ADDIS ABABA UNIVERSITY
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DEPARTMENT OF MICROBIAL, CELLULAR AND MOLECULAR BIOLOGY

Hydroponic Screening of Aluminium Tolerance and Genetic Diversity Analysis of Ethiopian finger millet (*Eleusine coracana* (L.) Gaertn) Genotypes as Revealed by Inter Simple Sequence Repeat (ISSR) Markers

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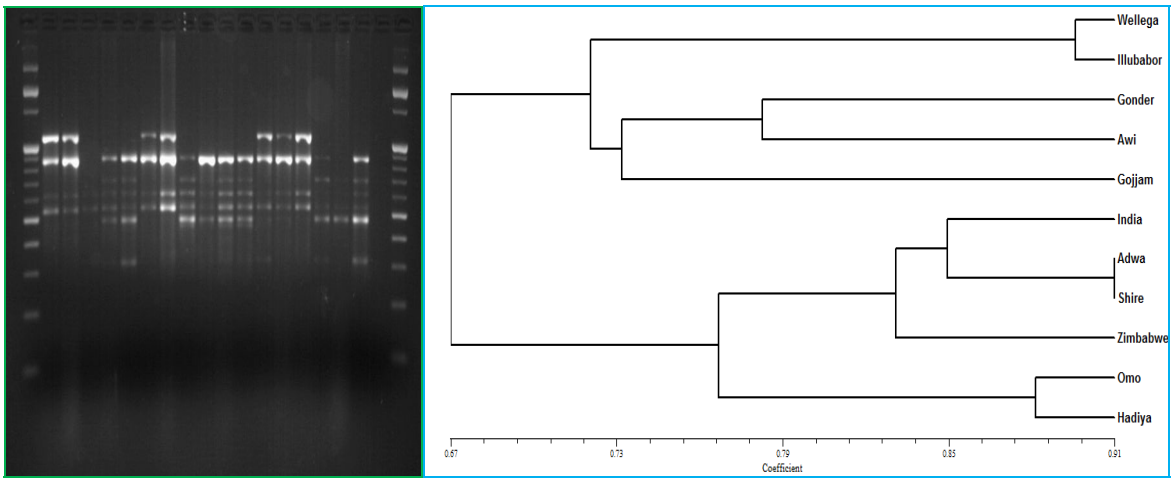
A Thesis Submitted to the School of Graduate Studies, Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Genetics)



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ABSTRACT

Eleusine coracana commonly called finger millet, is an annual allotetraploid (2n=4x=36), that belongs to grass family Poaceae, subfamily Chloridoideae. Biotic and abiotic stress combined with the use of less productive local cultivars cause low production in Ethiopia. This research was aimed to investigate acidity tolerance and genetic diversity of finger millet accessions from Ethiopia. The study was done on 288 finger millet accessions obtained from Ethiopian Institute of Biodiversity and six national varieties from NARs for optimization and preliminary screening, while 80 accessions were used for further characterization and molecular diversity work. The optimal Al toxicity level identified was 112.50 µM. Variety Gute was found to be Al tolerant and used as tolerant standard, while variety Necho was found susceptible and used as susceptible standard. Shoot and root length (SL, RL) as well as total fresh weight (FW) were measured after eight days of germination, and root length was found to be seriously affected. Screening was carried out on 288 accessions along with the standard checks in six batches, 75 (26.04 %) of them were Al tolerant, while 213 (73.95 %) were from medium to susceptible. After characterization 63 (78.75 %) out of 80 accessions showed significant Al induced stress in root length, while no distinct and visible symptom of aluminum toxicity were observed in the shoot of finger millet genotypes. According to RTI 21 genotypes were classified as tolerant, 35 genotypes as susceptible, and 24 as intermediate. Analysis was also carried out to estimate the genetic diversity among and within accessions of finger millet using six ISSR primers. The total genetic diversity (H) and Shannon's diversity information index (I) for the entire populations showed, 0.28, and 0.41, respectively. Individuals from Wellega, Gojam, Awi, and Zimbabwe showed the highest level of gene diversity similarly (H = 0.19, I = 0.28), while the lowest variability was shown by accessions from Omo (H = 0.05, I = 0.07). Variation within population was higher (58.54%) as compared to that of inter population (41.45%) based on AMOVA. Most individuals from all populations tended to form their own cluster, while only few of the individuals were distributed all over the tree. In PCO most of the individual accessions were clustered to their respective population. The result of the present study confirmed the presence of acid tolerant and genetically diversified accessions that can be used to improve the productivity as well as calls for a combined effort for the collection, conservation and sustainable use of finger millet.

Keywords: Acidity, *Eleusine coracana*, genetic diversity, hydroponics, ISSR

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
CTAB	Cetyl trimethyl ammonium bromide
ddH_2O	Double distilled water
2D	Two dimensions
3D	Three dimensions
EDTA	Ethylene diamine tetra acetic acid
FW	Fresh Weight
H	Nei"s Genetic diversity
I	Shannon information index
ISSR	Inter Simple Sequence Repeat
NJ	Neighbor Joining
NPL	Number of Polymorphic Loci
PAGE	Polyacrylamide Gel Electrophoresis
PCO	Principal Coordinate
PCR	Polymerase Chain Reaction
PPL	Percent of Polymorphic Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RL	Root Length
ROS	Reactive Oxygen Species
RTI	Relative Tolerance Index
SL	Shoot Length
SSR	Simple Sequence Repeat
TBE	Tris Borate EDTA
TE	Tris EDTA
UPGMA	Unweighted pair group method with arithmetic mean

1. INTRODUCTION

1.1. Background of the study

Eleusine coracana, commonly called finger millet, is an annual allotetraploid (2n=4x=36; genome constitution AABB), cultivated plant that belongss to the grass family Poaceae, subfamily Chloridoideae. It is an important cereal of semi-arid regions cultivated in parts of Africa and India, especially in dry areas for its grain. There are about nine species under the genus *Eleusine* Gaertn. Two species, *E. indica* and *E. floccifolia,* are believed to be the genome donors to the cultivated species, *E. coracana* (Bisht and Mukai, 2001).

