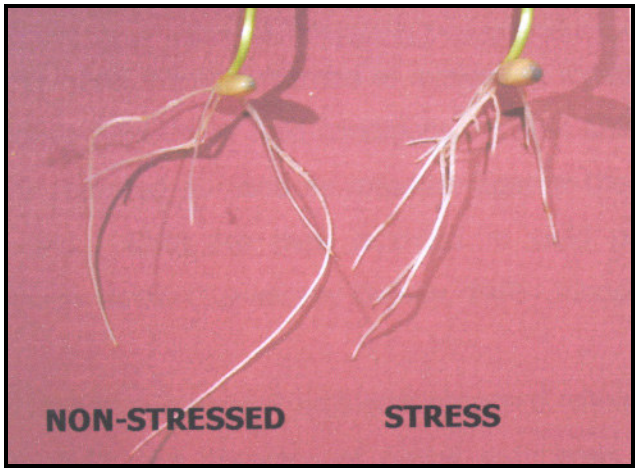

Figure 3.1 The difference between tolerance and sensitive aluminium genotypes after treatment in aluminum solution

The objectives of this study were to identify the most efficient screening method for aluminium tolerance in South African wheat cultivars and to screen known sources of tolerance in order to measure root re-growth and root tolerance index of wheat genotypes, in aluminium containing solutions, in order to establish good levels of aluminium tolerance in local wheat cultivars.

3.6 Materials and methods

3.6.1 Materials

Genotypes used were obtained from the Agricultural Research Council-Small Grain Institute's (ARC–SGI) gene bank, as well as entries from the acid soil-screening nursery obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico (Table 3.1).

Due to the genetic complexity of the trait and different gene frequencies for aluminium tolerance, the genotypes used in this study included a wide range of aluminium tolerance expression under field conditions. This is a valuable genetic resource to validate a

suitable screening and evaluation method, as well as to exploit the available genes to its full potential in a local pre-breeding programme.

Table 3.1 List of the tested genotypes and their aluminium status

Parental material	Aluminium status of genotypes	Total no of seeds incubated for germination	Total no of seeds evaluated
Elands	Local aluminium susceptible check	2400	275
Atlas 66	International aluminium tolerant check	1400	117
Tugela DN	Local aluminium tolerant variety	2200	180
ASSN1	Aluminium tolerant	52	14
ASSN5	Aluminium tolerant	234	34
ASSN7	Aluminium tolerant	152	5
ASSN12	Aluminium tolerant	126	28
ASSN15	Aluminium tolerant	138	4
ASSN16	Aluminium tolerant	168	34
ASSN2a	Aluminium tolerant	120	30
T96/6	Aluminium tolerant	168	2

ASSN = Acid Soil Screening Nursery (CIMMYT)
ASSN2a = selected population from ASSN1 original introduction

There was a large difference between the number of seeds incubated for germination and those eventually evaluated, as the initial parental seed samples were limited and the germination rate was very low. In certain instances, roots were too short so they were not in contact with the hematoxylin stain. In some seedlings where roots were broken and only one root could be evaluated, the seedlings were not counted in the final evaluation. T96/6 was evaluated for pre-harvest sprouting before it was used in the aluminium tolerance evaluation. Kernels that had a low pre-harvest sprouting score did not germinate well in the growth chamber.

3.6.2 Methods

3.6.2.1 Growing conditions and staining of material for aluminium tolerance testing

3.2.2.1.1 Preparation of planting trays and seeds for testing

The protocol followed, was that of Polle *et al.* (1978) with minor modification and was executed as follows:

Planting trays were surface sterilised in 40% sodium hypochlorite 3.5% m/v for 15 min to prevent contamination from fungi. The treatment of all the planting trays was done simultaneously for a specific cycle in the evaluation process. Each planting tray consisted of an external diameter of 28.5 x 10.5 cm and contained seven rows, 18 columns and 126 cubicles of 1.5 x 1.5 cm. The columns of the planting trays were numbered for data capturing purposes. One cultivar was placed in four columns with four seeds per cubicle if there was enough seed available and replaced in 40% sodium hypochlorite 3.5% m/v for another 10 min, to surface sterilise the seeds before the incubation process started for evaluation, to minimise fungi contamination. Four seeds were placed in each cubicle to allow seeds optimal contact with the water surface during the incubation period, to ensure optimal germination conditions. Tolerant and susceptible checks were included in each planting tray, to standardise the whole screening procedure over weeks, as well as between trays for a specific selection cycle. The susceptible check was placed in the first column of the planting tray, while the tolerant check was placed in the last column. After the surface sterilisation of the seeds, the planting trays with seeds were rinsed in distilled water for 15 min to remove excess sodium hypochlorite 3.5% m/v.

3.2.2.1.2 Incubation conditions for germinating seeds

Planting trays with seeds were placed in an incubator in complete darkness at 25ºC with aerated, distilled water for a 72 h period for seed germination. Seeds were constantly in contact with the aerated distilled water throughout the germination period, to optimise the germination rate. An air pump with bubble rods provided aeration during the germination period.

3.2.2.1.3 Conditions for stimulating plant growth before aluminium toxicity treatment

After 72 h of dark treatment, planting trays with germinated seeds were placed under a continuous fluorescent light source, in a nutrient medium solution pH4 (Table 3.2) at a constant temperature of 25ºC, for an incubation period of 32 h. The pH was adjusted by adding 4% NaOH or 0.5% HCl.

3.2.2.1.4 Incubation conditions during aluminium toxicity treatment

After the initial 32 h of incubation in the nutrient medium solution, this solution was replaced with fresh nutrient medium solution containing aluminium in the form of $AlCl_3$ to acidify the nutrient medium solution (pH4). Seedlings were incubated for an additional 17 h in the acidified solution under the same conditions as described in section 3.2.2.1.3.

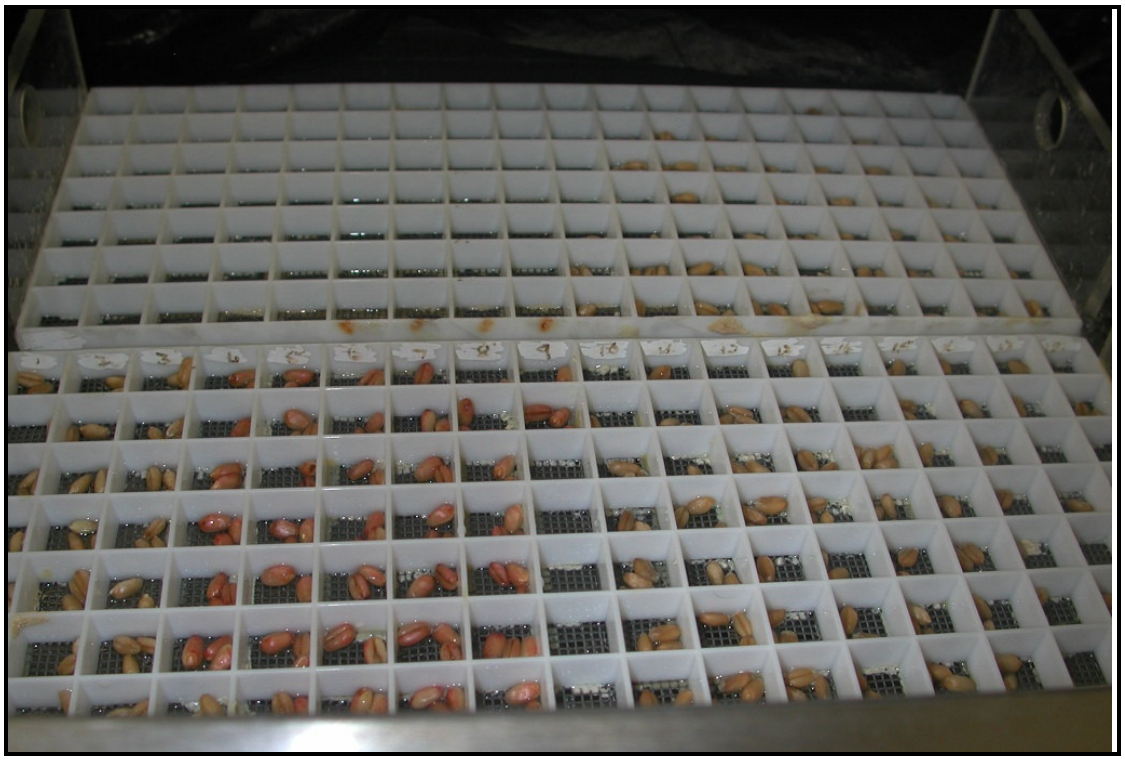

Figure 3.2 Planting trays with seeds

Table 3.2 Chemicals that were used to prepare the nutrient medium solution for seedlings (Polle *et al.,* 1978)

Chemicals	**4L of distilled water**
$CaCl_2.H_2O$	5 mM
KNO_3	6.5 mM
$MgCl_2$.6H2O	2.5 mM
$(NH4)_2SO_4$	0.1 mM
$(NH_4)NO_3$	0.4 mM
pH 4.0	

3.2.2.1.5 Staining of roots after the aluminium toxicity treatment

After growing the seedlings for 17 h in the nutrient solution containing $AlCl_3$, the roots were immersed in a solution of 0.2% (w/v) hematoxylin and 0.02% (w/v) $NaIO_3$ for 15 min. Excess dye was removed by rinsing the roots with running tap water for 5 min and seedlings returned to the nutrient solution without aluminium for 24 h.

3.6.2.2 Evaluation of seedlings

After 24 h exposure to hematoxylin, root re-growth was measured in millimeter to determine the length of root re-growth after aluminium treatment. Measurements were based on root re-growth beyond the hematoxylin stained layers. All seedlings where the primary and secondary roots were present, not broken and in contact with the hematoxylin staining solution, were used for the evaluation purposes.

Seedlings with well-developed roots were chosen for evaluating aluminium tolerance after staining. This ensured that the roots were in contact with the aluminium solution. Shorter roots tend to grow on the surface of the tray, without penetrating into the solution. Only roots that were not disturbed in any way e.g., broken or curled were used for the evaluation of the roots.

3.2.2.2.1 Modified pulse method

Measurements were done according to Aniol (1984) with minor modifications. After the sodium hypochlorite 3.5% m/v treatment, the step where seeds were germinated overnight on filter paper in Petri dishes was left out, and hematoxylin was used to stain the roots instead of Eriochrome cyanine R that was used in the original method.

3.2.2.2.2 Root re-growth method

The measurements were done according to Baier *et al*. (1995) with minor modifications. The aluminium tolerance of each genotype was expressed as the root tolerance index

where root tolerance index = $\frac{\text{(net growth in aluminium treatment solution)}}{\text{(net growth in the control)}} \times 100$

Baier *et al*. (1995), Ryan *et al.* (1995) and Kim *et al.* (2001) concluded that since root tolerance index is the relative growth of the genotype in aluminium solution compared to its potential growth without aluminium, this parameter is a measure of aluminium tolerance alone.

3.6.3 Statistical analysis

Descriptive statistics were calculated with Agrobase (2005). The descriptive statistics calculated the average value, minimum value, maximum value, the range, the standard deviation and the variance of the data.

3.7 Results

The root re-growth method indicated three categories of tolerance (no root growth was classified as susceptible, 1-5 mm as moderate, 5.1-10 mm as intermediate and 10.1-15 mm as tolerant). The rating system was that of (Miller *et al.,* 2002) with minor modification. The root tolerance index indicated the frequency of population distribution. The susceptible check, Elands, did not show root re-growth in any of the experiments.

Table 3.3 Root re-growth classes (percentage in parenthesis) of the primary (PR) and secondary (SR) roots of the ASSN1 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	44	44 (100)	0	0	0
ASSN1 (PR)	14	0	2 (14.29)	11 (78.57)	1 (7.14)
ASSN1 (SR)	14	0	3 (21.43)	10 (71.43)	1 (7.14)

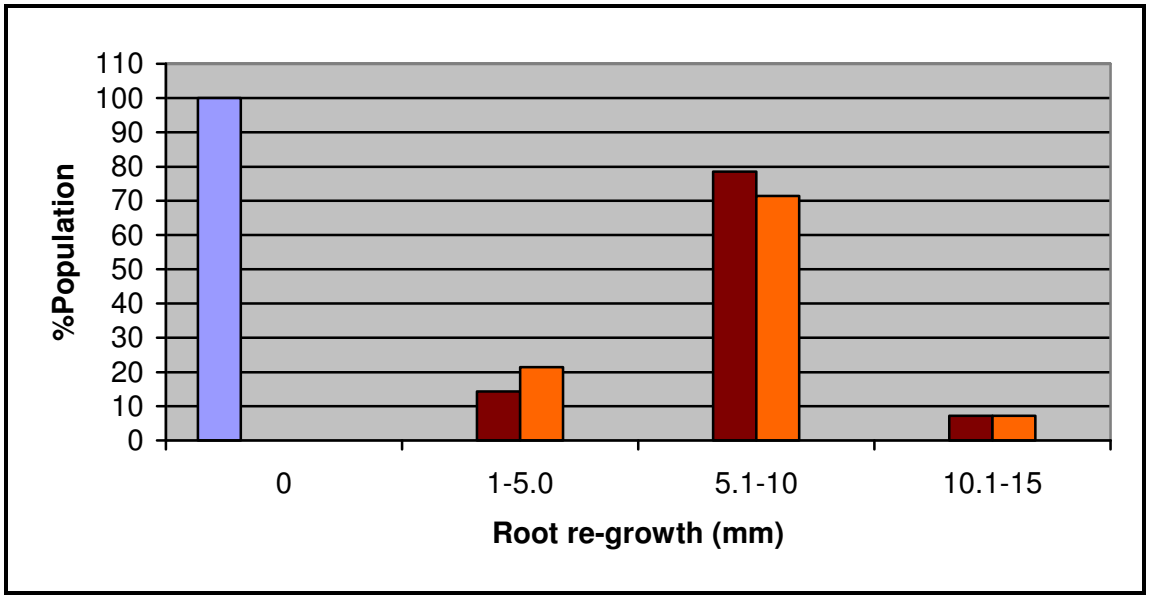

Figure 3.3 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN1 population

Table 3.4 Descriptive statistics of four variables measured on the ASSN1 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range(mm)	Std.dev.	Variance
RL (mm)	14	51.57	17.00	104.00	87.00	23.84	527.65
RG (mm)	14	7.43	4.00	11.00	7.00	2.14	4.26
S (mm)	14	5.64	2.00	8.00	6.00	1.85	3.17
RTI	14	0.19	0.05	0.59	0.54	0.15	0.02
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range(mm)	Std.dev.	Variance
RL (mm)	14	38.36	18.00	75.00	57.00	17.16	273.32
RG (mm)	14	7.64	2.00	15.00	13.00	3.09	8.86
S (mm)	14	5.86	4.00	8.00	4.00	0.99	0.90
RTI	14	0.24	0.07	0.48	0.41	0.14	0.02

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

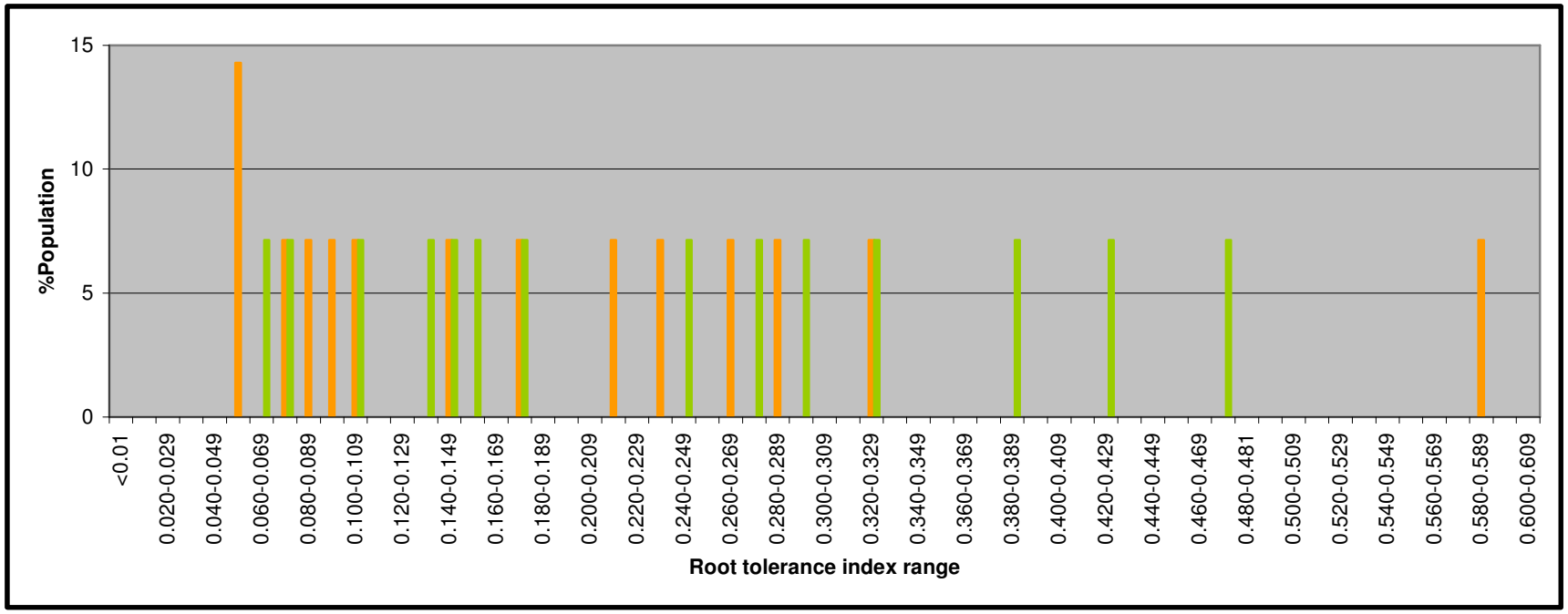

Figure 3.4 Frequency distribution of aluminium tolerance of the primary and secondary roots of the ASSN1 population

Table 3.5 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the ASSN5 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	56	56(100)	0	0	0
ASSN5 (PR)	34	0	14 (41.18)	18 (52.94)	2 (5.88)
ASSN5 (SR)	34	0	18 (52.94)	14 (41.18)	2 (5.88)

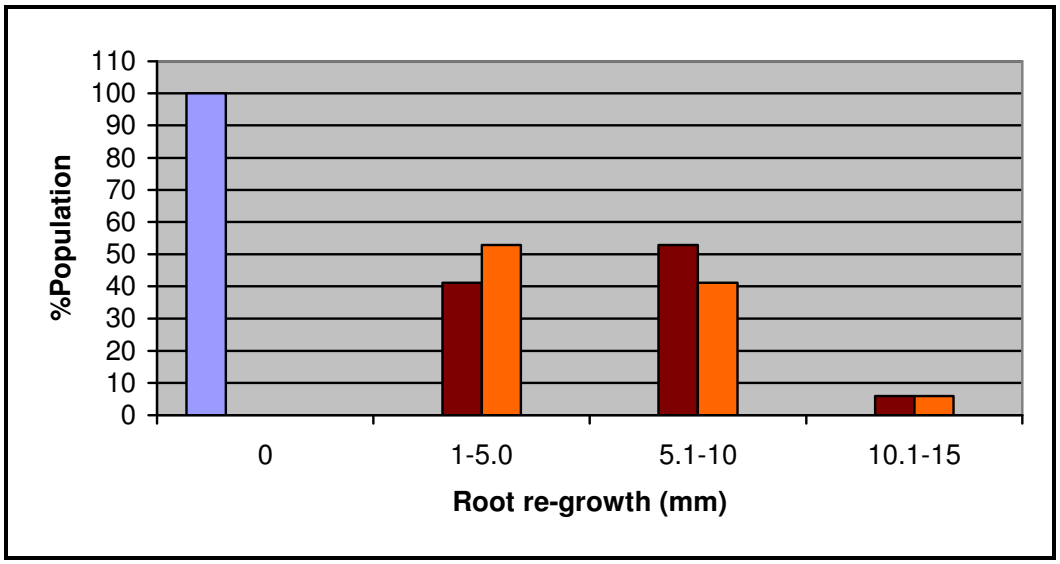

Figure 3.5 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN5 population

Table 3.6 Descriptive statistics of four variables measured on the ASSN5 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	61.77	22.00	119.00	97.00	22.49	491.09
RG (mm)	34	5.94	1.00	12.00	11.00	2.91	8.22
S (mm)	34	4.84	3.00	8.00	5.00	1.29	1.62
RTI	34	0.11	0.01	0.30	0.29	0.07	0.00
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	56.50	15.00	92.00	77.0	21.55	450.56
RG (mm)	34	5.53	1.00	12.00	11.00	3.25	10.23
S (mm)	34	4.74	2.00	8.00	6.00	1.66	2.69
RTI	34	0.13	0.01	0.73	0.72	0.15	0.02

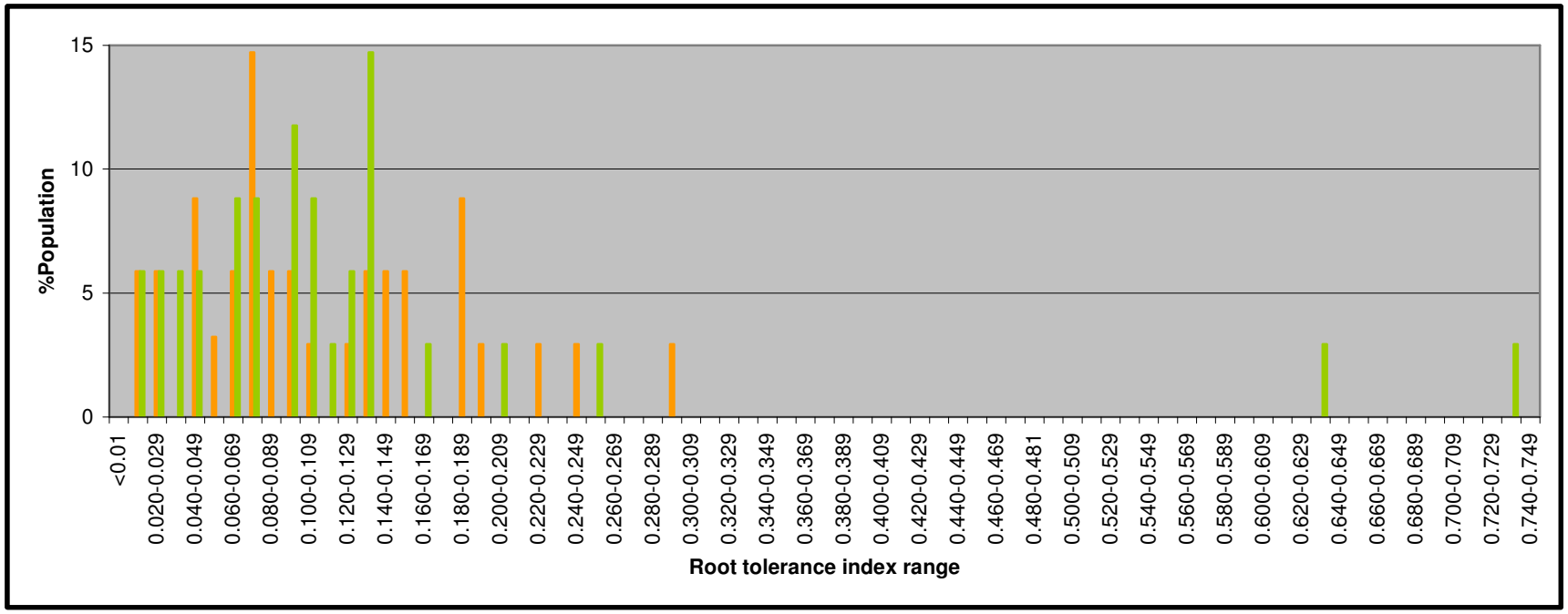

Figure 3.6 Frequency distribution of aluminium tolerance of the primary and secondary roots of the ASSN5 population

Table 3.7 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of Tugela DN population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	275	275 (100)	0	0	0
Tugela DN (PR)	180	0	133 (73.89)	46 (25.56)	1 (0.56)
Tugela DN (SR)	180	0	130 (72.22)	48 (26.67)	2 (1.11)

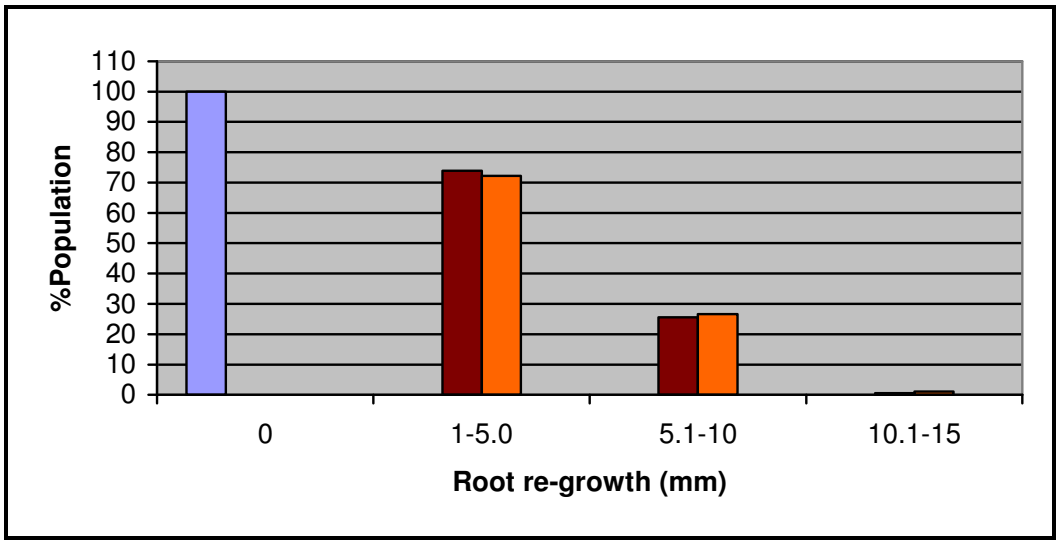

Figure 3.7 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the Tugela DN population

Table 3.8 Descriptive statistics of four variables measured on the Tugela DN population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range(mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	0.10	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range(mm)	Std.dev.	Variance
RL (mm)	180	53.34	8.00	117.00	109.0	19.78	389.17
RG (mm)	180	3.94	1.00	11.00	10.00	2.15	4.58
S (mm)	180	3.05	1.00	6.00	5.00	1.01	1.01
RTI	180	0.09	0.01	1.38	1.36	0.11	0.01

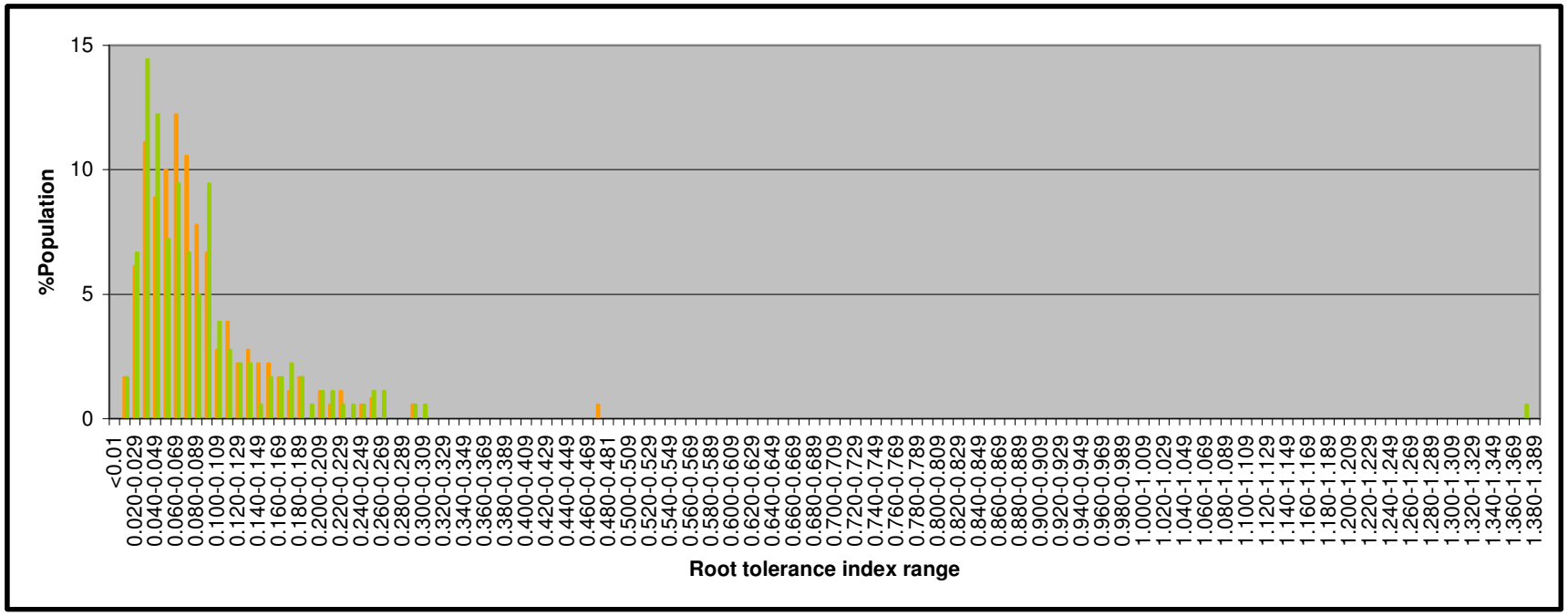

Figure 3.8 Frequency distribution of aluminium tolerance of the primary ■ and secondary ■ roots of the Tugela DN population

Table 3.9 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the ASSN16 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	84	84 (100)	0	0	0
ASSN16 (PR)	34	0	18 (52.94)	16 (47.06)	0
ASSN16 (SR)	34	0	23 (67.65)	11 (32.35)	0

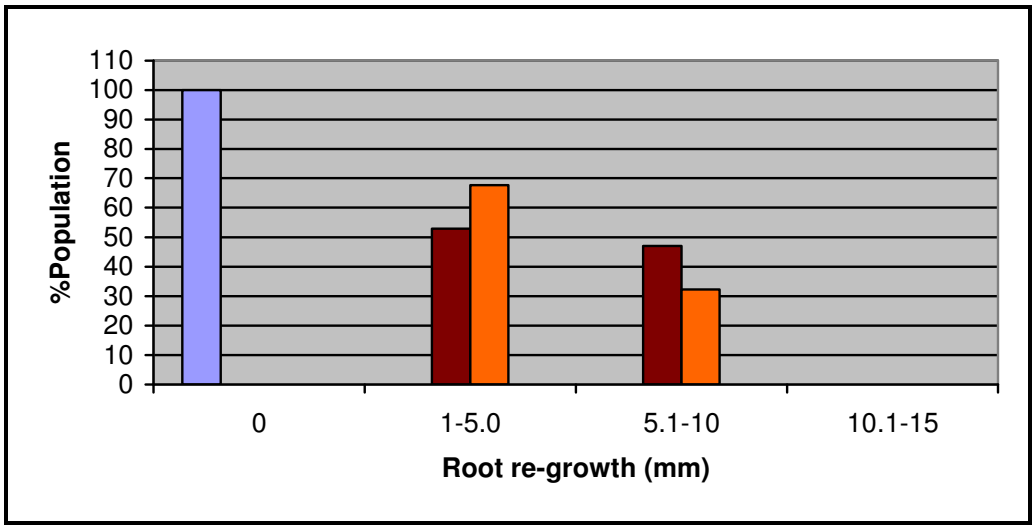

Figure 3.9 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN16 population

Table 3.10 Descriptive statistics of four variables measured on the ASSN16 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	72.94	9.00	105.00	96.00	28.90	810.48
RG (mm)	34	5.24	1.00	10.00	9.00	2.38	5.50
S (mm)	34	3.79	1.00	6.00	5.00	1.29	1.62
RTI	34	0.12	0.01	1.00	0.99	0.18	0.03
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	64.35	17.00	107.00	90.00	26.87	700.48
RG (mm)	34	4.21	1.00	9.00	8.00	2.44	5.79
S (mm)	34	3.54	2.00	5.50	3.50	1.06	1.10
RTI	34	0.10	0.02	0.53	0.51	0.13	0.02

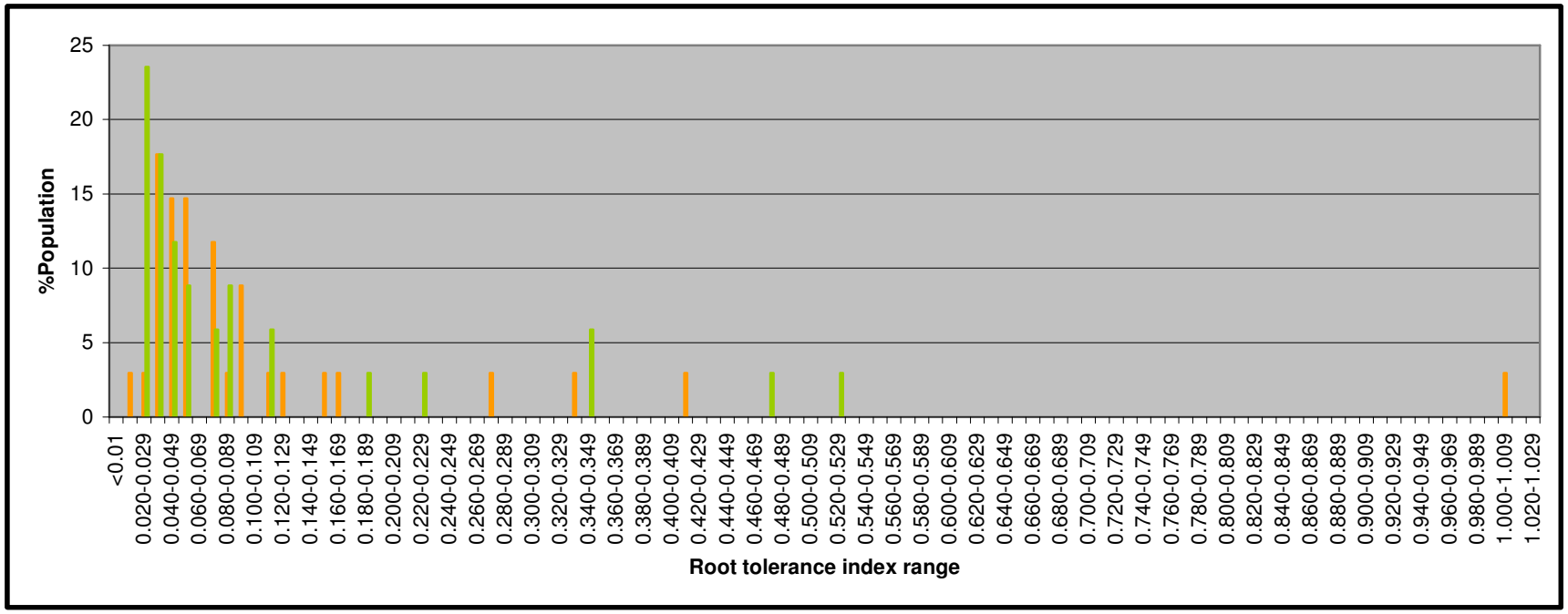

Figure 3.10 Frequency distribution of aluminium tolerance of the primary and secondary roots of the ASSN16 population

Table 3.11 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the ASSN12 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	58	58 (100)	0	0	0
ASSN12 (PR)	28	0	19 (67.86)	9 (32.14)	0
ASSN12 (SR)	28	0	23 (82.14)	5 (17.86)	0

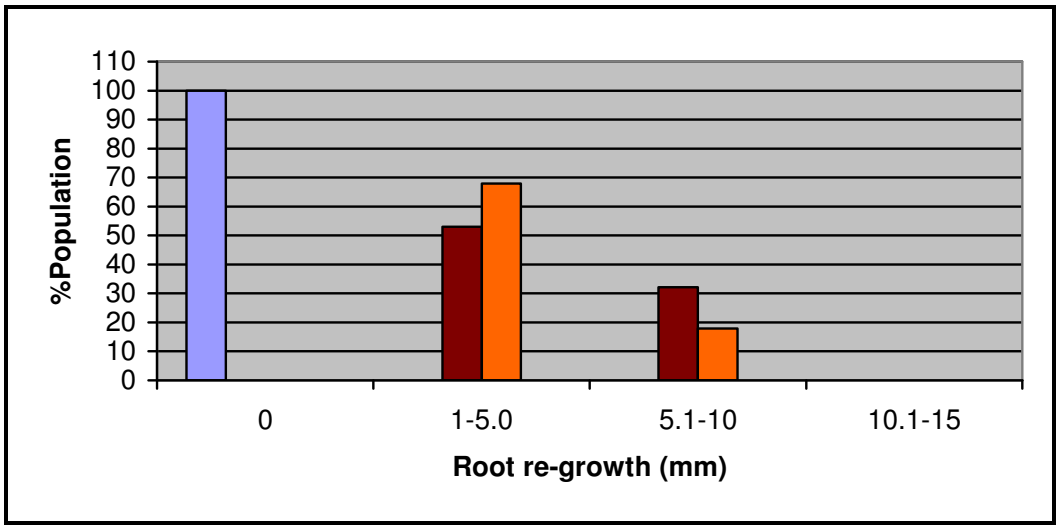

Figure 3.11 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN12 population

Table 3.12 Descriptive statistics of four variables measured on the ASSN12 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	72.18	12.00	107.00	95.00	31.02	927.93
RG (mm)	28	4.29	1.00	9.00	8.00	2.27	4.95
S (mm)	28	3.63	2.00	7.00	5.00	1.39	1.86
RTI	28	0.08	0.01	0.36	0.35	0.09	0.01
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	59.04	13.00	109.00	96.00	30.42	892.41
RG (mm)	28	3.61	1.00	9.00	8.00	2.00	3.86
S (mm)	28	2.98	1.00	6.00	5.00	1.35	1.75
RTI	28	0.11	0.01	0.69	0.68	0.15	0.02

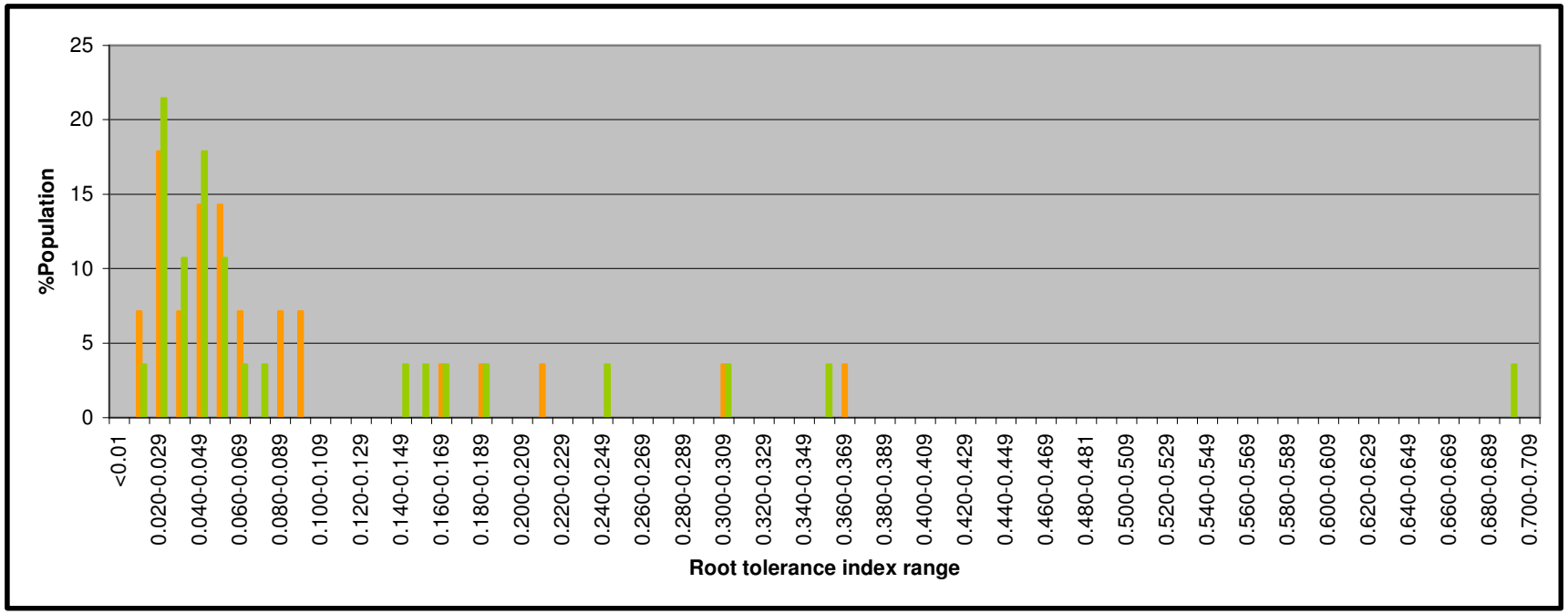

Figure 3.12 Frequency distribution of aluminium tolerance of the primary and secondary roots of the ASSN12 population

Table 3.13 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the ASSN7 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	76	76 (100)	0	0	0
ASSN7 (PR)	5	0	4 (80)	1 (20)	0
ASSN7 (SR)	5	0	4 (80)	1 (20)	0

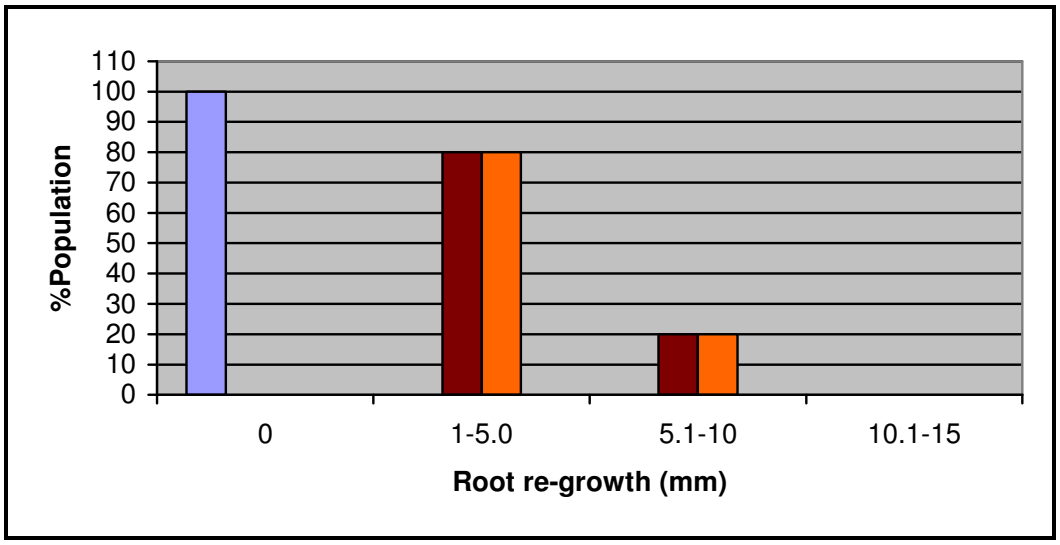

Figure 3.13 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN7 population

Table 3.14 Descriptive statistics of four variables measured on the ASSN7 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	5	40.60	21.00	60.00	39.00	19.18	294.30
RG (mm)	5	3.90	1.50	9.00	7.50	3.36	9.05
S (mm)	5	3.00	2.00	4.00	2.00	0.79	0.50
RTI	5	0.12	0.04	0.38	0.33	0.16	0.02
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	5	26.00	9.00	51.00	42.00	17.77	252.50
RG (mm)	5	3.70	1.00	10.00	9.00	4.14	13.70
S (mm)	5	2.40	2.00	3.00	1.00	0.61	0.30
RTI	5	0.30	0.03	1.11	1.08	0.52	0.21

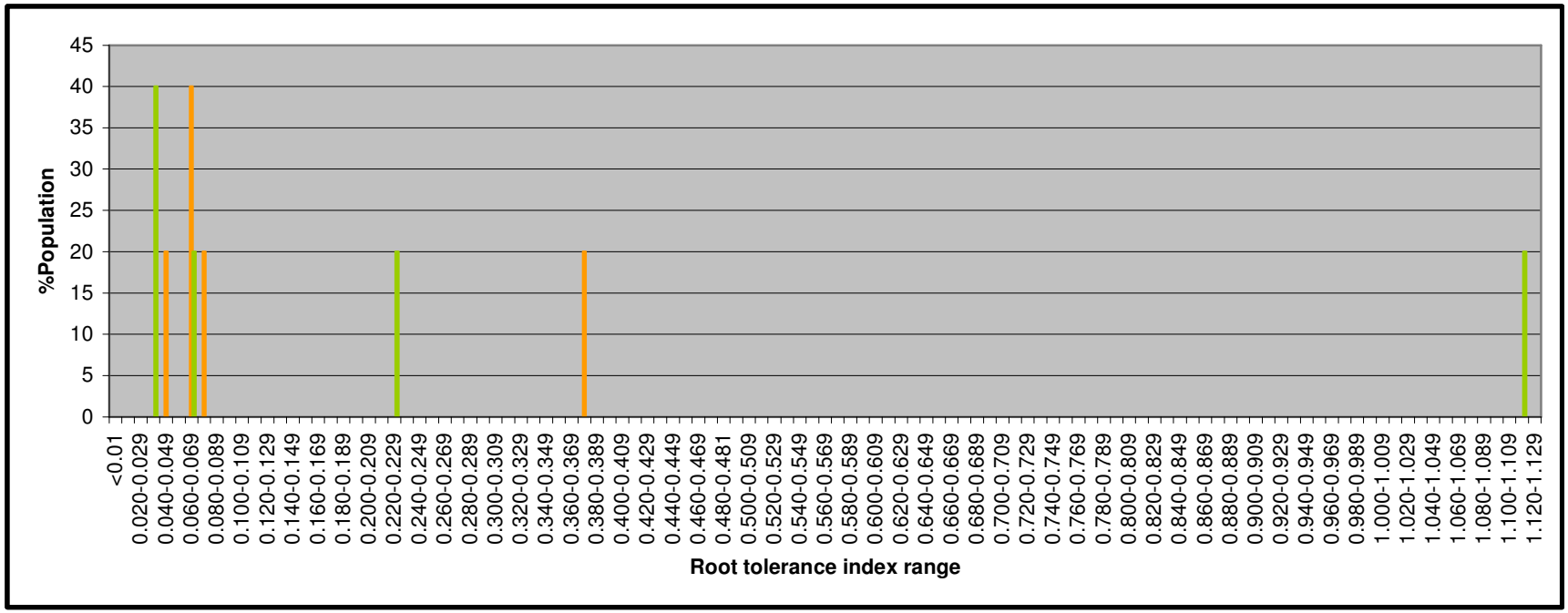

Figure 3.14 Frequency distribution of aluminium tolerance of the primary and secondary roots of the ASSN7 population

Table 3.15 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the Atlas 66 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	275	275 (100)	0	0	0
Atlas66 (PR)	117	0	102 (87.18)	15 (12.82)	0
Atlas 66 (SR)	117	0	105 (89.74)	12 (10.26)	0

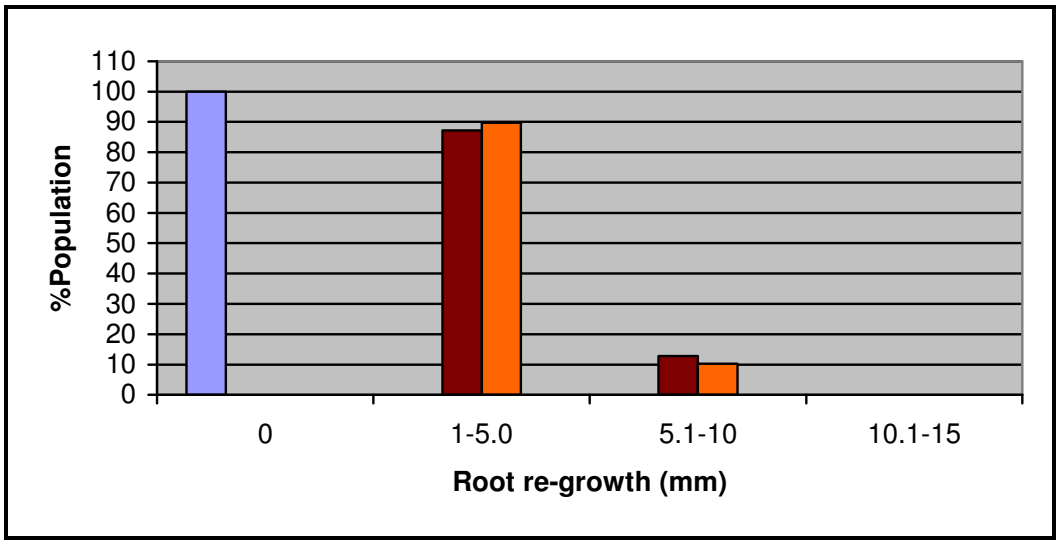

Figure 3.15 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the Atlas 66 population

Table 3.16 Descriptive statistics of four variables measured on the Atlas 66 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	55.07	12.00	105.00	93.00	18.08	324.15
RG (mm)	117	3.48	1.00	9.00	8.00	1.78	3.12
S (mm)	117	2.68	1.00	6.00	5.00	1.28	1.61
RTI	117	0.07	0.01	0.43	0.42	0.06	0.00
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	49.24	17.00	92.00	75.00	17.92	318.29
RG (mm)	117	2.77	1.00	8.00	7.00	1.73	2.95
S (mm)	117	2.56	1.00	6.00	5.00	1.24	1.52
RTI	117	0.07	0.01	0.35	0.34	0.06	0.00

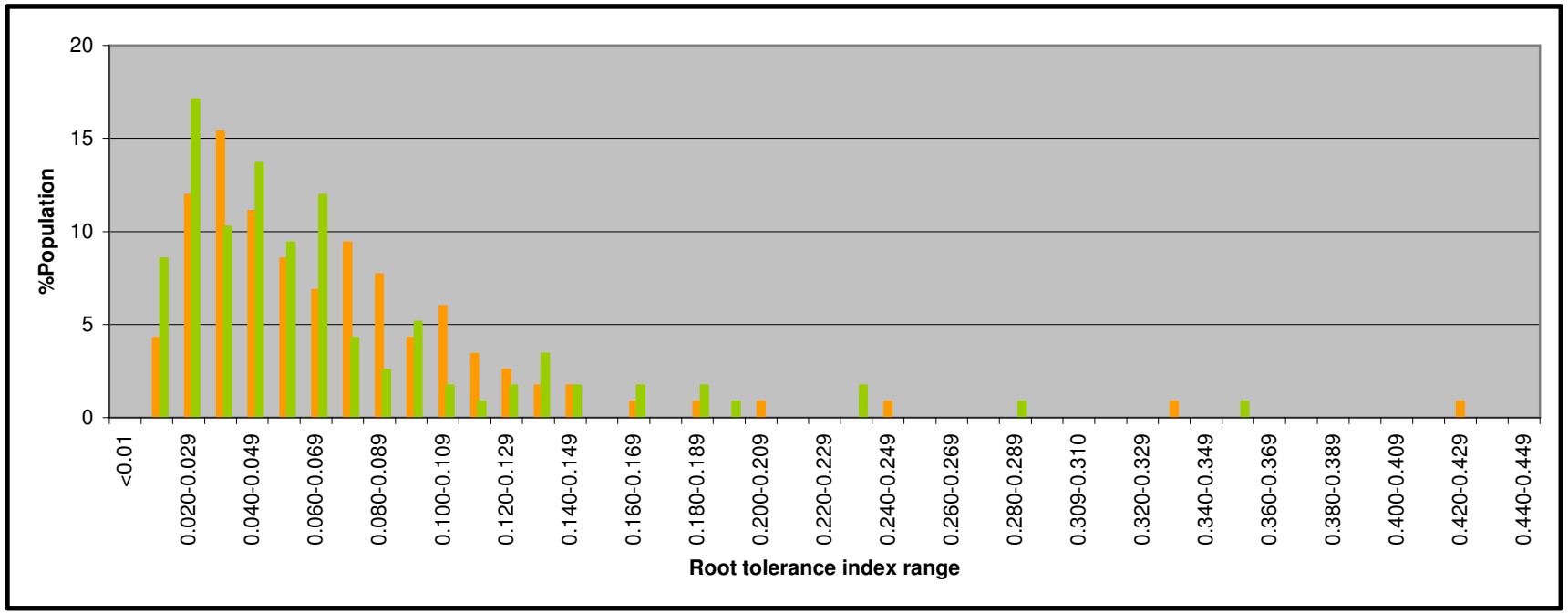

Figure 3.16 Frequency distribution of aluminium tolerance of the primary and secondary roots of the Atlas 66 population

Table 3.17 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the ASSN2a population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	70	70 (100)	0	0	0
ASSN2a (PR)	30	0	26 (86.67)	4 (13.33)	0
ASSN2a (SR)	30	0	27 (90)	3 (10)	0

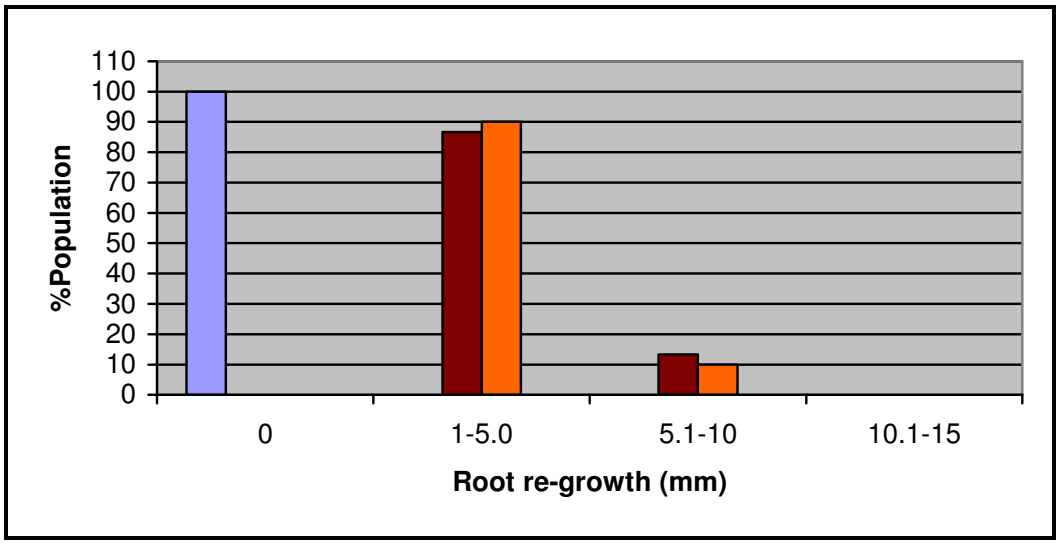

Figure 3.17 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN2a population

Table 3.18 Descriptive statistics of four variables measured on the ASSN2a population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	30	62.43	17.00	94.00	77.00	27.57	734.60
RG (mm)	30	3.85	2.00	7.00	5.00	1.43	1.99
S (mm)	30	3.77	2.00	8.00	6.00	1.27	1.56
RTI	30	0.10	0.02	0.37	0.35	0.10	0.01
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	30	59.13	18.00	91.00	73.00	25.26	617.02
RG (mm)	30	3.33	1.00	7.00	6.00	1.80	3.13
S (mm)	30	3.28	1.00	5.00	4.00	1.05	1.06
RTI	30	0.09	0.01	0.39	0.38	0.10	0.01

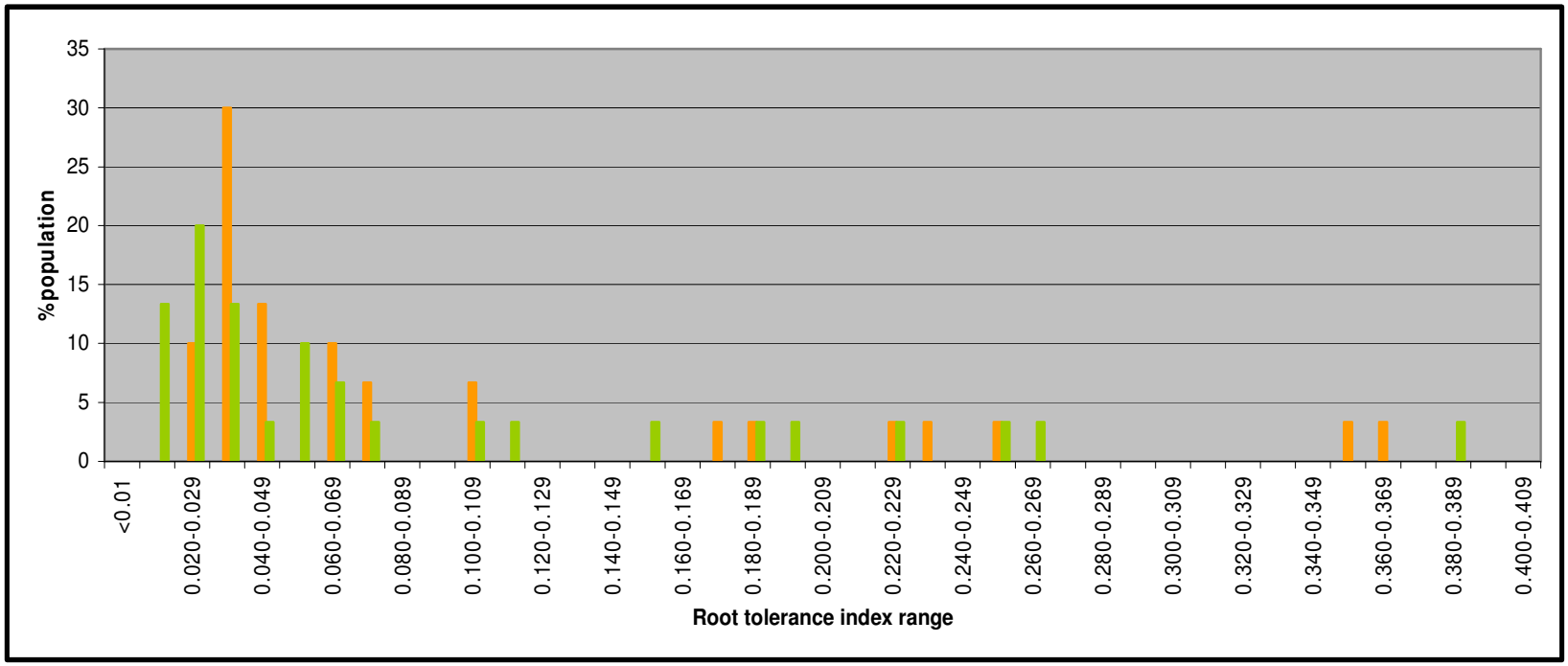

Figure 3.18 Frequency distribution of aluminium tolerance of the primary and secondary roots of the ASSN2a population

Table 3.19 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the T96/6 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	12	12 (100)	0	0	0
T96/6 (PR)	2	0	2 (100)	0	0
T96/6 (SR)	2	0	2 (100)	0	0

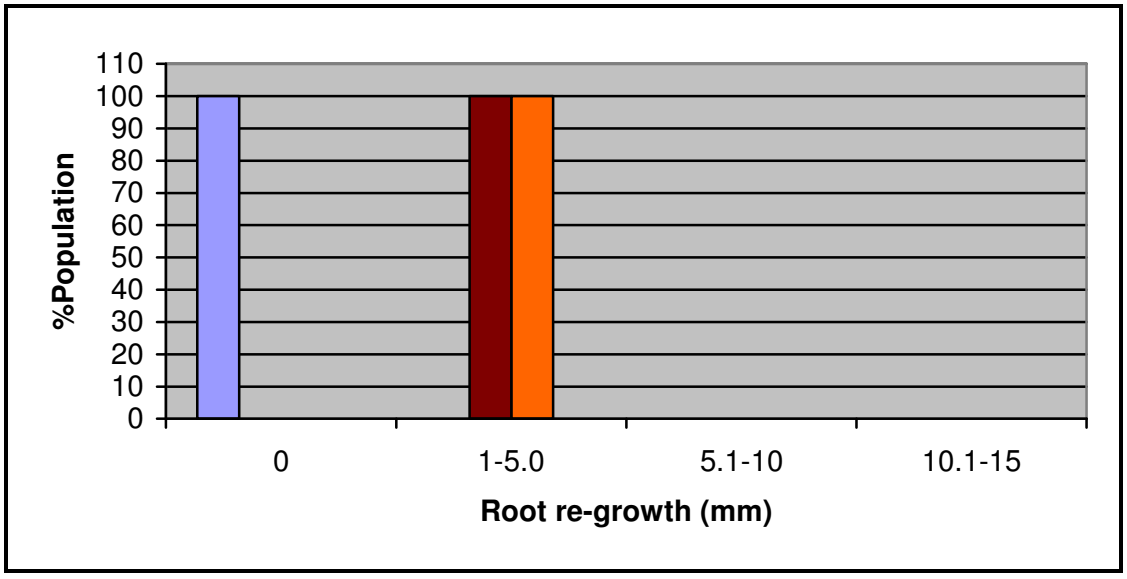

Figure 3.19 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the T96/6 population

Due to only two seedlings being used, no descriptive statistics and frequency distribution was done.

Table 3.20 Root re-growth classes (percentage in parenthesis) of the primary and secondary roots of the ASSN15 population

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	56	56 (100)	0	0	0
ASSN15 (PR)	4	0	4 (100)	0	0
ASSN15 (SR)	4	0	4 (100)	0	0

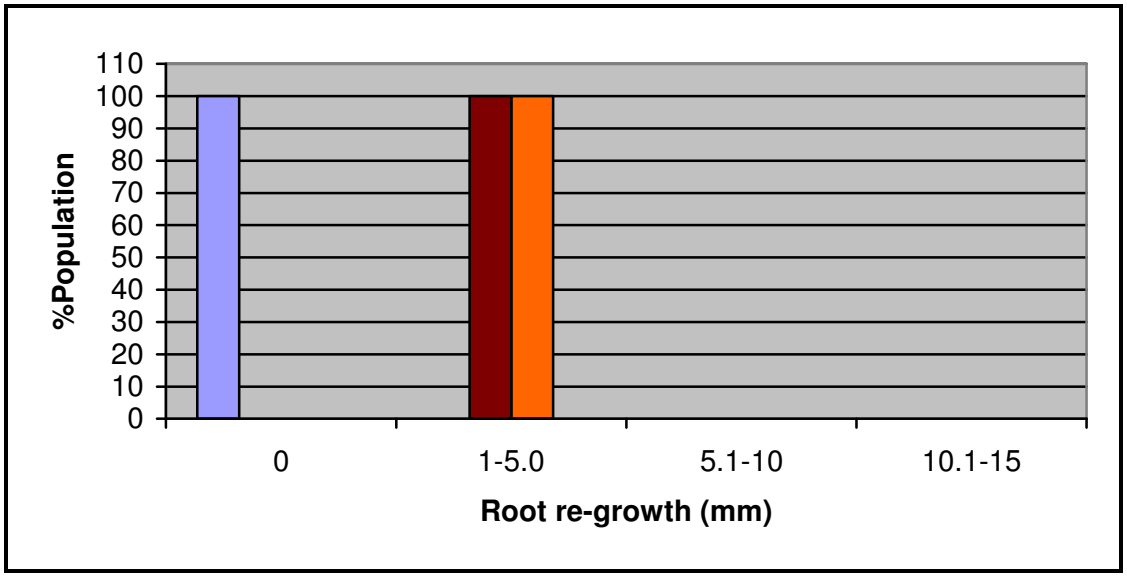

Figure 3.20 The frequency distribution of the root re-growth of Elands, primary and secondary roots of the ASSN15 population

Table 3.21 Descriptive statistics of four variables measured on the ASSN15 population

Primary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	4	56.25	25.00	78.00	53.00	26.86	540.92
RG (mm)	4	2.50	1.00	5.00	4.00	2.21	3.67
S (mm)	4	2.75	2.00	3.00	1.00	0.58	0.25
RTI	4	0.07	0.01	0.20	0.19	0.10	0.01
Secondary roots							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	4	54.50	23.00	95.00	72.00	35.72	957.67
RG (mm)	4	1.75	1.00	2.00	1.00	0.58	0.25
S (mm)	4	3.50	3.00	5.00	2.00	1.16	1.00
RTI	4	0.05	0.01	0.09	0.08	0.04	0.00

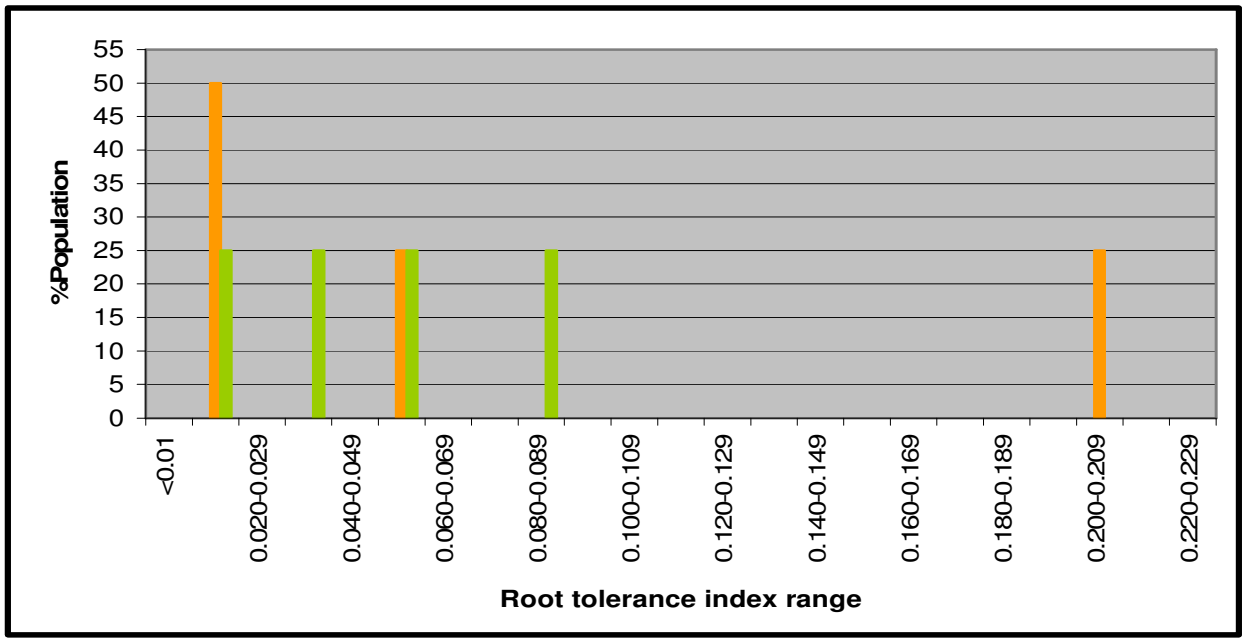

Figure 3.21 Frequency distribution of aluminium tolerance of the primary ■ and secondary ■ roots of the ASSN15 population

3.8 Discussion and conclusions

In this study, two screening methods for aluminium tolerance were used to investigate the best method to distinguish between different categories of tolerance in the tolerant sources as well as to identify a sensitive enough method to effectively evaluate and select individuals in the cross progeny. The primary and secondary roots responses to aluminium toxicity were evaluated with the root growth and the root tolerance index methods to determine the sensitivity of the methods in evaluation of roots of different ages, since this will determine the progress and success of an aluminium tolerance pre-breeding programme.

From the results it was evident, that with the root growth data, it was possible to clearly distinguish between three (moderate, intermediate and tolerant) different categories of tolerance. The genotypes were grouped into three groups. Group one were genotypes with all the three categories (moderate, intermediate and tolerant); group two, those with two categories (moderate and intermediate) and group three, those with only one category (moderate), which is the lowest tolerance class.

In group one, three categories of tolerance were observed. This group consisted of genotypes ASSN1, ASSN5 and Tugela DN (Figures 3.3, 3.5 and 3.7). It was also possible to discriminate between genotypes with a high possibility to identify individuals with a higher level of aluminium tolerance with the root re-growth method. Therefore, the genotypes were ranked from the best to the poorest aluminium tolerance level. This information is very important in a breeding programme to allow the reduction of the number of genotypes in the screening process to pinpoint parents that have the required genetic makeup for aluminium tolerance breeding.

Group two consisted of genotypes ASSN16, ASSN12, ASSN7, Atlas 66 and ASSN2a (Figures 3.9, 3.11, 3.13, 3.15 and 3.17) and this group consisted of two categories of tolerance. This group can be considered for use as parents, when tolerant parents are not available. Group three consisted of genotypes T96/6 and ASSN15 (Figures 3.19 and 3.21) which consisted of only one category of tolerance, which was the lowest level.

With the comparison of the primary root and secondary root growth, similar data were obtained for each genotype in the different groups. The data indicated that the root re-growth method is sensitive enough to discriminate between different levels of tolerance in genotypes and that the age of the root is not a limiting factor in obtaining reliable aluminium toxicity tolerance data.

The second method that was used was the root tolerance index. With this method, the root vigour was taken into consideration, as it gives a better indication of the aluminium tolerance levels in a specific genotype.

With the root tolerance index method it was also possible to identify genotypes with greater aluminium tolerance levels (Tugela DN, Figure 3.8; ASSN16, Figure 3.10 and ASSN7, Figure 3.14) and genotypes with smaller aluminium tolerance levels (T96/6, Figure 3.18). The root tolerance index method is a suitable approach for genetic and molecular studies in which a precise quantitative response for stress is needed. The method is also suitable for identifying genotypes with superior alleles for tolerance (Wang *et al.,* 2006a).

In this study, tolerant and sensitive genotypes displayed contrasting staining patterns; with very light stain on the root tips of aluminium tolerant cultivars and heavy stain on the roots of aluminium sensitive cultivars. Poor root re-growth in some seedlings, especially the susceptible seedlings, is the consequence of high accumulation of aluminium in the root caps and because tolerant cultivars have some mechanisms to avoid aluminium toxicity, there will always be root growth, though it might not be much.

Considerable cultivar variability appeared to exist among the nine wheat genotypes in comparison with the international tolerant standard Atlas 66 (Tables 3.22 and 3.23).

Multiple comparisons (Table 3.22) indicated that T96/6, ASSN16, ASSN12, ASSN2a, ASSN5, Tugela DN and ASSN15 were the most aluminium tolerant genotypes in comparison with Atlas 66 in terms of the root length. T96/6, a of South African cultivar, selected for moderate aluminium tolerance, as well as moderate pre-harvest sprouting, showed some aluminium tolerance, but less compared with Atlas 66. The susceptible check did not produce any root re-growth throughout the evaluation period.

The existence of considerable genetic diversity was shown for aluminium tolerance in the nine genotypes against Atlas 66. The range of the root length in the primary root was 93 mm and 75 mm for the secondary root, with an average of 55.07 mm for the primary root and 49.24 mm for the secondary root. The range of the root re-growth was 8 mm for the primary root and 7 mm for the secondary root. The average root tolerance index for the primary and secondary roots was 0.07, with a range of 0.42 for the primary root and 0.34 for the secondary root for Atlas 66 (Table 3.16).

The range of the root length for ASSN1 primary root (Table 3.4) was 87 mm with an average of 51.57 mm, while for the secondary root; the range was 57 mm, with an average of 38.36 mm. The root growth range for the primary root was 7 mm and 13 mm for the secondary root. In Table 3.6, the four parameters showed slight differences with a major difference in the range of root length and root growth in both roots. The range of both roots for root re-growth was 11 mm.

Within Tugela DN (Table 3.8), the range of the root length was 124 mm for the primary root and 109 mm for the secondary root. The root re-growth range was 11 mm for the primary root and 10 mm for secondary root, with an average root re-growth of 4.3 mm for the primary root and 3.94 mm for the secondary root. The range for the root tolerance index was 0.46 for the primary root, which was smaller compared with the range of 1.36 for the secondary root.

The range of the root length was 96 mm for the primary root and 90 mm for secondary root of ASSN16. The average root re-growth for the primary root was 5.24 mm with a range of 9 mm for primary root while for the secondary root, the average was 4.21 mm and a range of 8 mm was determined for the aluminium tolerance line ASSN16 (Table 3.10).

For ASSN12 (Table 3.12), the average root length for the primary root was 72.18 mm and 59.04 mm for the secondary root, with a range of 95 mm and 96 mm respectively. The range of the root re-growth in both roots was 9 mm. The range for root staining in both roots was also the same with a value of 5 mm. The root tolerance index range was smaller in the primary root and greater in the secondary root.

Considering the number of seedlings that were evaluated for ASSN7 (Table 3.14) in both roots, it can clearly be seen that there is significant genetic variability for this genotype compared with the other genotypes. The range of the root length, root re-growth and root tolerance index was smaller in the primary root than the secondary root.

In Table 3.18 (ASSN2a), the average and range for root length was greater in the primary than the secondary root. The average of root tolerance index was greater in the primary root than in the secondary root, with a range of 0.35 for the primary root and 0.38 for the secondary root.

The average root length for ASSN15 (Table 3.21) was 56.25 mm for the primary root and 54.5 mm for the secondary root. The range of the primary root was 53 mm for root length and 72 mm for the secondary root. The range of root re-growth and root tolerance index was 2.21 mm in the primary root and the 0.58 of the secondary root.

Many researchers using modifications of the nutrient culture of Polle *et al.* (1978) and other screening systems, have performed screening for apparent aluminium tolerance in wheat. Based on the irreversible damage to the apical meristem of roots at the seedling stage, root re-growth following aluminium shock can be easily observed (Aniol, 1991; Zhang & Jessop, 1998) and the differential aluminium response of given genotypes could thus be effectively evaluated. The test gives reproducible results, provided that conditions such as temperature, pH value, aluminium concentration and time of exposure to aluminium shock, are controlled (Aniol, 1983; Carver & Ownby, 1995).

Varietal tolerance to aluminium toxicity is apparently relative, rather than absolute (Aniol, 1991; Zhang & Jessop, 1998; Camargo *et al.,* 2004), since differing root growth and/or root re-growth may occur, depending on the level of aluminium stress imposed in the nutrient culture solution used and a range of other factors, including pH level, temperature and the nutrient content of the culture solution (Cosic *et al.,* 1994; Camargo *et al.,* 2004), in the early development of wheat. This may also explain the discrepancies of results reported in some working reports, even though similar materials were used.

Considerable genetic variability of tolerance to aluminium toxicity was present in all genotypes included in this study, which should lay a sound foundation for further improvement of aluminium tolerance in wheat breeding in South Africa. Better information concerning varietal aluminium tolerance, yield stability and grain quality characteristics is also needed to facilitate the expansion of wheat production in South Africa.

In conclusion, a rapid and reliable screening method to distinguish between aluminium tolerant and sensitive genotypes is necessary to select and breed crops for aluminium tolerance. It is important to screen all genotypes to ensure that genotypes are selected, which have the probability to give higher levels of aluminium tolerance for future hybridisation. The system is especially useful for initial screening tests.

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CHAPTER 4

Genetic response of F_2 progeny for aluminium tolerance

4.1 Introduction

Genetic diversity is the foundation of genetic improvement in plants. Genetic variation in response to aluminium toxicity has been found not only among plant species but also among cultivars within species, which differ greatly in their susceptibility to aluminium toxicity in acid soils. Some of these differences are genetically controlled (Tang *et al.,* 2001; 2003; Gustafson, 2005; Ma, 2005; Zhou *et al.,* 2007). This genetic variability within species provides an opportunity to develop and select desirable genotypes with improved tolerance to aluminium toxicity (Bona *et al.,* 1993). The most effective strategy for the production of economically important crops in acidic soils is the use of aluminium tolerant cultivars (Zhang *et al.,* 2007).

Breeding and selection of wheat with higher aluminium tolerance will be more effective if the genetic control of this trait is better understood. Quantitative genetic data of root re-growth is a useful and reliable indicator of relative aluminium tolerance in wheat (Parker, 1995; Zhang *et al.,* 2007).

Beside aluminium tolerance, root growth during the development of wheat in the laboratory, as well as in the field, is very important to allow for good crop establishment (Camargo *et al.,* 2004). With the combination of genes, it would be possible to select plants with high levels of tolerance to aluminium toxicity for future use in breeding for aluminium tolerance.

The objective of this study was to cross selected genotypes with high and low root re-growth in the presence of aluminium, in order to enhance aluminium tolerance.

4.2 Material and methods

Tolerant parental genotypes Atlas 66, Tugela DN, ASSN1, ASSN5, ASSN7, ASSN12, ASSN16, ASSN2a and one susceptible parental line, Elands, were crossed in the

glasshouse to produce F_1 seeds. Atlas 66 was selected as the international check. Tugela DN and Elands were selected as local tolerant and susceptible checks respectively. The F_1 seeds were harvested, hand threshed and selfed in the glasshouse to produce F_2 seeds, which were used in subsequent studies (Table 4.1).

Although 10 of the original entries were classified as tolerant, they had different levels of tolerance, due to different genes or gene combination which was shown in Chapter 3. Due to the genetic variability of the trait, specific crosses were made between the tolerant genotypes to explore the ability of different genes to combine that may result in a higher aluminium tolerance response in the progeny.

Table 4.1 List of total number of seeds incubated for germination and evaluated for aluminium tolerance

Combination ♀x♂	Total no of seeds Incubated for germination	Total no. of seedlings evaluated
Tugela DNxASSN16	692	129
Atlas 66xASSN16	608	52
ElandsxASSN16	708	111
Tugela DNxASSN12	307	91
ASSN7xASSN12	252	107
ASSN1xASSN5	557	75
ASSN12xASSN16	469	124
ASSN2axASSN7	276	66
ASSN7xTugela DN	234	51

Seed dormancy of F_2 seeds was broken by placing seeds in an envelope, which was then placed in an incubator that was set at 35ºC. After 12 h of heat treatment, the seeds were placed in the freezer for another 12 h. The heat and cold treatment were alternated at 12 h intervals for 4 days. After seed dormancy was broken, seeds were stored in the freezer until needed.

The F_2 seeds were evaluated for aluminium tolerance, using the nutrient solution cultures as described in Section 3.2.2 in Chapter 3.

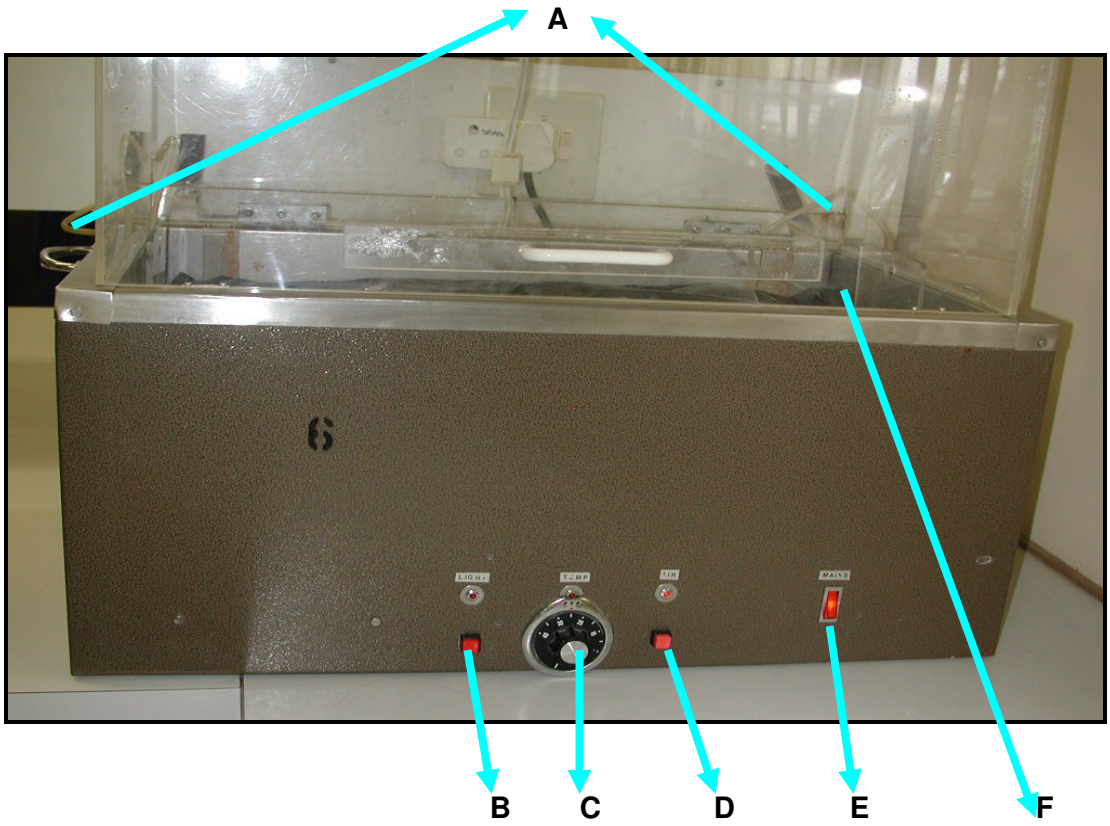

Figure 4.1 Growth chamber in which seeds were germinated

A= Two valves that create aeration in the water bath, B= Light switch, C= Temperature control knob, D= Air switch, E= main switch of the growth chamber and F= black plastic bag covering seed trays containing seeds in the growth chamber.

The effect of aluminium toxicity on parents and their progeny was studied through the root re-growth and root tolerance index method. Individual plants were measured for the longest root length, root re-growth, root staining and root tolerance index. Descriptive statistics was performed as in chapter 3, under statistical analysis.

4.3 Results

The root re-growth method showed the increase of aluminium tolerance from crossing parental materials with aluminium toxicity tolerance (Figures 4.2 - 4.4). The level of aluminium tolerance of parents is represented by **X** in tables. The classifications come from the previous chapter.

Table 4.2 Root re-growth classes (percentage in parenthesis) of susceptible Elands and the F_2 progeny of ASSN7xASSN12 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	35	35 (100)	0	0	0
ASSN7	5	0	4 (80)	1 (20)	0
ASSN12	28	0	19 (67.86)	9 (32.14)	0
F_2	107	0	86 (80.37)	13 (12.15)	8 (7.48)
ASSN7			**X**		
ASSN12			**X**		

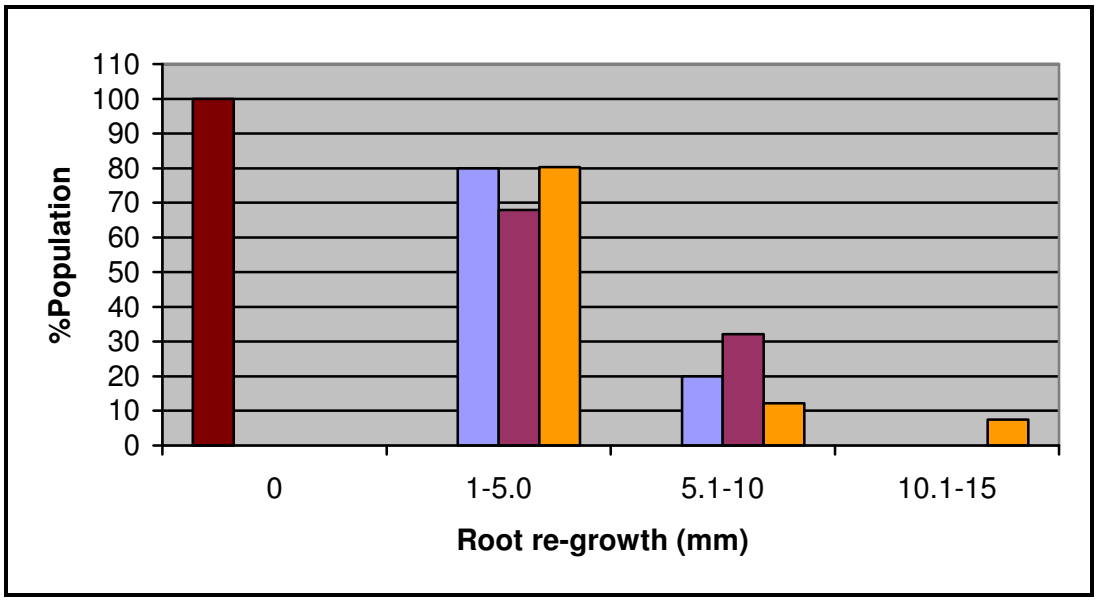

Figure 4.2 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes ASSN7 and ASSN12 after aluminium tolerance testing (Elands)

Table 4.3 Descriptive statistics of four variables measured for the parental genotypes ASSN7 and ASSN12 as well as the derived F_2 population

ASSN7							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	5	40.60	21.00	60.0	39.00	19.18	294.30
RG (mm)	5	3.90	1.50	9.00	7.50	3.36	9.05
S (mm)	5	3.00	2.00	4.00	2.00	0.791	0.50
RTI	5	0.12	0.04	0.38	0.33	0.158	0.02
ASSN12							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	72.18	12.00	107.00	95.00	31.02	927.93
RG (mm)	28	4.29	1.00	9.00	8.00	2.27	4.95
S (mm)	28	3.63	2.00	7.00	5.00	1.39	1.86
RTI	28	0.08	0.01	0.36	0.35	0.09	0.01
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	107	67.94	20.00	127.00	107.00	32.94	1075.15
RG (mm)	107	4.15	1.00	13.00	12.00	2.94	8.57
S (mm)	107	3.22	1.00	8.00	7.00	1.59	2.50
RTI	107	0.08	0.01	0.46	0.45	0.08	0.01

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.4 Root re-growth classes (percentage in parenthesis) of the F_2 of ASSN2axASSN7 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	31	31 (100)	0	0	0
ASSN2a	30	0	26 (86.67)	4 (13.33)	0
ASSN7	5	0	4 (80)	1 (20)	0
F_2	66	0	48 (72.73)	17 (25.76)	1 (1.52)
ASSN2a			**X**		
ASSN7				**X**	

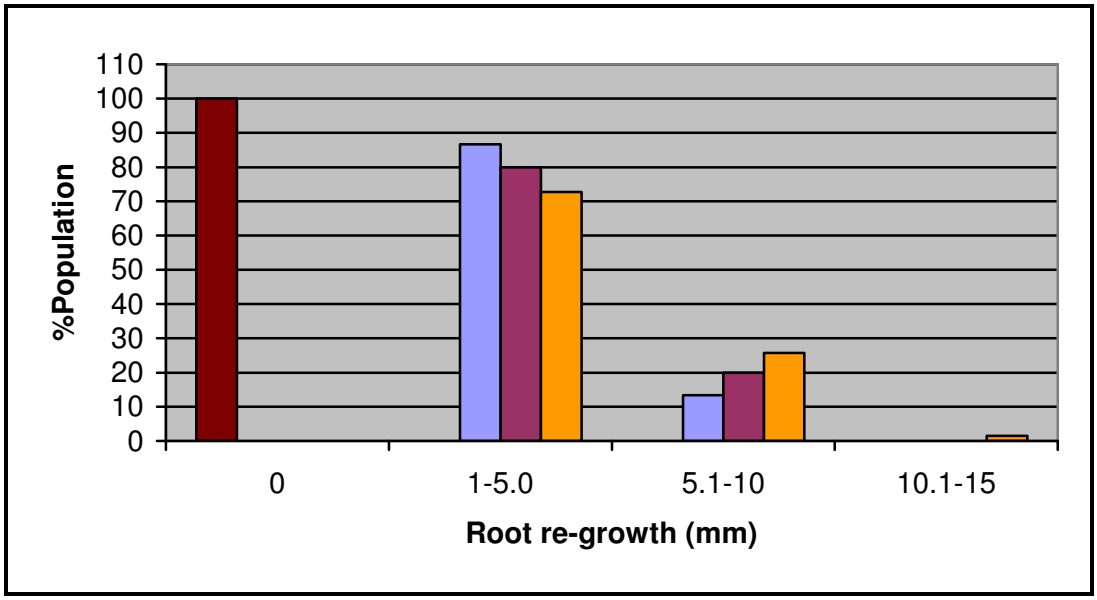

Figure 4.3 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes ASSN2a and ASSN7 after aluminium tolerance testing (Elands)

Table 4.5 Descriptive statistics of four variables measured for the parental genotypes ASSN2a and ASSN7 as well as the derived F_2 population

ASSN2a							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	30	62.43	17.00	94.00	77.00	27.57	734.60
RG (mm)	30	3.85	2.00	7.00	5.00	1.43	1.99
S (mm)	30	3.77	2.00	8.00	6.00	1.27	1.56
RTI	30	0.10	0.02	0.37	0.35	0.10	0.01
ASSN7							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	5	40.60	21.00	60.0	39.00	19.18	294.30
RG (mm)	5	3.90	1.50	9.00	7.50	3.36	9.05
S (mm)	5	3.00	2.00	4.00	2.00	0.79	0.50
RTI	5	0.12	0.04	0.38	0.33	0.16	0.02
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	66	57.91	22.00	119.00	97.00	29.04	830.24
RG (mm)	66	4.27	1.00	11.00	10.00	2.17	4.62
S (mm)	66	2.94	2.00	5.00	3.00	0.81	0.64
RTI	66	0.09	0.02	0.19	0.17	0.04	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.6 Root re-growth classes (percentage in parenthesis) of the F_2 of Tugela DNxASSN16 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	39	39 (100)	0	0	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.55)
ASSN16	34	0	18 (52.94)	16 (47.06)	0
F_2	129	0	76 (58.91)	52 (40.31)	1 (0.78)
Tugela DN			**X**		
ASSN16				**X**	

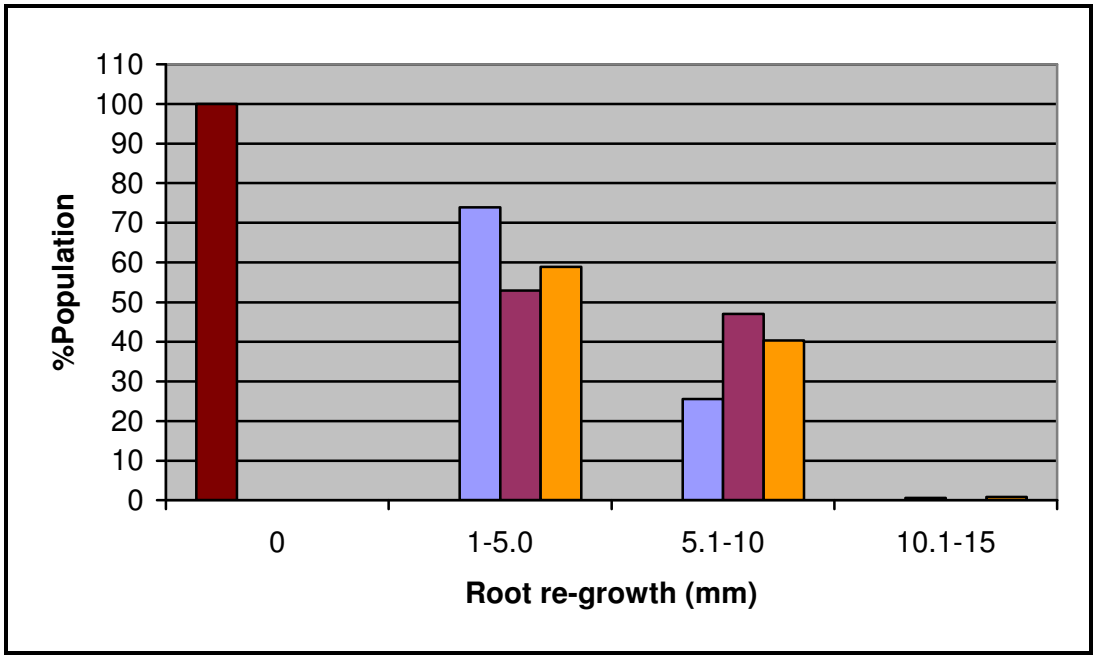

Figure 4.4 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Tugela DN and ASSN16 after aluminium tolerance testing (Elands)

Table 4.7 Descriptive statistics of four variables measured for the parental genotypes Tugela DN and ASSN16 as well as the derived F_2 population

Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.05	4.16
S (mm)	180	3.22	1.00	6.00	5.00	0.10	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
ASSN16							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	72.94	9.00	105.00	96.00	28.90	810.48
RG (mm)	34	5.24	1.00	10.00	9.00	2.38	5.50
S (mm)	34	3.79	1.00	6.00	5.00	1.29	1.62
RTI	34	0.12	0.01	1.00	0.99	0.18	0.03
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	129	44.36	15.00	88.00	73.00	14.59	211.16
RG (mm)	129	4.48	1.00	11.00	10.00	2.54	6.41
S (mm)	129	2.87	1.00	5.00	4.00	0.77	0.58
RTI	129	0.11	0.01	0.30	0.29	0.06	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.8 Root re-growth classes (percentage in parenthesis) of the F_2 of ASSN1xASSN5 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	32	32 (100)	0	0	0
ASSN1	14	0	2 (14.29)	11 (78.57)	1 (7.14)
ASSN5	34	0	14 (41.18)	18 (52.94)	2 (5.88)
F_2	75	0	71 (94.67)	2 (2.67)	2 (2.67)
ASSN1			**X**		
ASSN5			**X**		

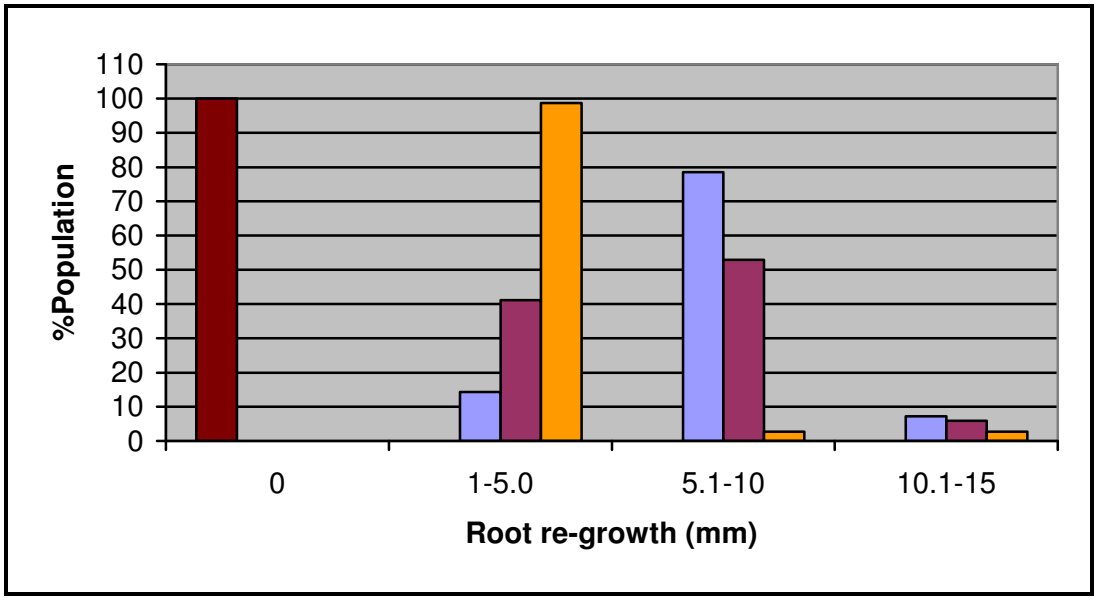

Figure 4.5 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes ASSN1 and ASSN5 after aluminium tolerance testing (Elands)

Table 4.9 Descriptive statistics of four variables measured for the parental genotypes ASSN1 and ASSN5 as well as the derived F_2 population

ASSN1							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	14	51.57	17.00	104.00	87.00	23.84	527.65
RG (mm)	14	7.43	4.00	11.00	7.00	2.14	4.26
S (mm)	14	5.64	2.00	8.00	6.00	1.85	3.17
RTI	14	0.19	0.05	0.59	0.54	0.150	0.02
ASSN5							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	61.77	22.00	119.00	97.00	22.49	491.09
RG (mm)	34	5.94	1.00	12.00	11.00	2.91	8.22
S (mm)	34	4.84	3.00	8.00	5.00	1.29	1.62
RTI	34	0.11	0.01	0.30	0.29	0.07	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	75	72.29	18.00	124.00	106.00	24.46	590.10
RG (mm)	75	3.03	1.00	6.00	5.00	1.50	2.21
S (mm)	75	2.76	2.00	5.00	3.00	0.77	0.59
RTI	75	0.05	0.01	0.21	0.02	0.04	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.10 Root re-growth classes (percentage in parenthesis) of the F_2 of Tugela DNxASSN12 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	39	39 (100)	0	0	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.55)
ASSN12	28	0	19 (67.86)	9 (32.14)	0
F_2	91		44 (48.35)	47 (51.65)	0
Tugela DN				**X**	
ASSN12				**X**	

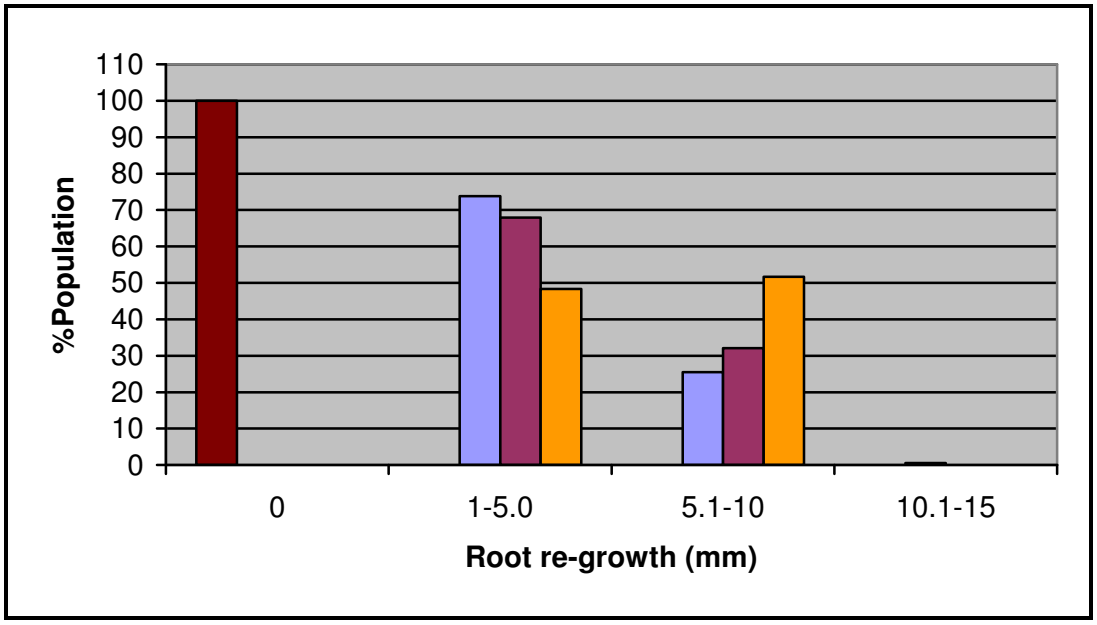

Figure 4.6 Frequency distribution of the root re-growth response of the F_2 population ■ in comparison with the two parental genotypes Tugela DN ■ and ASSN12 ■ after aluminium tolerance testing (■ Elands)

Table 4.11 Descriptive statistics of four variables measured for the parental genotypes Tugela DN and ASSN12 as well as the derived F_2 population

Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	0.70	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
ASSN12							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	72.18	12.00	107.00	95.00	31.02	927.93
RG (mm)	28	4.29	1.00	9.00	8.00	2.27	4.95
S (mm)	28	3.63	2.00	7.00	5.00	1.39	1.86
RTI	28	0.08	0.01	0.36	0.35	0.09	0.01
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	91	39.00	21.00	65.00	44.00	10.78	115.00
RG (mm)	91	5.62	1.00	9.00	8.00	1.59	2.49
S (mm)	91	3.21	1.00	5.00	4.00	0.70	0.48
RTI	91	0.15	0.03	0.28	0.25	0.05	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.12 Root re-growth classes (percentage in parenthesis) of the F_2 of ASSN7xTugela DN and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	35	35 (100)	0	0	0
ASSN7	5	0	4 (80)	1 (20)	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.55)
F_2	51	0	48 (94.12)	3 (5.88)	0
ASSN7			**X**		
Tugela DN				**X**	

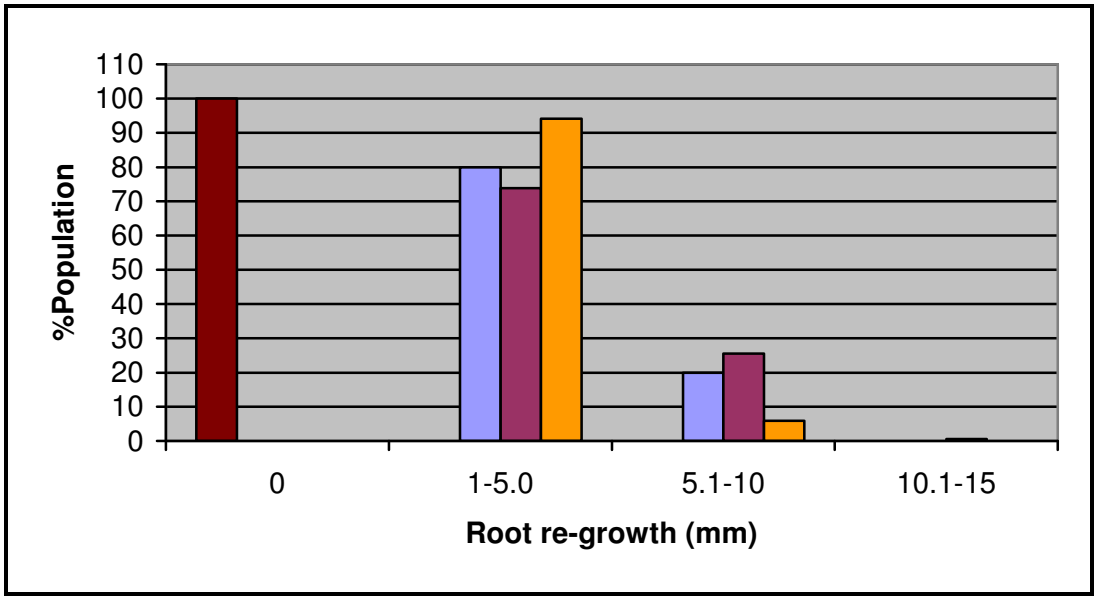

Figure 4.7 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes ASSN7 and Tugela DN after aluminium tolerance testing (Elands)

Table 4.13 Descriptive statistics of four variables measured for the parental genotypes ASSN7 and Tugela DN as well as the derived F_2 population

ASSN7							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	5	40.60	21.00	60.0	39.00	19.18	294.30
RG (mm)	5	3.90	1.50	9.00	7.50	3.36	9.05
S (mm)	5	3.00	2.00	4.00	2.00	0.79	0.50
RTI	5	0.12	0.04	0.38	0.33	0.16	0.02
Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	0.10	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	51	50.78	19.00	114.00	95.00	21.52	454.21
RG (mm)	51	3.08	1.00	5.00	4.00	1.53	2.29
S (mm)	51	2.81	2.00	4.00	2.00	0.46	0.21
RTI	51	0.07	0.02	0.14	0.13	0.04	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.14 Root re-growth classes (percentage in parenthesis) of the F_2 of ASSN12xASSN16 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	48	48 (100)	0	0	0
ASSN12	28	0	19 (67.86)	9 (32.14)	0
ASSN16	34	0	18 (52.94)	16 (47.06)	0
F_2	124	0	117 (94.35)	7 (5.65)	0
ASSN12			**X**		
ASSN16			**X**		

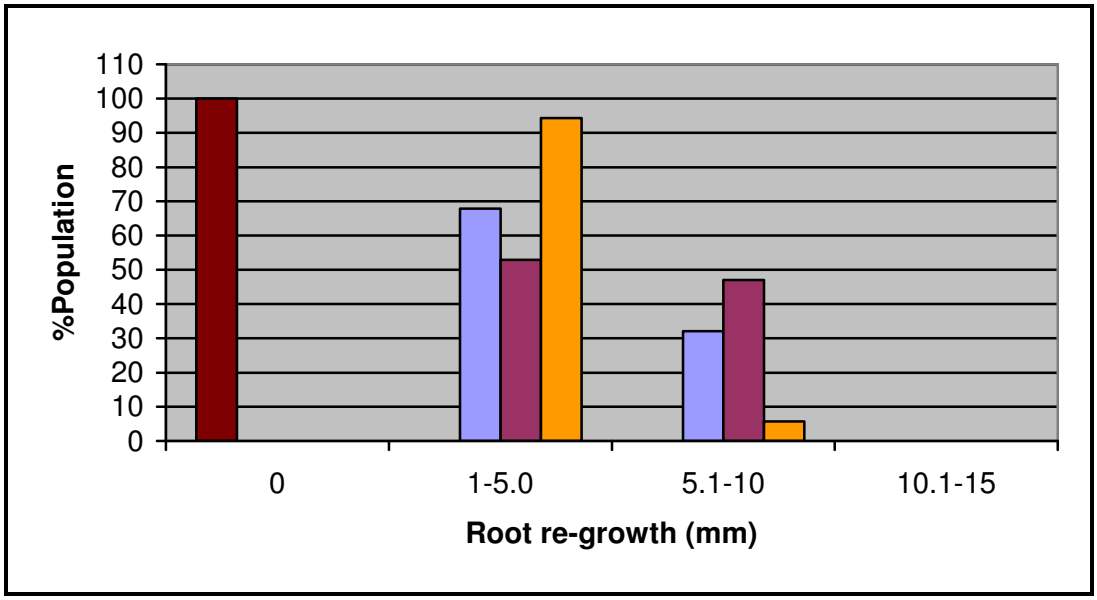

Figure 4.8 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes ASSN12 and ASSN16 after aluminium tolerance testing (Elands)

Table 4.15 Descriptive statistics of four variables measured for the parental genotypes ASSN12 and ASSN16 as well as the derived F_2 population

ASSN12							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	72.18	12.00	107.00	95.00	31.02	927.93
RG (mm)	28	4.29	1.00	9.00	8.00	2.27	4.95
S (mm)	28	3.63	2.00	7.00	5.00	1.39	1.86
RTI	28	0.08	0.01	0.36	0.35	0.09	0.01
ASSN16							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	72.94	9.00	105.00	96.00	28.90	810.48
RG (mm)	34	5.24	1.00	10.00	9.00	2.38	5.50
S (mm)	34	3.79	1.00	6.00	5.00	1.29	1.62
RTI	34	0.12	0.01	1.00	0.99	0.18	0.03
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	124	64.95	18.00	138.00	120.00	24.46	593.33
RG (mm)	124	3.24	1.00	10.00	9.00	1.85	3.40
S (mm)	124	2.73	1.00	5.00	4.00	0.78	0.61
RTI	124	0.06	0.01	0.31	0.30	0.05	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.16 Root re-growth classes (percentage in parenthesis) of the F_2 of Atlas 66xASSN16 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	35	35 (100)	0	0	0
Atlas 66	117	0	102 (87.18)	15 (12.82)	0
ASSN16	34	0	18 (52.94)	16 (47.06)	0
F_2	52	0	45 (86.54)	7 (13.46)	0
Atlas 66			**X**		
ASSN16			**X**		

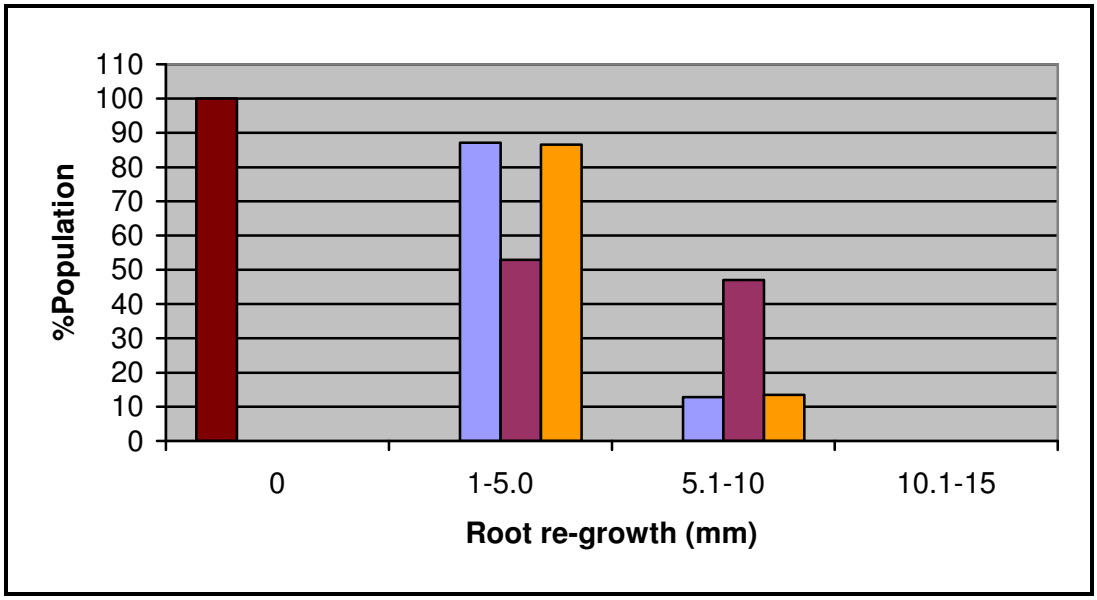

Figure 4.9 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Atlas 66 and ASSN16 after aluminium tolerance testing (Elands)

Table 4.17 Descriptive statistics of four variables measured for the parental genotypes Atlas 66 and ASSN16 as well as the derived F_2 population

Atlas 66							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	55.07	12.00	105.00	93.00	18.08	324.15
RG (mm)	117	3.48	1.00	9.00	8.00	1.78	3.12
S (mm)	117	2.68	1.00	6.00	5.00	1.28	1.61
RTI	117	0.07	0.01	0.43	0.42	0.06	0.00
ASSN16							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	34	72.94	9.00	105.00	96.00	28.90	810.48
RG (mm)	34	5.24	1.00	10.00	9.00	2.38	5.50
S (mm)	34	3.79	1.00	6.00	5.00	1.29	1.62
RTI	34	0.12	0.01	1.00	0.99	0.18	0.03
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	52	49.02	23.00	113.00	90.00	23.19	527.47
RG (mm)	52	2.88	1.00	9.00	8.00	2.04	4.07
S (mm)	52	2.95	2.00	5.00	3.00	0.72	0.51
RTI	52	0.08	0.02	0.28	0.27	0.07	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 4.18 Root re-growth classes (percentage in parenthesis) of the F_2 of ElandsxASSN16 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	45	45 (100)	0	0	0
Elands	275	275 (100)	0	0	0
ASSN16	34	0	18 (52.94)	16 (47.06)	0
F_2	111	54 (48.65)	54 (48.65)	3 (2.70)	0
Elands		**X**			
ASSN16			**X**		

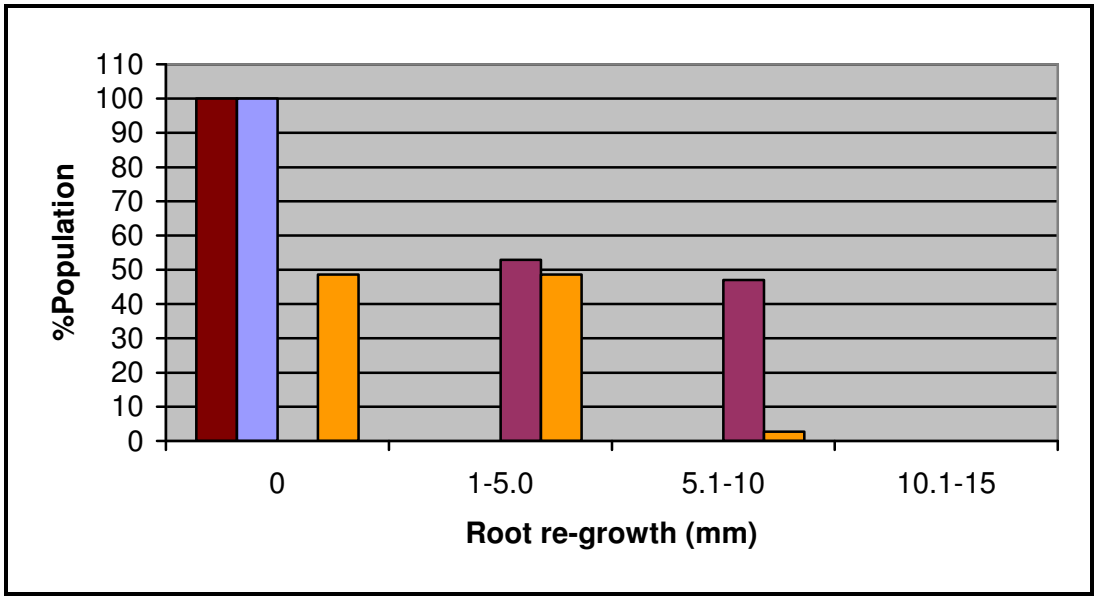

Figure 4.10 Frequency distribution of the root re-growth response of F_2 population in comparison with the two parental genotypes Elands and ASSN16 after aluminium tolerance testing (Elands)

Table 4.19 Descriptive statistics of four variables measured for the parental genotype ASSN16 as well as the derived F_2 population

ASSN16							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range(mm)	Std.dev.	Variance
RL (mm)	34	72.94	9.00	105.00	96.00	28.90	810.48
RG (mm)	34	5.24	1.00	10.00	9.00	2.38	5.50
S (mm)	34	3.79	1.00	6.00	5.00	1.29	1.62
RTI	34	0.12	0.01	1.00	0.99	0.18	0.03
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range(mm)	Std.dev.	Variance
RL (mm)	111	58.05	19.00	119.00	100.00	25.79	653.34
RG (mm)	111	1.98	1.00	7.00	6.00	1.45	2.07
S (mm)	111	2.71	1.00	4.00	3.00	0.61	0.37
RTI	111	0.04	0.01	0.28	0.27	0.05	0.00

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

F_2 results with the root tolerance index method indicated normal distribution curves and a shift of the parental population to the F_2 population. Figures 4.11 - 4.19 indicated normal distribution.

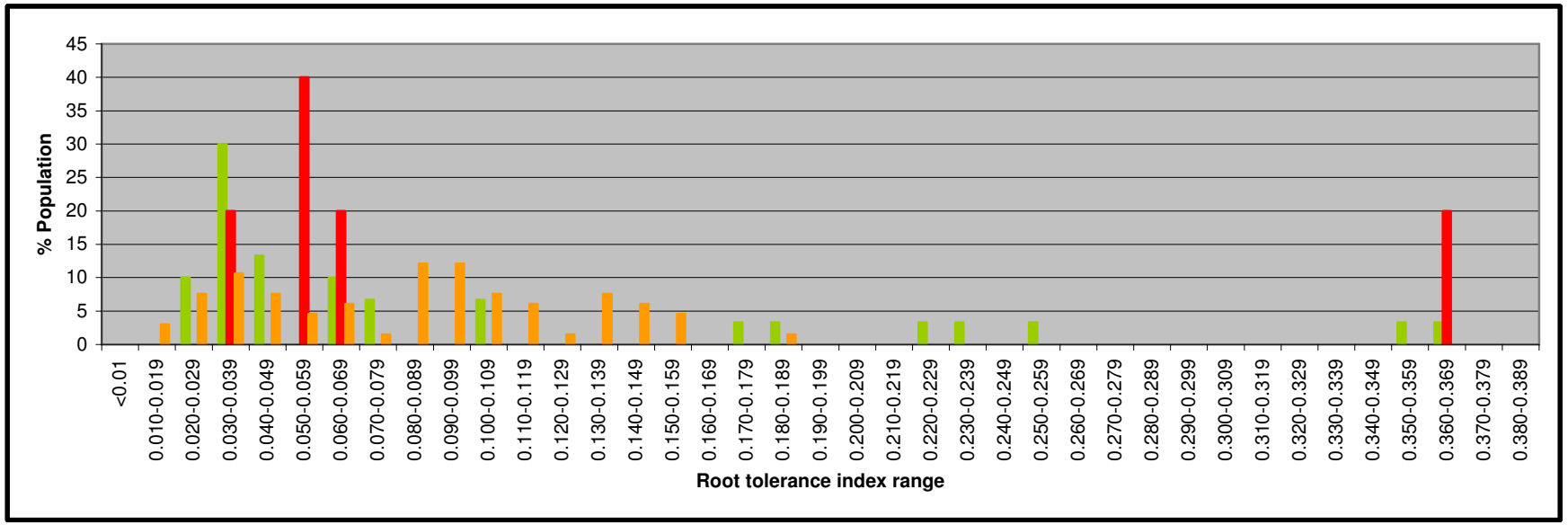

Figure 4.11 Frequency of aluminium tolerance index distribution of the F_2 population in comparison with the two parental genotypes ASSN2a and ASSN7 after aluminium tolerance testing (F_2, n= 66)

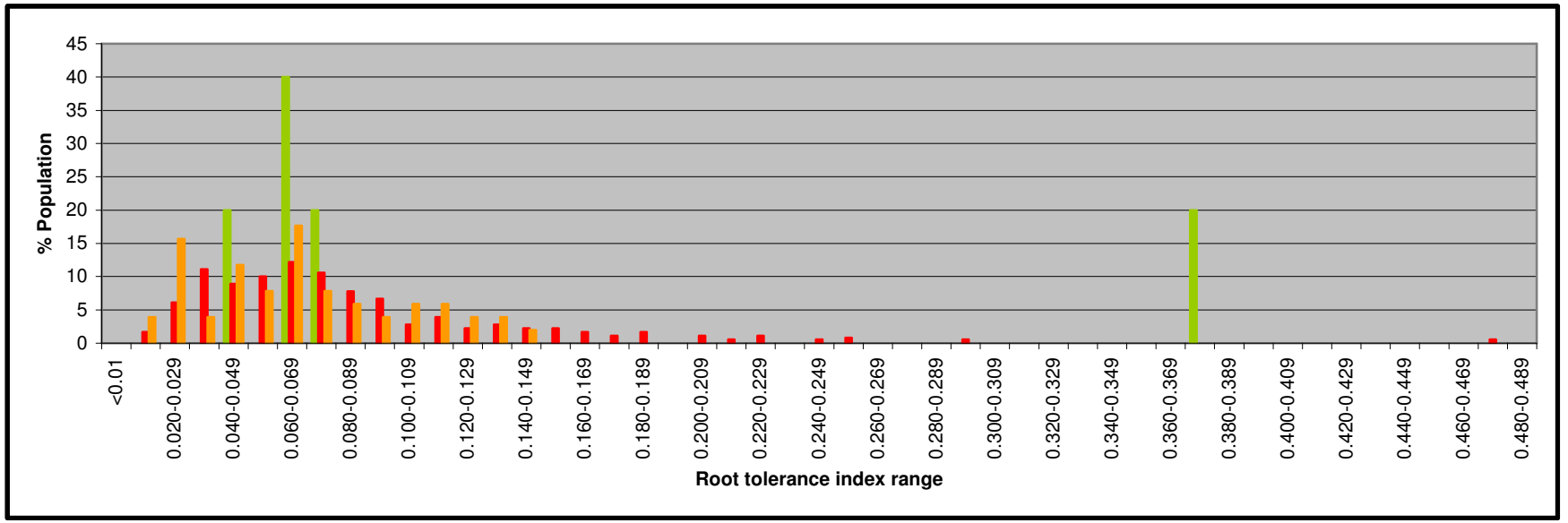

Figure 4.12 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes ASSN7 ■ and Tugela DN ■ after aluminium tolerance testing (F_2, n= 51)

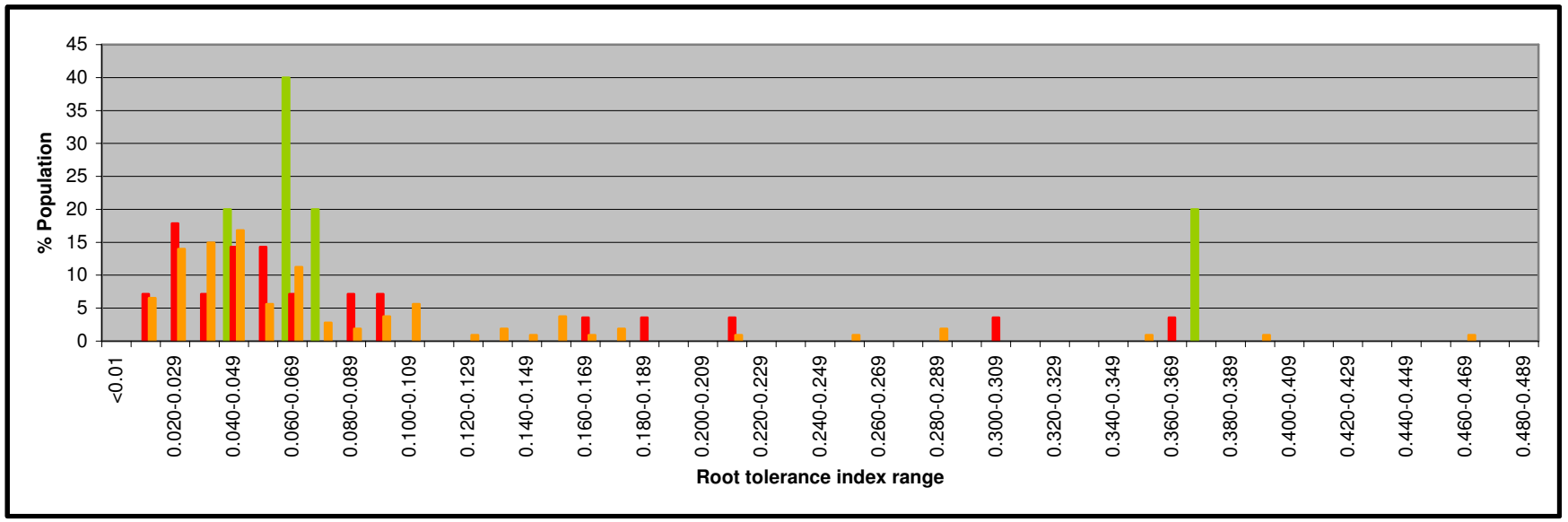

Figure 4.13 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes ASSN7 ■ and ASSN12 ■ after aluminium tolerance testing (F_2, n= 107)

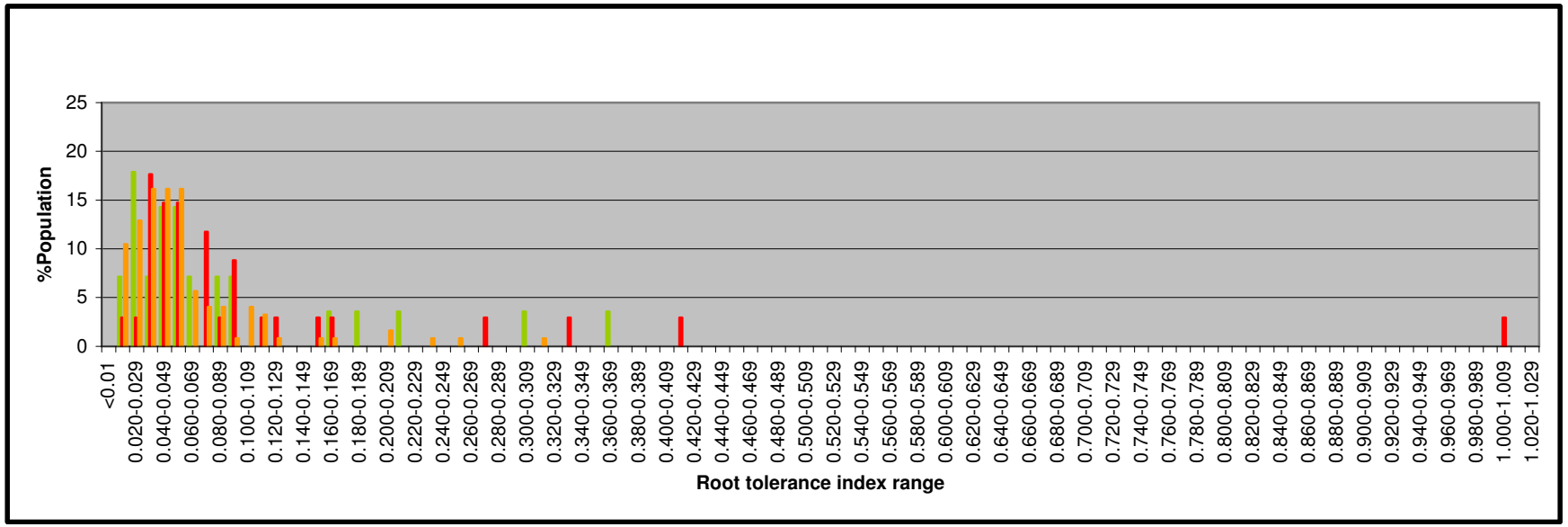

Figure 4.14 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes ASSN12 ■ and ASSN16 ■ after aluminium tolerance testing (F_2, n= 124)

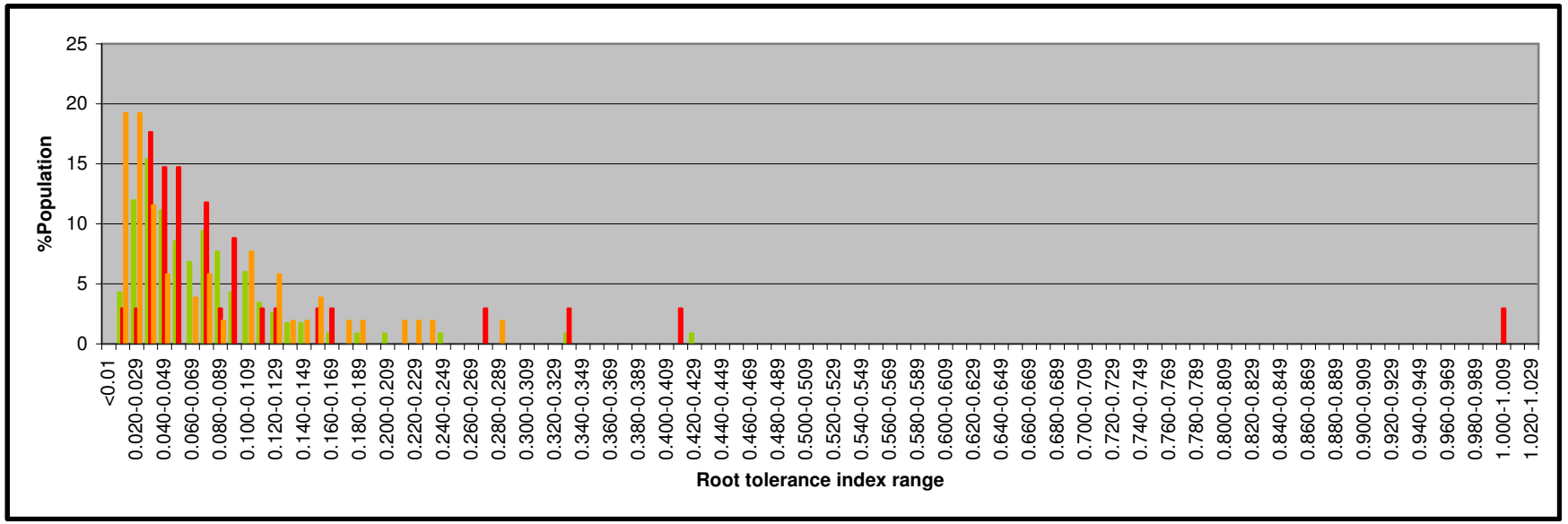

Figure 4.15 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Atlas 66 ■ and ASSN16 ■ after aluminium tolerance testing (F_2, n= 52)

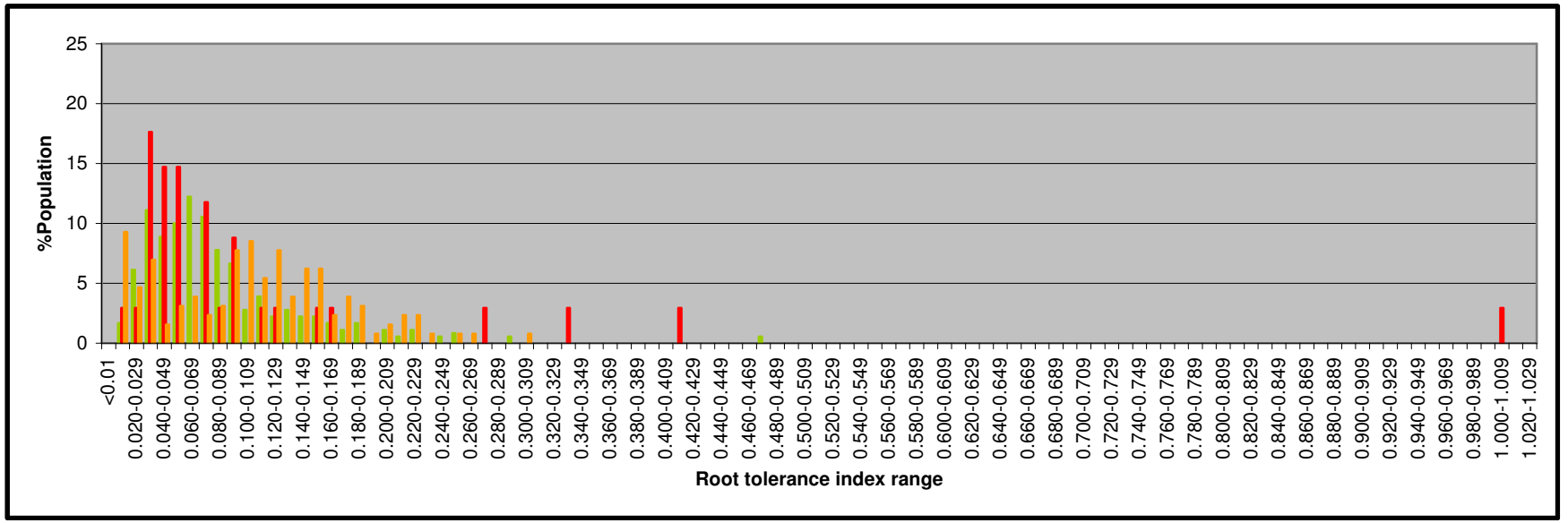

Figure 4.16 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Tugela DN ■ and ASSN16 ■ after aluminium tolerance testing, F_2 (n= 129)

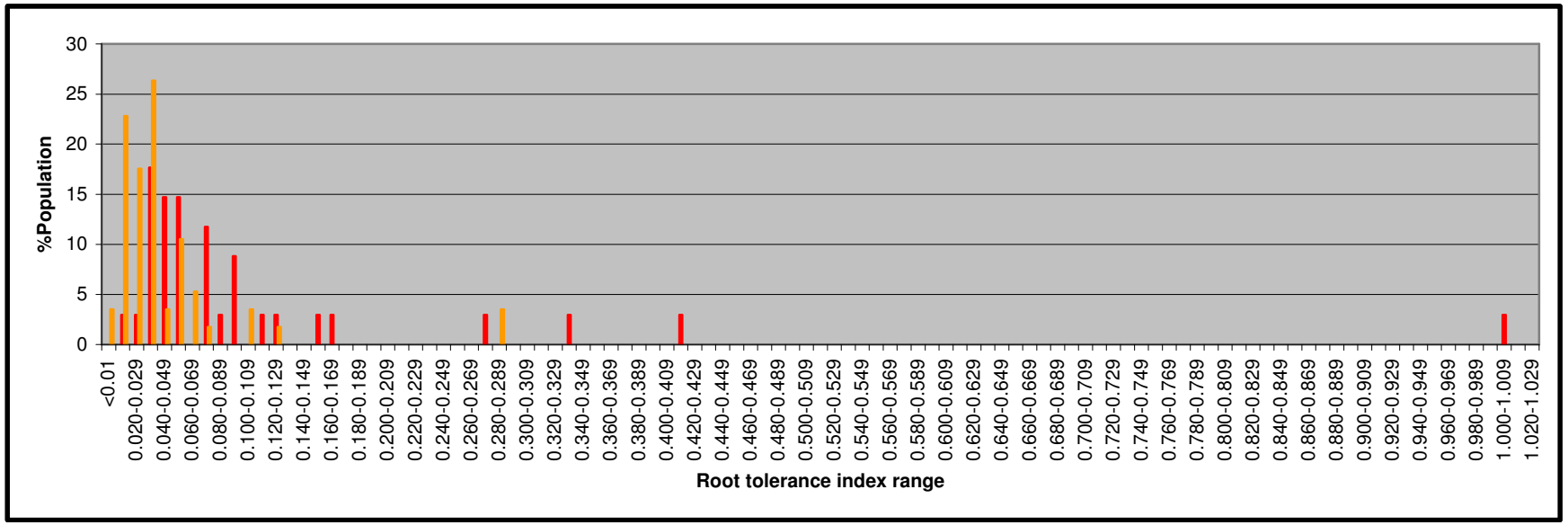

Figure 4.17 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Elands ■ and ASSN16 ■ after aluminium tolerance testing (F_2, n= 57)

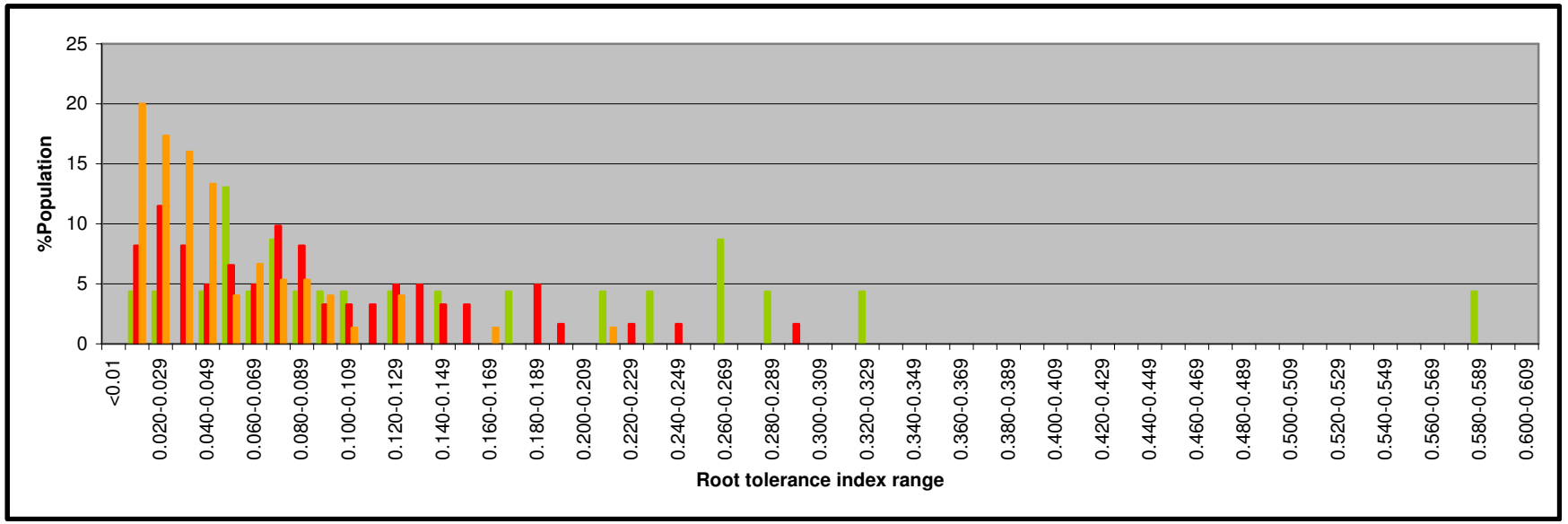

Figure 4.18 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes ASSN1 ■ and ASSN5 ■ after aluminium tolerance testing (F_2, n= 75)

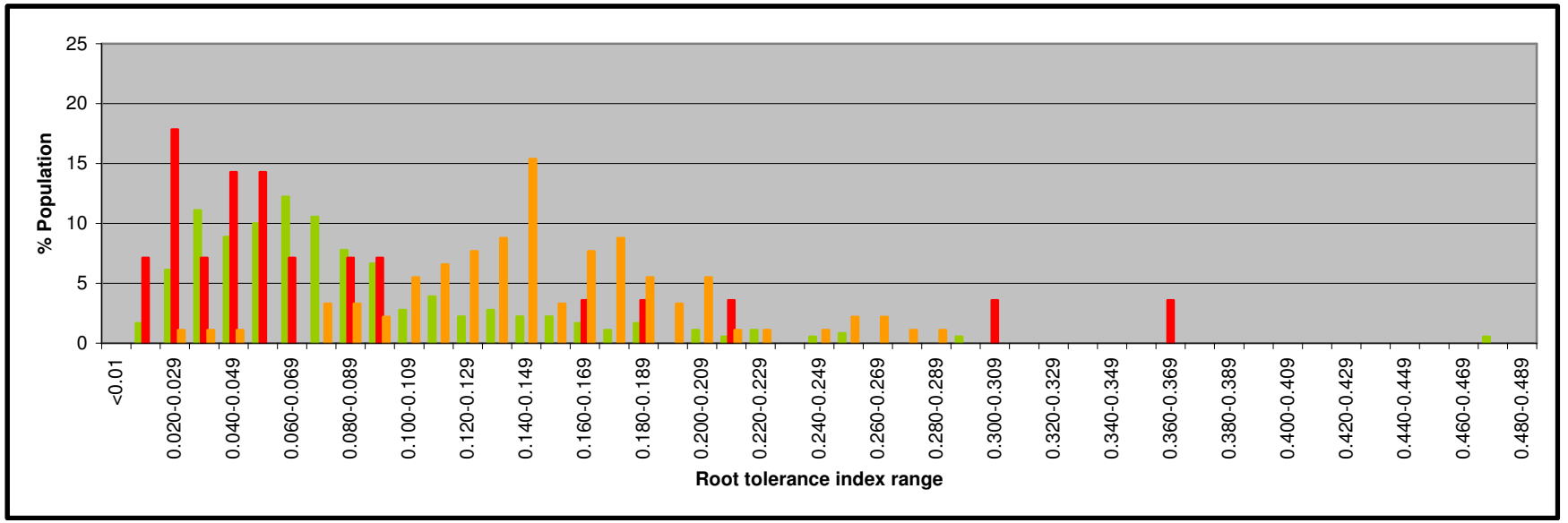

Figure 4.19 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Tugela DN ■ and ASSN12 ■ after aluminium tolerance testing (F_2, n= 91)

4.4 Discussion and conclusions

From the data obtained with the root re-growth method, it is evident that there are different genes involved in the donor sources that were used. The root re-growth results of the F_2 population clearly distinguish two groups regarding the tolerance responses obtained. Increased aluminium tolerance responses of the F_2 progeny in comparison to the parents used were shown in specific crossing combination. This reaction could be explained by the fact that the donor sources used as parents for aluminium tolerance, have different genetic backgrounds for tolerance that were combined during the crossing process, or there was dominance for expression of tolerance genes. The genetic contribution of the parents used in this study may be due to the presence of single or more genes present for aluminium tolerance.

This data is in accordance with literature, which indicated that genes influencing tolerance to aluminium toxicity in wheat, could be monogenic or polygenic (Aniol & Gustafson, 1984; Riede & Anderson, 1996; Aniol, 1990; Luo & Dvořák, 1996; Ma *et al*., 2000, 2005; Matos *et al*., 2005; Fontecha *et al.,* 2007; Guo *et al.,* 2007; Zhou *et al.,* 2007; Cai *et al.,* 2008; Navakode *et al.,* 2009).

The effect of increased aluminium tolerance by gene combination in the F_2 progeny can be seen in Figures 4.2 - 4.5. The F_2 progeny involved were the combination between the acid soil screening nursery entries ASSN7xASSN12, ASSN2a (an initial selection for the greatest tolerance individuals in the entries)xASSN7, Tugela DNxASSN16 and ASSN1xASSN5. In Figures 4.2 and 4.3, tolerance to aluminium toxicity in the F_2 progeny was greater than in the parental material. The Tugela DNxASSN16 cross (Figure 4.4) indicated that the effect of one donor parent might have been more pronounced than the other.

There was no increase in tolerance in the F_2 progeny, resulting in the same aluminium tolerance in the progeny as the donor parents (Figures 4.6 - 4.10), which indicated additive gene effects. Alternatively the genes of the donor parents might be so closely linked that during crossover, the effect of the cross over is not effective. These results confirm those of Minella and Sorrells (2002), who reported that failure to detect tolerance in progeny where parents are tolerant, indicates that tolerance in these genotypes is

either controlled by the same locus or by tightly linked loci and that this is also an indication of either the absence of new gene combinations or that those different genes had no additive effects.

When intermediate tolerant parents were crossed (Figures 4.6), there were no susceptible F_2 progenies and the failure of parents with the longest root re-growth to produce F_2 progeny similar to the tolerant parents, indicated that inheritance was more complex than a single gene with incomplete dominance. In Figures 4.6 and 4.7, the F_2 plants produced shorter roots than either parent. Lafever and Campbell (1978) reported that this dispersion could have been caused by segregation of aluminium tolerance genes or segregation for factors affecting root length, but not related to aluminium response.

With the root tolerance index method, it was possible to determine normal distribution curves, to indicate the relationship of parents with F_2 progeny in terms of aluminium tolerance. Simple pedigree or mass selection could be used to select from the progeny of the best combiners for larger root re-growth to improve aluminium stress tolerance.

Figures 4.11-4.19 indicated normal frequency distribution curves. The F_2 population values in Figure 4.11 was smaller than the two parental populations in the root tolerance index range, with the smallest F_2 population value of 1.52% at the root tolerance index range of 0.070-0.079, 0.120-0.129 and 0.180-0.189 respectively. The greatest F_2 population value was 12.12% at the root tolerance index range of 0.080-0.089 and 0.090-0.099. ASSN7 had the greatest population value of 40% at the root tolerance index range of 0.060-0.069, while ASSN2a had the greatest population value of 30% at the root tolerance index range of 0.030-0.039.

Figure 4.12 showed the highest F_2 population value of 17.65% at the root tolerance index range of 0.060-0.069, which was between the parental populations. The smallest population percentage for the F_2 was 1.96% at the root tolerance index range of 0.140-0.149. ASSN7 had the highest population value of 40% at the root tolerance index range of 0.060-0.069 and for Tugela DN it was 12.22% at the root tolerance index range of 0.060-0.069. The F_2 population fell between the parental populations for root tolerance index range.

The F_2 population (Figure 4.13) had a high value of 16.82% at the root tolerance index range of 0.040-0.049, which was below the values of ASSN7 (40%) and ASSN12 (17.86%). The greatest F_2 population value in Figure 4.14 was 16.13%, smaller than the greatest population of both parents. ASSN16 had the greatest population value of 17.65% at the root tolerance index range of 0.030-0.039, while for ASSN12 the greatest population was 17.86% at the root tolerance index range of 0.020-0.029. There was an increase in the values of progeny in relation to parents.

In Figure 4.15, the F_2 population had the greatest population value of 19.23% at the root tolerance index range of 0.010-0.019 and 0.020-0.029, which was above the two parental population percentages. The smallest value for the F_2 population was 1.92%. Atlas 66 had the greatest population value of 15.38%, while for ASSN16 it was 17.65% at the root tolerance index ranges of 0.030-0.039 respectively, with an increased progeny value in relation to parents.

Figure 4.16 indicated a decrease in the progeny value in relation to the parents, with the F_2 having the greatest population value of 8.53% at the root tolerance index range of 0.100-0.109, which was below the greatest population value of both parents. The smallest population percentage of the F_2 was 0.78% greater than the smallest population of Tugela DN, which was 0.56%.

In Figure 4.18, the greatest population value for the F_2 was 26.32% at the root tolerance index range of 0.030-0.039 falling above the parental population value. Combination ASSN1xASSN5 had the greatest F_2 population of 20% at the root tolerance index range of 0.010-0.019, which was greater than both parental populations and with an increased progeny value in relation to parents. The greatest population for ASSN1 was 14.29% at the root tolerance index range of 0.050-0.059 and 14.71% for ASSN5 at the root tolerance index range of 0.070-0.079.

The greatest F_2 population in Figure 4.19 was 15.38%, at the root tolerance index range of 0.140-0.149, which was in between the parental population's greatest percentages. ASSN12 had the greatest population value of 17.86% at the root tolerance index range of 0.020-0.029, while Tugela DN had the greatest population value of 12.22% at the root

tolerance index range of 0.060-0.069. The smallest F_2 population percent was 1.1% greater than the smallest population percentage of Tugela DN, which was 0.56%.

The greatest genetic gain of the F_2 population for tolerance was shown in the combinations Atlas 66xASSN16, ElandsxASSN16 and ASSN1xASSN5 (Figures 4.15, 4.17 and 4.18). The F_2 population percentage was above the population percentage of both parents.

The greatest population percentage of the F_2 found in combinations ASSN7xTugela DN, Atlas 66xASSN16 and Tugela DNxASSN12 (Figures 4.12, 4.15 and 4.19) fell in between the population percentage of the parents for aluminium tolerance, showing no genetic gain.

The lowest genetic gain of a F_2 population was shown in Figures 4.11, 4.13, 4.14 and 4.16, combinations ASSN2xASSN7, ASSN7xASSN12, ASSN12xASSN16 and Tugela DNxASSN16, with the highest aluminium tolerance level of the F_2 falling below that of both parents.

From these results, it can be concluded that ASSN1, ASSN5 and ASSN16 can be used as parents to improve aluminium tolerance. Genetic variation in response to aluminium toxicity has been found not only among plant species, but also among cultivars which differ significantly in their susceptibility to aluminium toxicity in acid soils and these differences are genetically controlled (Aniol, 2004). Plants differ in their reaction to aluminium toxicity and these differences are largely genetically controlled. While most cultivars are sensitive to aluminium, tolerant genotypes can be found in most species. Despite the abundance of information on genetic variability of plant response to aluminium toxicity amongst wild and cultivated species, the information on genetic systems controlling these responses is limited and fragmentary (Aniol & Gustafson, 1984; Aniol, 1990; Tang *et al.,* 2001; 2003; Aniol, 2004; Gustafson, 2005; Ma, 2005).

The root re-growth of the F_2 populations generally reflected the same pattern of root re-growth length of the parents, which indicated additive genetic effects. There were two exceptions; the combination of ASSN7xASSN12 and ASSN2axASSN7, which had a root re-growth greater than the best parent. This result suggests dominance effects of genes

which control aluminium tolerance or the complementary role of aluminium tolerance genes present in the two parents.

The range of the F_2 progeny root length (Table 4.3) was 44 mm, greater than that of ASSN7 and smaller than that of ASSN12. The root re-growth range for the F_2 was 8 mm, equal to the root re-growth range of ASSN12 and greater than that of ASSN7. The root tolerance index range of the F_2 was the lowest with a value of 0.25, 033 for ASSN7 and 0.35 for ASSN12.

The range of the root length and root re-growth for ASSN2a and ASSN7 (Table 4.5) was smaller than that of the F_2. The F_2 root length was 97 mm, with an average of 57.97 mm, while ASSN2a had a range of 77 mm, with an average of 62.43 mm and the range of ASSN7 was 39 mm, with an average of 57.91 mm. The root tolerance index range was greater for ASSN2, with a value of 0.35 followed by 0.33 for ASSN7 and 0.17 for the F_2.

The F_2 root length range was 73 mm, smaller than the 124 mm of Tugela DN and 96 mm for ASSN16. Though the number of seedlings evaluated for the F_2 was higher than that of ASSN16, the average root length was 44.36 mm for the F_2, smaller than 72.94 mm of ASSN16. The ranges of the root re-growth were very close, 10 mm for the F_2, 11 mm for Tugela DN and 9 mm for ASSN16 (Table 4.7).

The F_2 population (Table 4.9) root length range was 106 mm, greater than that of ASSN1 and ASSN5. The range of the other three parameters was small for the F_2 and high for ASSN1 and ASSN5. The root length range of the F_2 progeny (Table 4.11) was small, compared to the root length range of Tugela DN and ASSN12. The range of root re-growth for the F_2 and ASSN12 was 8 mm and for Tugela DN the range was 11 mm. Beside the range of the root re-growth in the F_2, which was equal to the range of ASSN12 root re-growth, the rest of the parameters were greater for Tugela DN and ASSN12, than for the F_2.

ASSN7 had the smallest root length range of 39 mm, with an average of 40.60 mm. The range of the root length for the F_2 was 95 mm, with an average of 50.78 mm (Table 4.13). The range of the root re-growth and root tolerance index for the F_2 was smaller

than that of ASSN7 and Tugela DN. The root staining of the F_2 was 2 mm, equal to that of ASSN7 and smaller to that of Tugela DN.

The average root length for the F_2 was 64.95 mm, smaller than 72.18 mm of ASSN7 and 72.94 mm for ASSN16, but the range of the F_2 was 120 mm, greater than that of ASSN12 and ASSN16. The root re-growth range for the F_2 was 9 mm, equal to the root re-growth range of ASSN16 (Table 4.15). The root staining and root tolerance index for the F_2 were smaller than those of ASSN12 and ASSN16.

The root length range of the F_2 was 90 mm, smaller when compared to the 93 mm of Atlas 66 and the 96 mm of ASSN16 (Table 4.17). The root re-growth range of the F_2 was 8 mm, equal to the root re-growth range of Atlas 66. The root tolerance index range was 0.42 for Atlas 66 and 0.99 for ASSN16, while for the F_2 the root tolerance index range was 0.27.

The F_2 root length range was 100 mm, greater than the 96 mm of ASSN16 (Table 4.19). Though the range of the root length was high in the F_2, the average root length of the F_2 was 58.05 mm, smaller than the 72.94 mm of ASSN16. The ranges of the other three parameters for the F_2 were smaller, when compared to ASSN16. These results confirm the results represented in the root re-growth and root tolerance index graphs.

From this study it can be concluded, that within the genotypes involved, there is considerable variation in response to aluminium toxicity, suggesting that aluminium tolerance in wheat may not be simply inherited and is sometimes a complex character, controlled by several major genes (Aniol, 1990; Zhang & Jessop, 1998). Also, complex traits often reflect the cumulative effects of many minor alleles and not several major alleles as indicated. A lack of segregation in hybrids from some parents suggests that they have the same genetic background for aluminium tolerance (Minella & Sorrells, 2002). The root re-growth values indicated that selecting for aluminium tolerance would be effective in early segregating populations. Actual selection response of aluminium tolerance in both degree and range warrants a thorough examination of a range of wheat varieties with diversified genetic backgrounds.

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CHAPTER 5

Reciprocal effects in wheat for aluminium tolerance

5.1 Introduction

Plants differ in their reaction to aluminium toxicity and variability is found between cultivars and within species (Kerridge & Kronstad, 1968; Aniol, 1984; Bona *et al.,* 1993; Kochian, 1995; Aniol, 2004; Ma *et al.,* 2005). Due to their immense influence on plant development, investigation of plant root systems is important (Sharma & Lafever, 1992; Tahira & Salam, 2006) as aluminium toxicity is a limiting factor restricting rooting and branching in plants (Zhang *et al.,* 2007). In wheat, genes governing traits can be transmitted from parents to their progeny via the nucleus or the cytoplasm. In most crops, maternal effects are important, because it can bias the means and variances of families and mislead the breeders in their attempts to understand the genetics of a given quantitative trait (Yildirim *et al.,* 2008).

In wheat, the inheritance of aluminium tolerance is usually determined from F_2 populations instead of the more sophisticated mating designs needed to detect gene interactions (Carver & Ownby, 1995). For this reason, a simple mating design, such as the diallel mating design is useful since it allows suitable genetic analysis to be carried out after one generation and serves as a basis for predicting and selecting promising genotypes in a breeding program (Zhang *et al.,* 2007). The feasibility of improving aluminium tolerance through enhanced root re-growth using parents to combine desirable aluminium tolerance genes focusing on parental lines expressing long root re-growth roots was indicated in Chapter 4.

Not much work has been reported on reciprocal effects for aluminium tolerance. The use of reciprocals to further evaluate the inheritance of aluminium tolerance in wheat would provide more information on the nature of aluminium tolerance and its inheritance. The main objective of this study was to determine the reciprocal effects of aluminium tolerance in wheat using three F_2 cross combinations and their reciprocals.

5.2 Materials and methods

5.2.1 Materials

Three F_2 cross combinations and their reciprocals were used in this study (Table 5.1)

Table 5.1 List of total number of seeds incubated for germination and evaluated for aluminium tolerance

Combination	Total no of seeds incubated for germination	Total no of seedlings evaluated
Tugela DNxElands	289	123
ElandsxTugela DN	729	177
Atlas 66xASSN12	247	59
ASSN12xAtlas 66	477	92
Atlas 66xTugela DN	3221	574
Tugela DNxAtlas 66	5115	888

5.2.2 Methods

The F_2 seeds were germinated and evaluated for aluminium tolerance, using the nutrient solution cultures as described in Section 3.2.2 in Chapter 3.

Individual plants were measured for the longest root length of the primary root, root re-growth, root staining and root tolerance index after chapter 3.

5.3 Results

In this study, aluminium tolerance was measured as the primary root re-growth after exposure to aluminium toxicity. The results indicated two groups of tolerance to aluminium toxicity. Group one, are those with three levels of tolerance and group two are those with two levels of tolerance to aluminium toxicity.

Table 5.2 Root re-growth classes (percentage in parenthesis) of the Atlas 66xTugela DN F_2 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	89	89 (100)	0	0	0
Atlas 66	117	0	102 (87.18)	15 (12.82)	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.56)
F_2	574	0	367 (63.94)	202 (35.19)	1 (0.17)
Atlas 66			**X**		
Tugela DN			**X**		

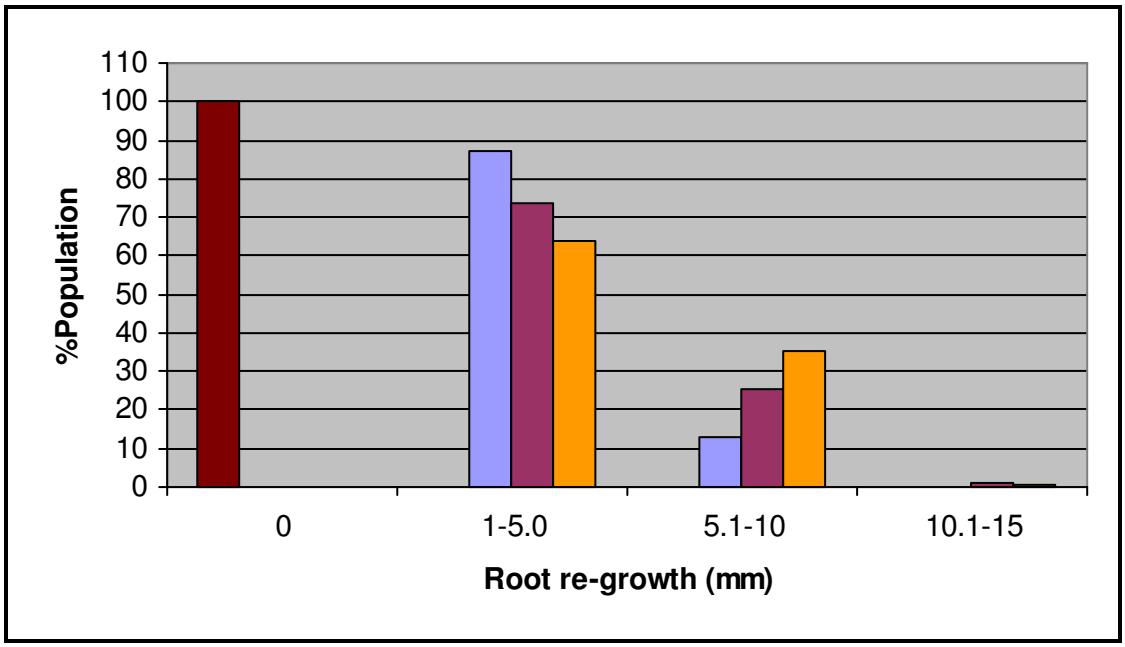

Figure 5.1 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Atlas 66 (♀) and Tugela DN (♂) after aluminium tolerance testing (Elands)

Table 5.3 Descriptive statistics of four variables measured for the parental genotypes Atlas 66 and Tugela DN, as well as the derived F_2 population

Atlas 66							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	55.07	12.00	105.00	93.00	18.08	324.15
RG (mm)	117	3.48	1.00	9.00	8.00	1.78	3.12
S (mm)	117	2.68	1.00	6.00	5.00	1.28	1.61
RTI	117	0.07	0.01	0.43	0.42	0.06	0.00
Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	1.00	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	574	41.00	13.00	103.00	90.00	17.65	310.90
RG (mm)	574	4.73	1.00	16.00	15.00	2.65	7.00
S (mm)	574	3.50	1.00	7.00	6.00	1.12	1.26
RTI	574	0.14	0.01	0.89	0.88	0.09	0.01

RL is the root length before aluminium treatment

RG is the root re-growth after aluminium treatment

S is the portion of the root affected by aluminium treatment, stained with hematoxylin

RTI is the RG/RL x 100

Table 5.4 Root re-growth classes (percentage in parenthesis) of the Tugela DNxAtlas 66 F_2 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	104	104 (100)	0	0	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.56)
Atlas 66	117	0	102 (87.18)	15 (12.82)	0
F_2	888		610 (68.69)	274 (30.86)	4 (0.45)
Tugela DN				**X**	
Atlas 66			**X**		

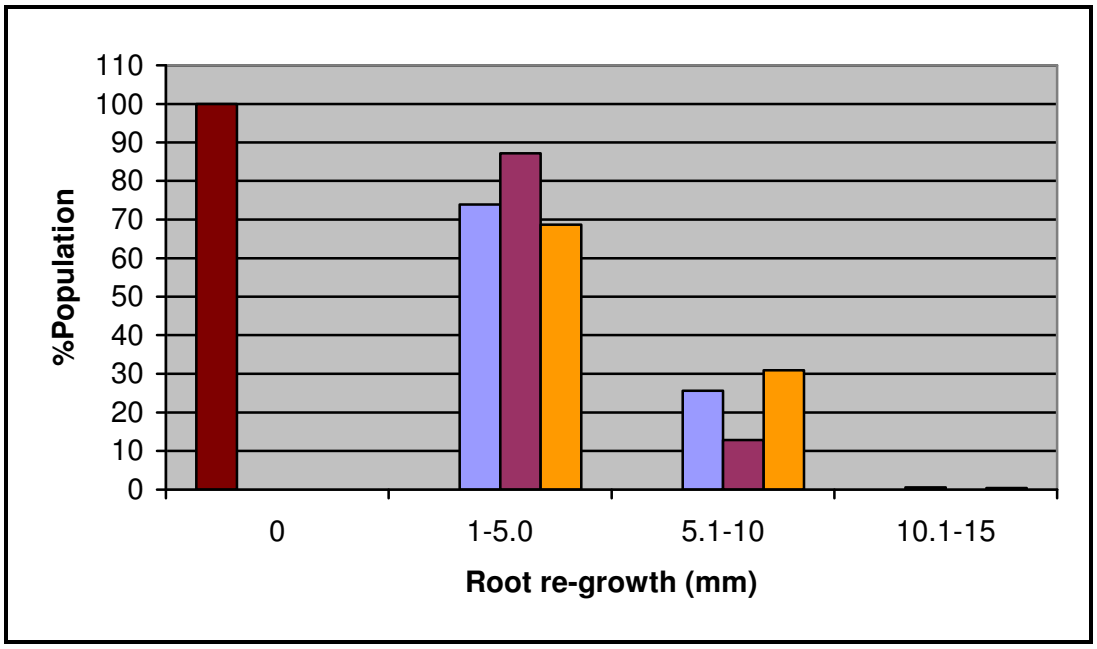

Figure 5.2 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Tugela DN (♀) and Atlas 66 (♂) after aluminium tolerance testing (Elands)

Table 5.5 Descriptive statistics of four variables measured for the parental genotypes Tugela DN and Atlas 66, as well as the derived F_2 population

Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	1.00	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
Atlas 66							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	55.07	12.00	105.00	93.00	18.08	324.15
RG (mm)	117	3.48	1.00	9.00	8.00	1.78	3.12
S (mm)	117	2.68	1.00	6.00	5.00	1.28	1.61
RTI	117	0.07	0.01	0.43	0.42	0.06	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	888	45.68	14.00	129.00	115.00	21.63	467.27
RG (mm)	888	4.30	1.00	13.00	12.00	2.62	6.85
S (mm)	888	3.15	1.00	6.00	0.98	0.98	0.96
RTI	888	0.11	0.01	0.46	0.45	0.08	0.01

Table 5.6 Root re-growth classes (percentage in parenthesis) of the ASSN12xAtlas 66 F_2 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	76	76 (100)	0	0	0
ASSN12	28	0	19 (67.86)	9 (32.14)	0
Atlas 66	117	0	102 (87.18)	15 (12.82)	0
F_2	92	0	64 (69.57)	20 (21.74)	8 (8.7)
ASSN12			**X**		
Atlas 66			**X**		

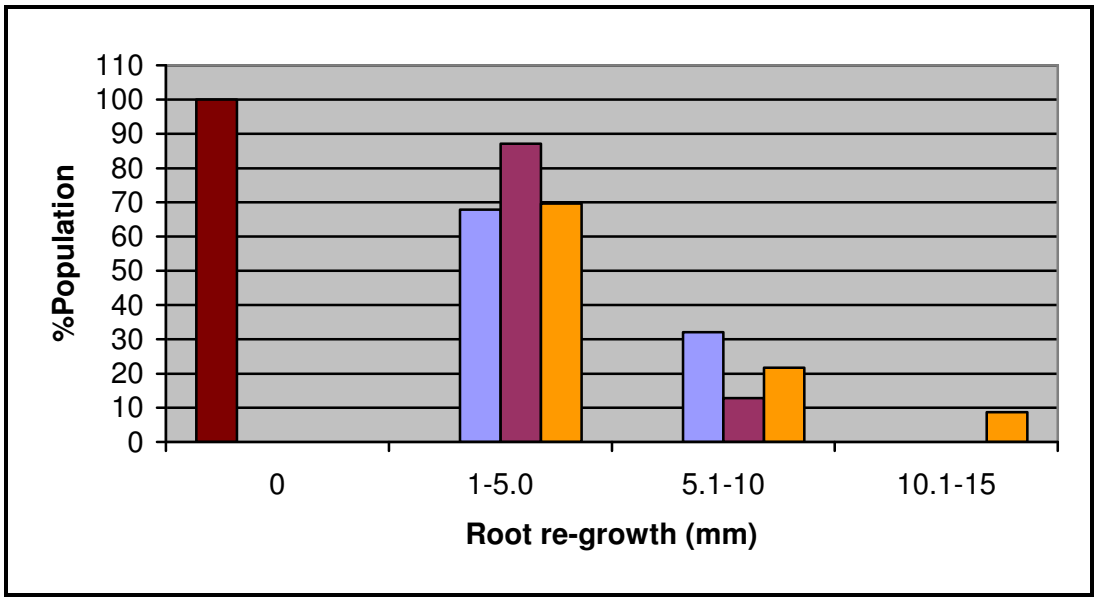

Figure 5.3 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes ASSN12 (♀) and Atlas 66 (♂) after aluminium tolerance testing (Elands)

Table 5.7 Descriptive statistics of four variables measured for the parental genotypes ASSN12 and Atlas 66, as well as the derived F_2 population

ASSN12							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	72.18	12.00	107.00	95.00	31.02	927.93
RG (mm)	28	4.29	1.00	9.00	8.00	2.27	4.95
S (mm)	28	3.63	2.00	7.00	5.00	1.39	1.86
RTI	28	0.08	0.01	0.36	0.35	0.09	0.01
Atlas 66							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	55.07	12.00	105.00	93.00	18.08	324.15
RG (mm)	117	3.48	1.00	9.00	8.00	1.78	3.12
S (mm)	117	2.68	1.00	6.00	5.00	1.28	1.61
RTI	117	0.07	0.01	0.43	0.42	0.06	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	92	71.59	22.00	131.00	109.00	24.55	595.89
RG (mm)	92	4.71	1.00	14.00	13.00	3.40	11.45
S (mm)	92	2.71	1.00	7.00	6.00	1.18	1.39
RTI	92	0.07	0.01	0.52	0.51	0.07	0.01

Table 5.8 Root re-growth classes (percentage in parenthesis) of the Atlas 66xASSN12 F_2 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	84	84 (100)	0	0	0
Atlas 66	117	0	102 (87.18)	15 (12.82)	0
ASSN12	28	0	19 (67.86)	9 (32.14)	0
F_2	59	0	42 (71.19)	17 (28.81)	0
Atlas 66			**X**		
ASSN12			**X**		

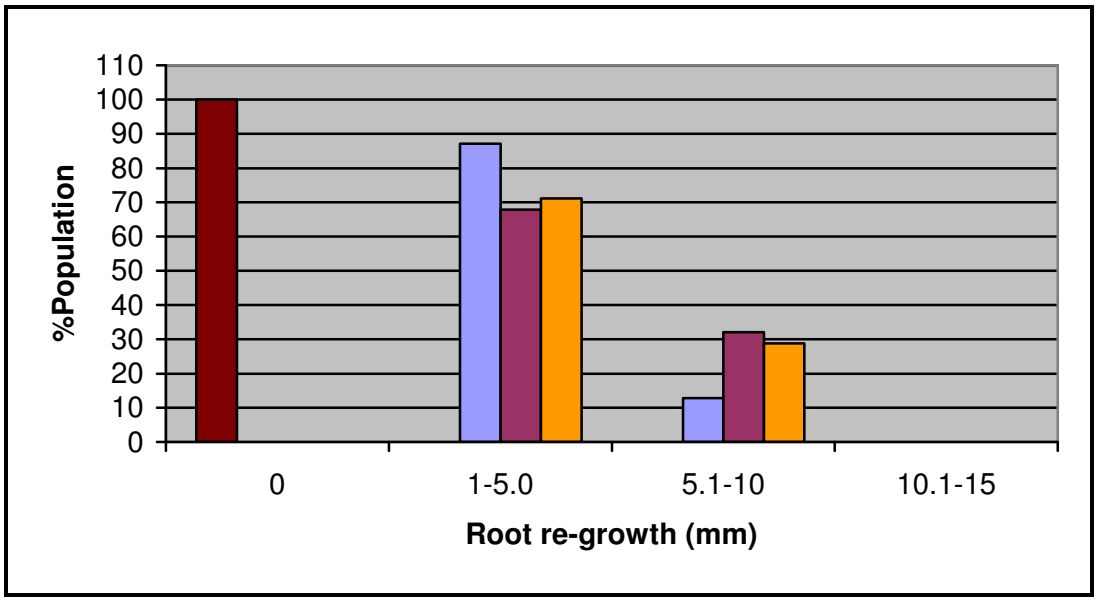

Figure 5.4 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Atlas 66 (♀) and ASSN12 (♂) after aluminium tolerance testing (Elands)

Table 5.9 Descriptive statistics of four variables measured for the parental genotypes Atlas 66 and ASSN12, as well as the derived F_2 population

Atlas 66							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	117	55.07	12.00	105.00	93.00	18.08	324.15
RG (mm)	117	3.48	1.00	9.00	8.00	1.78	3.12
S (mm)	117	2.68	1.00	6.00	5.00	1.28	1.61
RTI	117	0.07	0.01	0.43	0.42	0.06	0.00
ASSN12							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	28	72.18	12.00	107.00	95.00	31.02	927.93
RG (mm)	28	4.29	1.00	9.00	8.00	2.27	4.95
S (mm)	28	3.63	2.00	7.00	5.00	1.39	1.86
RTI	28	0.08	0.01	0.36	0.35	0.09	0.01
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	59	43.37	17.00	66.00	49.00	12.25	147.44
RG (mm)	59	3.17	1.00	8.00	7.00	2.28	5.10
S (mm)	59	2.63	1.00	4.00	3.00	0.62	0.38
RTI	59	0.08	0.02	0.32	0.30	0.07	0.00

Table 5.10 Root re-growth classes (percentage in parenthesis) of the Tugela DNxElands F_2 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	76	76 (100)	0	0	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.56)
Elands	275	275 (100)	0	0	0
F_2	123	40 (32.52)	71 (57.72)	12 (9.76)	0
Tugela DN			**X**		
Elands		**X**			

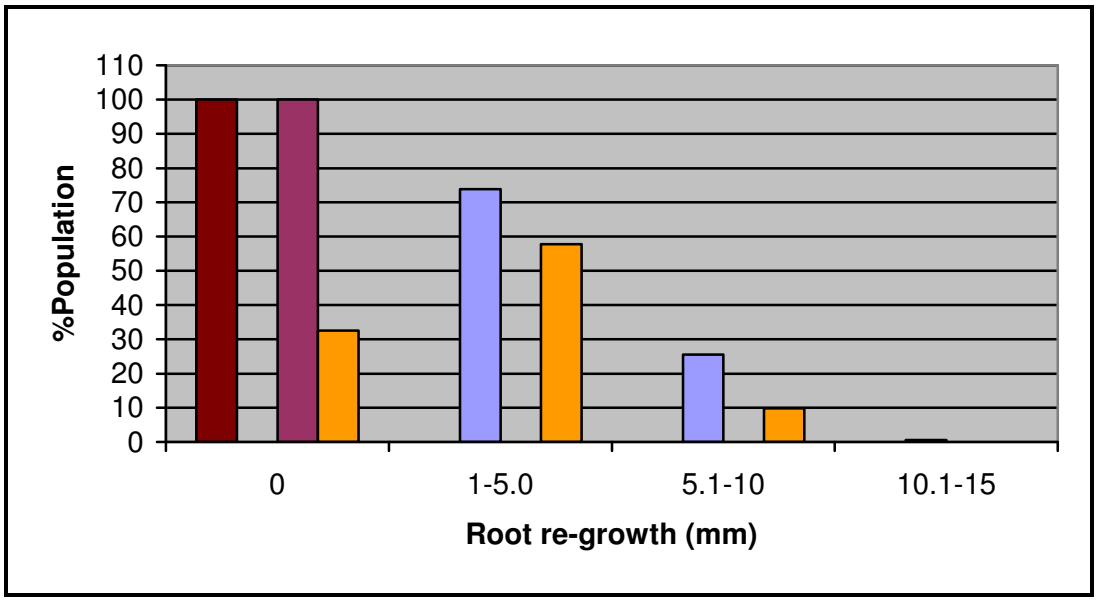

Figure 5.5 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Tugela DN (♀) and Elands (♂) after aluminium tolerance testing (Elands)

Table 5.11 Descriptive statistics of four variables measured for the parental genotype Tugela DN, as well as the derived F_2 population

Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	1.00	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	83	50.93	21.00	117.00	96.00	25.91	663.41
RG (mm)	83	3.08	1.00	9.00	8.00	2.12	4.43
S (mm)	83	3.06	1.00	6.00	5.00	1.02	1.03
RTI	83	0.07	0.01	0.29	0.28	0.06	0.00

Table 5.12 Root re-growth classes (percentage in parenthesis) of the ElandsxTugela DN F_2 and parental genotypes' primary roots

Genotype	n	Susceptible	Moderate	Intermediate	Tolerant
Elands	58	58 (100)	0	0	0
Elands	275	275 (100)	0	0	0
Tugela DN	180	0	133 (73.89)	46 (25.56)	1 (0.56)
F_2	177	53 (29.94)	117 (66.10)	7 (3.95)	0
Elands		**X**			
Tugela DN			**X**		

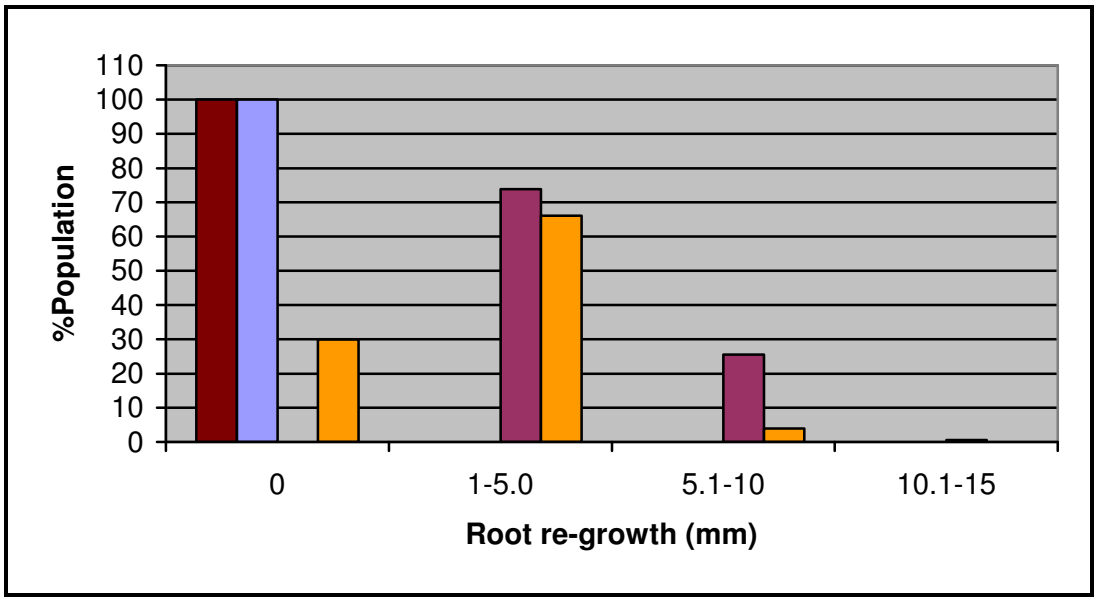

Figure 5.6 Frequency distribution of the root re-growth response of the F_2 population in comparison with the two parental genotypes Elands (♀) and Tugela DN (♂) after aluminium tolerance testing (Elands)

Table 5.13 Descriptive statistics of four variables measured for the parental genotype Tugela DN, as well as the derived F_2 population

Tugela DN							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	180	59.14	16.00	140.00	124.00	21.07	441.44
RG (mm)	180	4.30	1.00	12.00	11.00	2.04	4.16
S (mm)	180	3.22	1.00	6.00	5.00	1.00	0.99
RTI	180	0.08	0.01	0.47	0.46	0.06	0.00
F_2							
Variable	n	Ave (mm)	Min (mm)	Max (mm)	Range (mm)	Std.dev.	Variance
RL (mm)	124	51.00	15.00	119.00	104.00	26.34	687.92
RG (mm)	124	2.46	1.00	8.00	7.00	1.68	2.79
S (mm)	124	2.80	1.00	7.00	6.00	0.98	0.96
RTI	124	0.06	0.01	0.33	0.33	0.05	0.00

The root re-growth index method results indicate normal distribution frequencies of the F_2 and parental populations.

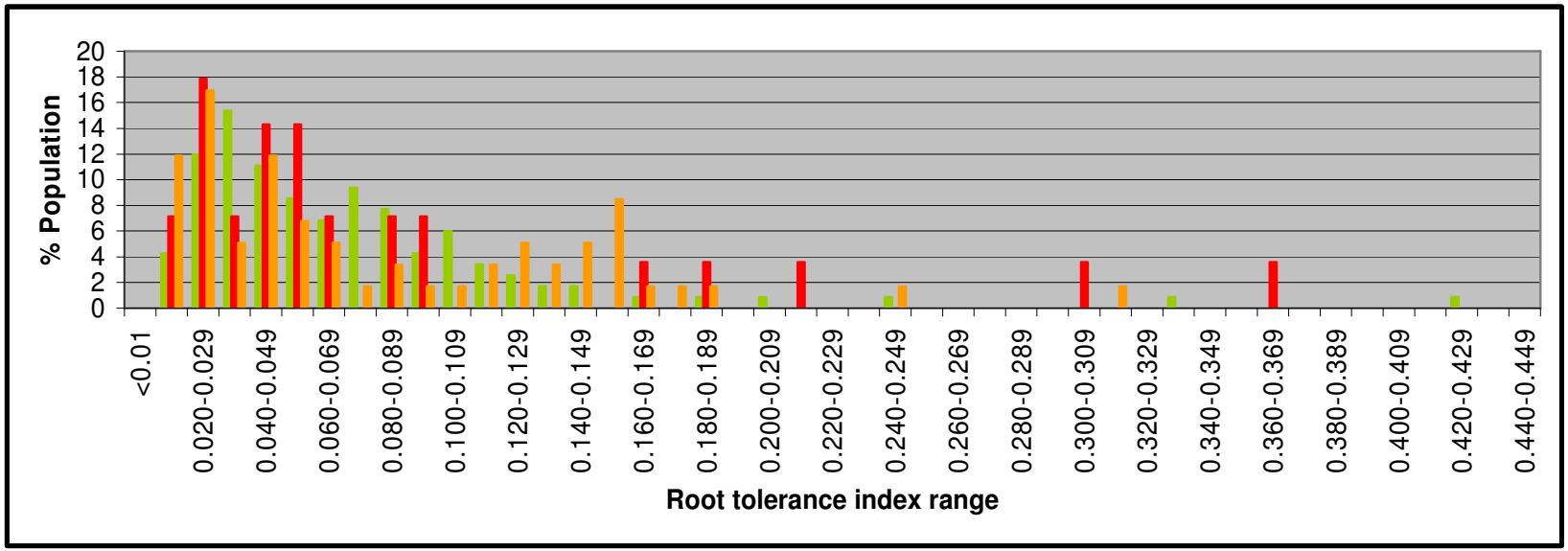

Figure 5.7 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Atlas 66 ■ (♀) and ASSN12 ■ (♂) after aluminium tolerance testing (F_2, n = 59)

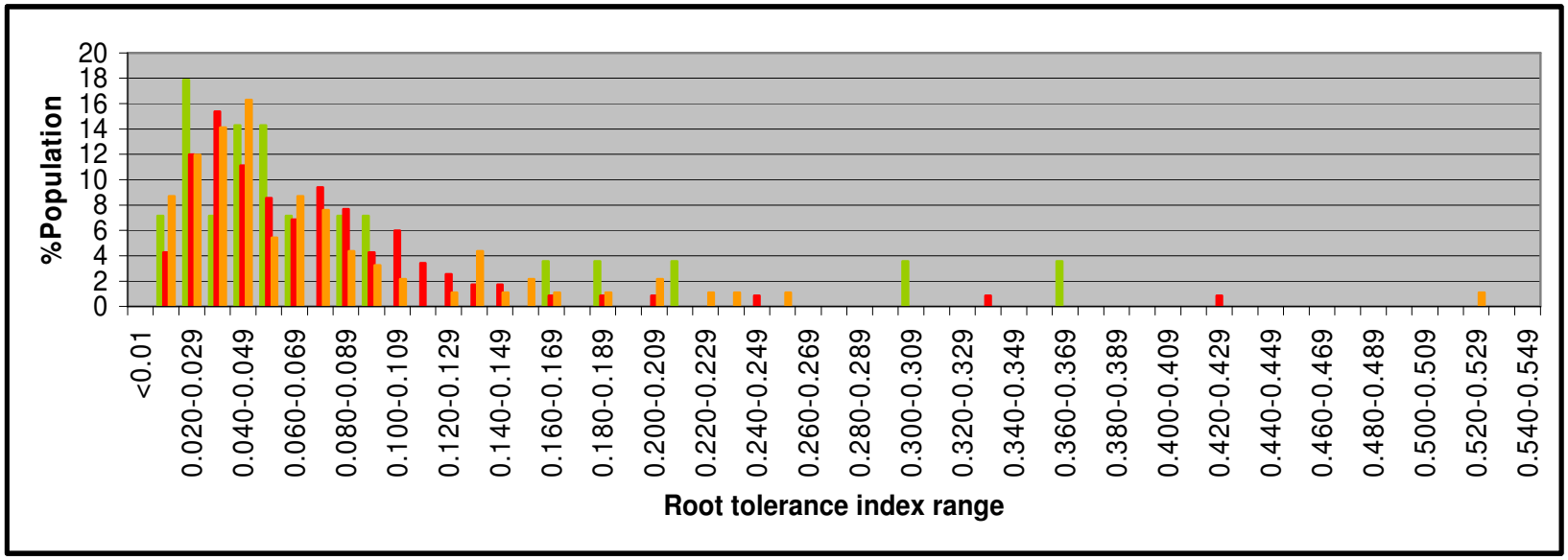

Figure 5.8 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes ASSN12 ■ (♀) and Atlas 66 ■ (♂) after aluminium tolerance testing (F_2, n = 92)

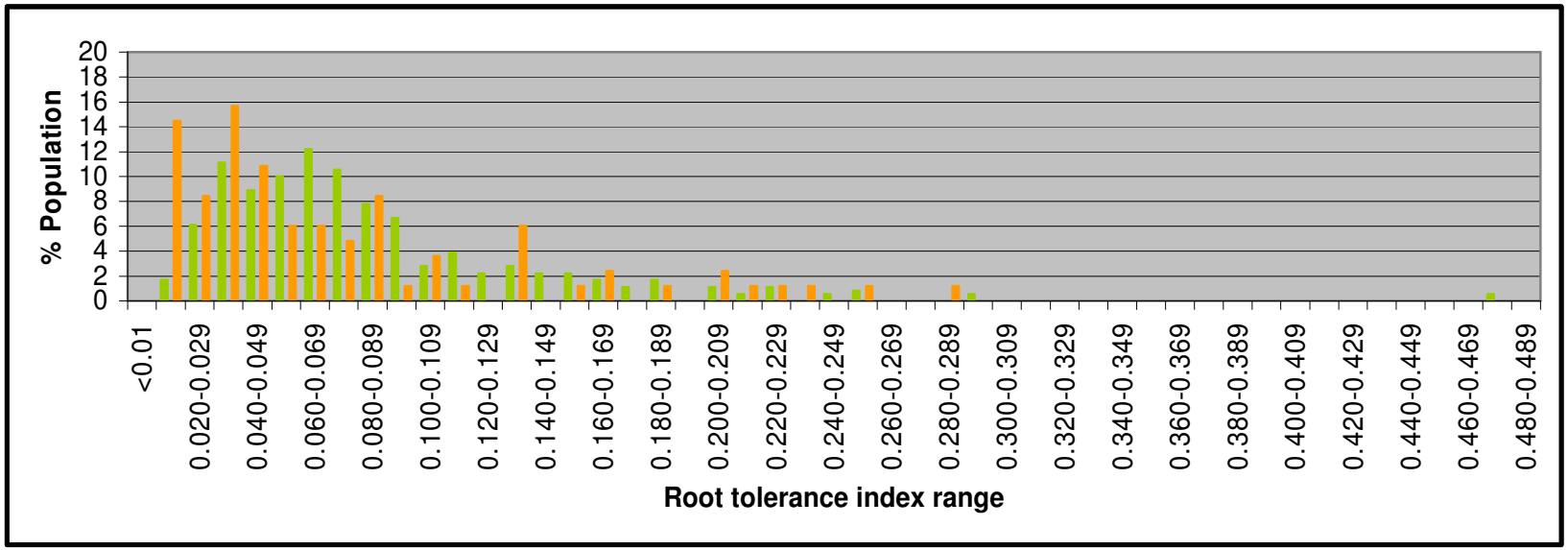

Figure 5.9 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Tugela DN ■ (♀) and Elands ■ (♂) after aluminium tolerance testing (F_2, n = 83)

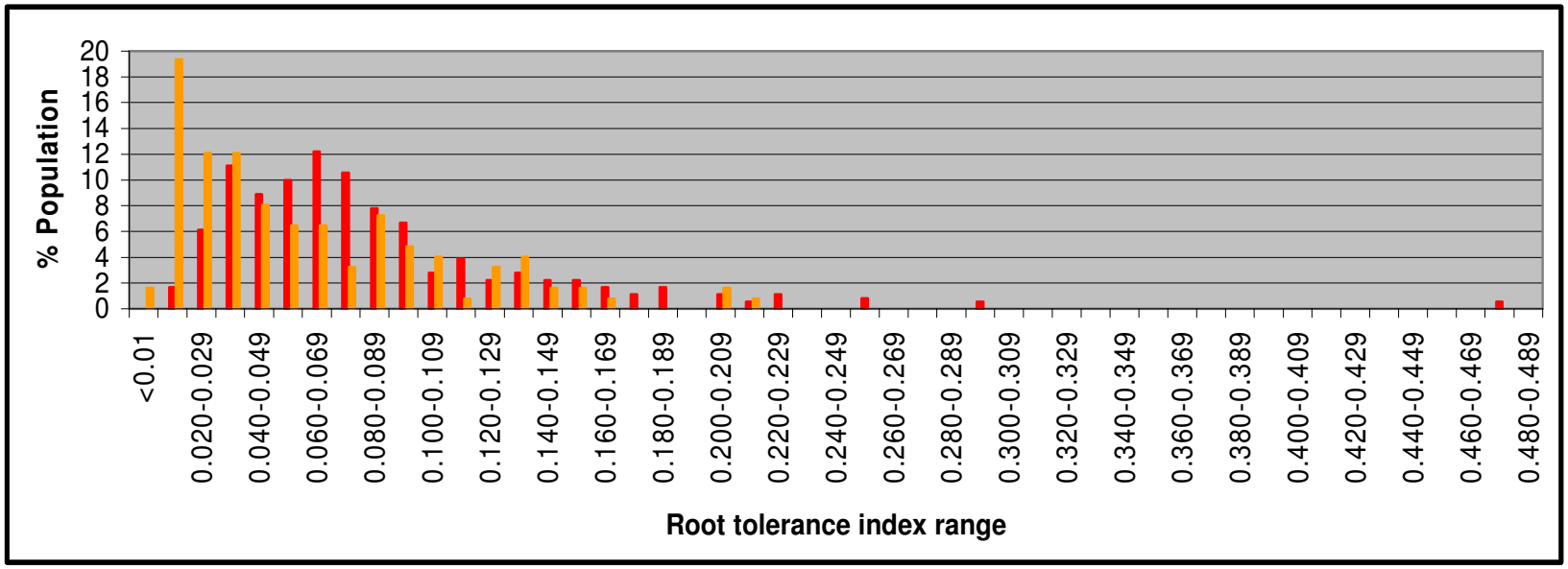

Figure 5.10 Frequency of aluminium tolerance index distribution of the F_2 population in comparison with the two parental genotypes Elands (♀) and Tugela DN (♂) after aluminium tolerance testing (F_2, n = 124)

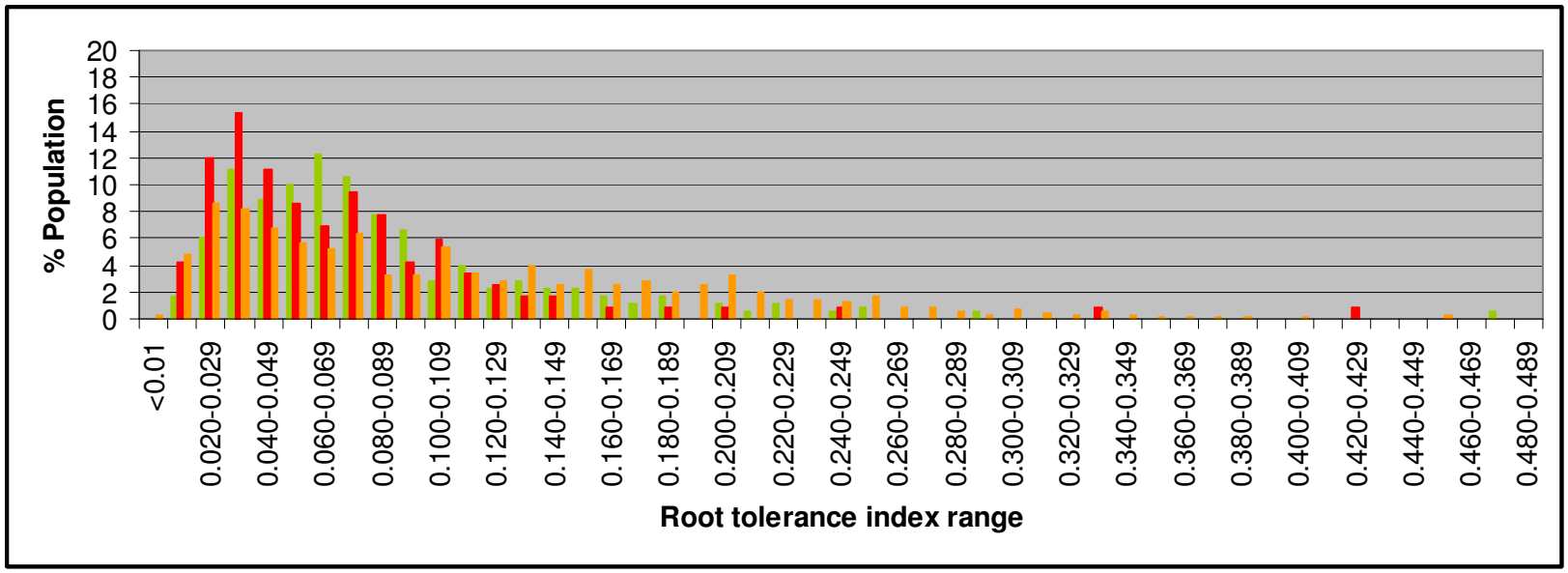

Figure 5.11 Frequency of aluminium tolerance index distribution of the F_2 population in comparison with the two parental genotypes Tugela DN (♀) and Atlas 66 (♂) after aluminium tolerance testing (F_2, n = 888)

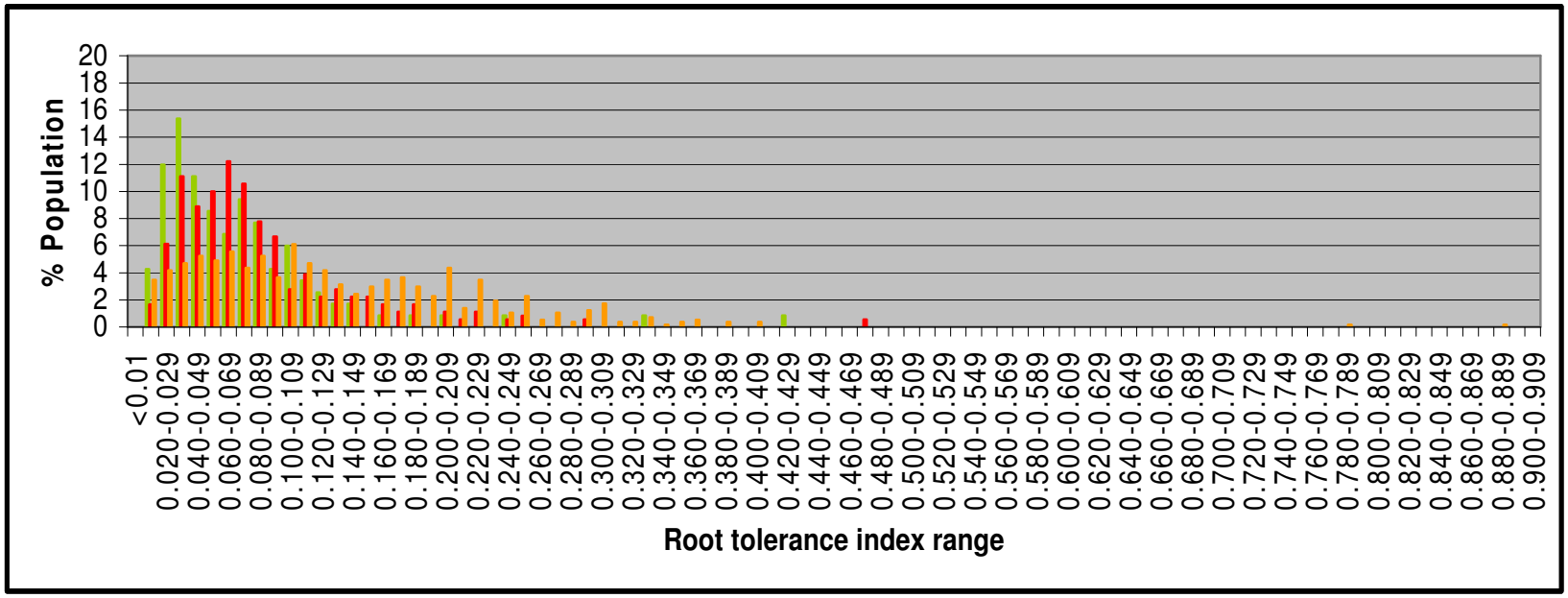

Figure 5.12 Frequency of aluminium tolerance index distribution of the F_2 population ■ in comparison with the two parental genotypes Atlas 66 ■ (♀) and Tugela DN ■ (♂) after aluminium tolerance testing (F_2, n = 574)

5.4 Discussion and conclusion

From the data obtained with the root re-growth method, combinations Tugela DNx Atlas 66 and Atlas 66xTugela DN showed three categories (moderate, intermediate and tolerant) of tolerance to aluminium toxicity in the F_2 population, which was equal to the tolerance categories of one of the donor parents, Tugela DN. The highest percentage of the F_2 population in both combinations fell in the moderate category, with a small amount of the population falling in the tolerant category, indicating some genetic gain.

Combination ASSN12xAtlas 66 showed an increase of tolerance to aluminium toxicity in the F_2 population, with three categories of tolerance, while combination Atlas 66xASSN12 showed an intermediate and moderate tolerance in the F_2 population for aluminium toxicity, which is the same as the parental donors. There was an increased tolerance in the F_2 population of combination ASSN12xAtlas 66, but not in Atlas 66xASSN12, indicating that ASSN12 should be used as female rather than male.

Figures 5.5 and 5.6 indicated that in combinations Tugela DNxElands and ElandsxTugela DN, 33% and 30% of the F_2 population was susceptible, with a high population percentage of the F_2 falling within the moderate tolerance category. It was expected that a certain percentage of the F_2 population would be susceptible because of Elands, the susceptible parent that was used.

In both Atlas 66xASSN12 and ASSN12xAtlas 66 (Figures 5.7 and 5.8) there was an increase in the values of progeny in relation to the parents. In Atlas 66xASSN12 the highest F_2 population mean fell below the parental population mean value, with 16.33% of the F_2 population at the root tolerance index range of 0.020-0.029, 16.67% of the population for Atlas 66 at the root tolerance index range of 0.030-0.039 and 21.96% for ASSN12 at the root tolerance index range of 0.020-0.029. For the combination ASSN12xAtlas 66, the highest F_2 population mean value fell in between the donor parents, with 16.3% at the root tolerance index range of 0.040-0.049, indicating no genetic gain.

The data for combinations Tugela DNxElands and ElandsxTugela DN (Figures 5.9 and 5.10) indicated a normal distribution with the root tolerance index method. Of the F_2 population, 16.05% fell above the parental mean values for Tugela DNxElands and 19.83% above that of ElandsxTugela DN.

Of the F_2 population1.61% for ElandsxTugela DN (Figure 5.10) fell below the parental root tolerance index range, indicating poor recombination or the presence of the same genes for aluminium tolerance in these two parents. The lowest F_2 population value for ElandsxTugela DN was 0.81% and 1.25 for Tugela DNxElands. The highest F_2 population percentage in both combinations was greater than those of the parents, indicating some genetic gain in the F_2 population.

Atlas 66xTugela DN and Tugela DNxAtlas 66 (Figure 5.11 and 5.12), indicated a normal distribution. The F_2 population had the highest root tolerance index range of 0.780-0.789 and 0.880-0.889 with a population of 0.19%. For Tugela DNxAtlas 66, the F_2 population fell below the parents' root tolerance index range, with the root tolerance index range of <0.01 with 0.24% of the population. The highest F_2 population for the combination Atlas 66xTugela DN was 6.13% at the root tolerance index range of 0.100-0.109, while for Tugela DNxAtlas 66 it was 8.95% at the root tolerance index range of 0.020-0.029. For Atlas 66, the highest population mean was 16.67% at the root tolerance index range of 0.030-0.039 and 12.78% at the root tolerance index range of 0.070-0.079 for Tugela DN. The F_2 population mean was below the donor parental population mean, indicating no genetic gain in the F_2 population. This might be because the aluminium tolerance genes in the parents are the same or the genes are difficult to combine.

Gupta (1997) reported, that maternal inheritance is not involved in aluminium tolerance control in hexaploid wheat. Dominance was reported to play a major role in the inheritance of aluminum tolerance in barley (Gupta, 1997). No differences were found among the reciprocals. The progeny of Tugela DNxAtlas 66 (Figure 5.11) and Atlas 66xTugela DN (Figure 5.12) showed no differences in the distribution frequencies of the root re-growth index. Carver and Ownby (1995) also reported that maternal effect does not influence the inheritance of aluminium tolerance in wheat.

The susceptible x tolerant crosses and tolerant x susceptible crosses had similar F_2 root re-growth distributions (Figures 5.5 and 5.6). The progeny appeared to be similar to the susceptible parent, indicating that susceptibility was conditioned by a single recessive gene. In this study the parents used as the female, did not influence the level of tolerance of the progeny, as it was reported by Lafever and Campbell (1978). Rehman *et al.* (2006) reported that reciprocal effects for hybrid morphology for plant height, spike length, flag leaf length, flag leaf width and number of spike were non-significant, indicating that these characteristics were not affected by the direction of the cross.

The greatest root length range of Atlas 66 and Tugela DN were 93 mm and 124 mm, greater than the 90 mm for the F_2 population (Table 5.3). The range of the root re-growth, root staining and root tolerance index for the $F_{2,}$ were greater than those of Atlas 66 and Tugela DN.

The greatest root length range for Tugela DN was 124 (Table 5.5), greater than the 115 for the F_2 and the F_2's range was greater than the 93 for Atlas 66. The root re-growth range for the F_2 was 12, greater than the 8 of Atlas 66 and the 11 of Tugela DN. The range of the stained portion parameter for Tugela DN and Atlas 66 was 5 mm greater than 0.98 for the F_2. The root tolerance index range for the $F_{2,}$ was 0.45 greater than 0.42 for Atlas 66, but smaller than 0.47 for Tugela DN.

In Table 5.7 the range of the four parameters for the F_2 was greater than that of ASSN12 and Atlas 66. The average of the root length for ASSN12 was 72.18 mm greater than the average of 71.59 mm for the F_2.

The range of the four parameters (Table 5.9) for Atlas 66 and ASSN12, were greater than that of the F_2, while the average root tolerance index for the F_2 was 0.080, greater than 0.072 of Atlas 66 and smaller than 0.083 of ASSN12.

The root length, root re-growth and root tolerance index ranges (Table 5.11) were high for Tugela DN and small for the $F_{2,}$ while the range of the stained portion for Tugela DN was 5 mm, equal to that of the F_2. The averages of the four parameters for Tugela DN were greater than those of the F_2.

The range of the root length, root re-growth and root tolerance index parameters for the F_2, were smaller than that of Tugela DN, while the range of the F_2 stained portion (Table 5.13) was 6 mm, greater than the 5 mm for Tugela DN. The standard deviation of the F_2 was 26.34, greater than the 21.07 for Tugela DN and the other three parameters' standard deviations were smaller for the F_2 and higher for Tugela DN.

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Chapter 6
General conclusions

Selection of wheat cultivars with diversified genetic backgrounds to screen for aluminium tolerance is important and a large number of materials should be screened. In this study the direct measurements of individual seedlings allowed for a precise score of each plant without subjective analysis. Three levels (moderate, intermediate and tolerant) of tolerance to aluminium toxicity were identified with the root re-growth method. All three levels of tolerance were seen for genotypes ASSN1, ASSN5 and Tugela DN. ASSN16, ASSN12, ASSN7, ASSN2a and Atlas 66 were found to have two levels (moderate and intermediate) of tolerance whereas T96/6 and ASSN15 had only the lowest level (moderate) of tolerance to aluminium toxicity. Although Atlas 66 was used as an international aluminium tolerance check, it performed far poorer than the local aluminium tolerance check, Tugela DN. The ASSN genotypes also performed much better than Atlas 66 and T96/6 which was the poorest performing genotype.

The root tolerance index method identified seedlings at a specific root tolerance index range, which varied in the seedlings. The genotypes that had three levels of tolerance did not necessarily have the highest root tolerance index range. Combining of favourable genes for aluminium tolerance seemed to be much successful between ASSN's. When crosses were made between different ASSN's the F_2 progeny had three levels of tolerance, indicating that the ASSN's can be used for hybridisation. For breeding, genotypes that would be highly recommended for crossing would be ASSN1, ASSN5 and ASSN16, because of their mean root re-growth, which is an important tolerance measure for aluminium toxicity. ASSN1 was the best performing genotype.

No reciprocal effects were seen for root re-growth length of the F_2 progeny. Genetic improvement of crops for acid soil tolerance can be accelerated by screening cultivars for aluminium tolerance using the nutrient solution culture method. More work is needed in this area, as soils in the most wheat producing areas in South Africa are becoming more acidic and the potential use of aluminium tolerance genotypes to breed highly tolerant genotypes is less cost effective to use in acidic soils.

Chapter 7
Summary

This study was undertaken to evaluate 11 wheat genotypes for aluminium tolerance using three laboratory based evaluation methods. Four parameters namely the root length before aluminium treatment, the root re-growth after aluminium treatment, the portion of the root affected by aluminium treatment, stained with hematoxylin and root tolerance index were measured on the two longest (primary and secondary) roots of each seedling to determine the effect of aluminium toxicity on the physiological development of the seedling roots.

With the root re-growth method it was possible to distinguish between three categories of tolerance (moderate, intermediate and tolerant) that will be very helpful in future resistance breeding for aluminium tolerance. With this method it is possible to discriminate between individuals in a population for aluminium tolerance.

Similar data was obtained for the primary and secondary roots, which indicated that the age of the roots are not a limiting factor for aluminium tolerance screening with the nutrient bioassay. Although the root re-growth method discriminated between the different aluminium tolerance categories, a better indication of aluminium tolerance categories was achieved with the root tolerance index method. With the above methodology in place it was possible to observe an increase in aluminium tolerance in some progeny after gene recombination and it was possible to discriminate between good aluminium tolerant progeny and progeny showing no genetic gain from the hybridisation. It was also shown that there were no reciprocal effects for aluminium tolerance in wheat.

There were genetic differences for aluminium tolerance between the genotypes used in this study and this methodology can be successfully implemented in an aluminium tolerance-breeding programme for wheat. This study indicated that there is useful methodology to effectively follow the genetic gains during gene-recombination for aluminium tolerance and, secondly that there are different genetic resources available in wheat that can be utilised to increase aluminium tolerance.

Opsomming

Hierdie studie is onderneem om 11 koring genotipes te evalueer vir aluminium toleransie met die gebruik van drie laboratorium gebaseerde evaluasie metodes. Vier parameters naamlik die wortellengte voor aluminium behandeling, die hergroei na aluminium behandeling, die gedeelte van die wortel wat deur aluminium behandeling beïnvloed is en gekleur is met hematoksilien en wortel toleransie indeks wat gemeet is op die twee langste (primêre en sekondêre) wortels van elke saailing, is gebruik om die effek van aluminium toksisiteit op elke saailing se fisiologiese ontwikkeling van hulle wortels te bepaal.

Dit was moontlik om te onderskei tussen drie kategorië van toleransie (laag, intermediêr en tolerant) met die wortel hergroei metode. Dit sal baie nuttig wees vir toekomstige weerstandsteling vir aluminium toleransie. Met hierdie metode was dit moontlik om tussen individue te onderskei vir aluminium toleransie binne ‘n populasie.

Die data vir primêre en sekondêre wortels was baie dieselfde, wat aandui dat die ouderdom van die wortels nie ‘n beperkende faktor is wanneer daar met die voedings biotoets vir aluminium toleransie getoets word met nie. Alhoewel die wortel hergroei metode onderskei het tussen verskillende aluminium toleransie kategorië, is ‘n beter aanduiding van aluminium toleransie verkry met die wortel toleransie indeks metode. Met bogenoemde metodes was dit moontlik om die toename van aluminium toleransie in die nageslag te sien na kruisings en dit was moontlik om te onderskei tussen nageslag met goeie toleransie, en die wat geen genetiese verbetering na gee-herkombinering getoon het nie. Daar is ook gewys dat daar geen resiproke effekte vir aluminium toleransie in koring is nie.

Daar was genetiese verskille vir aluminium toleransie tussen die genotipes wat gebruik is in hierdie studie en hierdie metodes kan dus suksesvol gebruik word in ‘n aluminium toleransie teelprogram vir koring. Die metodes is dus beskikbaar om genetiese verbetering in toleransie te volg na kruisings vir aluminium toleransie en tweedens is die genetiese bronne beskikbaar in koring wat gebruik kan word om aluminium toleransie te verbeter.