Most of the species of *Eeusine* are distributed in tropical and subtropical parts of Africa, Asia and South America (Hilu *et al.,* 1979). *E. indica* (goose grass) is the most successful cosmopolitan grass distributed in most part of the globe (Kennedy-O'Byrne, 1957). *E. floccifolia* is found in Ethiopia and the Eritrea region. *E. africana,* progenitor of *E. coracana,* is mainly found in Africa and some parts of India (Philips, 1972).

Archeological studies confirmed that domestication of *E. coracana* started around 5000 B.C. in western Uganda and highlands of Ethiopia; it arrived in India much earlier, probably more than 3000 years ago (Hilu *et al.,* 1979). Most of the reports favor that the wild species *E. africana* originated first and then *E. coracana* was selected as a large grain mutant for cultivation from the wild species (Hilu and Johnson, 1992).

Finger millet was described as a robust free tillering annual grass. The inflorescence is terminal and digitate with 3-9 sessile spikes. The finger-like appearance of the terminal

inflorescence is the reason why the crop is known as “finger millet”. The shape of the head can be classified in to three as top curved, incurved and open (Phillips, 1995).

The significance of the crop lies in the wide array of values. The grain can be used in many types of foods, some of which are not quite common. Its several major uses include porridge, bread, malt, beverage, fodder and popped products. Finger millet is preferred to other grains as a food in difficult terrain and times of famine and fasting (National Research Council, 1996). Even though it is an important crop as a food security, production of the crop is inconsistent due to biotic and abiotic stresses. Among this, soil acidity is a major abiotic constraint to crop productivity especially in arid and semiarid regions.

The rate of soil quality degradation depends on land use systems, soil types, topography and climatic conditions. Land uses have significant influences on soil quality indicators. Soil pH, cation exchange capacity, total nitrogen and organic compounds, different forms of phosphorus, exchangeable bases and available micronutrients were affected due to intensive cultivation and use of acid forming inorganic fertilizers for the past three decades in western Ethiopia (Wakene Negassa and Heluf Gebrekidan, 2003).

The aim of this study is therefore, to carry out comparative evaluation of acid tolerance ability of different finger millet cultivars and to analyze their responses to acidity stress with regard to root growth, shoot growth and total fresh weight characteristics. In addition, this study aims to investigate the level of genetic diversity of *E. coracana* populations collected from different parts of Ethiopia using ISSR markers. This will provide information on the overall genetic variability of *E. coracana* populations which

may assist in the identification and selection of the genetic materials for conservation and use in acid affected regions of Ethiopia.

1.2. Significance of the study

According to Abdenna Deressa *et al.*, (2007), acidity affected soils are prevalent in the Western and Southern parts of Ethiopia, areas such as Nedjo, Diga, Gimibi and Bedi in Oromiya, Chencha and Sodo in SNNP, and Gozamin and Senan Woreda in Eastern Gojam and Awi zone in West Amhara region. In the Western and Eastern Wellega zones in particular, the large proportion exchangeable acidity was due to exchangeable aluminum while at West Showa zone it was due to exchangeable hydrogen. Therefore, as *E. coracana* grows in most of the acidity prone regions of the country, this research will attempt to investigate its acid tolerance ability, an important abiotic stress that affects its productivity and genetic diversity study using ISSR marker to shed some light on both aspects which could be important in selective improvement of some of the varieties and lines for acid its tolerance in the future.

2. LITERATURE REVIEW

2.1. Origin and distribution of *Eleusine coracana*

Eleusine coracana commonly called finger millet, is an annual allotetraploid (2n=4x=36; with genome constitution AABB), cultivated for its grain in many parts of Africa and India (Hilu and Johnson, 1992). Morphological studies confirmed domestication of *Eleusine coracana* started around 5000 B.C. in Western Uganda and highlands of Ethiopia; and it arrived in India much earlier, probably more than 3000 years ago (Hilu *et al.,* 1979). The oldest domesticated *Eleusine coracana* was found in the archaeological record of a prehistoric site in Axum, Ethiopia, dating back some 5000 years and it resembles race plana (a highly evolved race) which is the principal *Eleusine coracana* still grown in Ethiopia *(*Hilu *et al.,* 1979). However, Morphological, cytogenetic and molecular reports favor that the wild species *Eleusine africana* originated first and then *Eleusine coracana* was selected as a large grain mutant for cultivation from the wild species (Hilu and Johnson, 1992).

2.2. Description of *Eleusine coracana*

Finger millet belongs to the grass family Poaceae, subfamily Chloridoideae. Finger millet is described as a robust free tillering annual grass and it develops an extensive but shallow root system from the base of the main Culm. The lower nodes have been described as semi-procumbent and the upper part erect, stout, compressed smooth, 30-120 cm in height, 4-12cm thick and bearing numerous distichously leaves. The inflorescence is terminal and digitate with 3-9 sessile spikes. The finger-like appearance

of the terminal inflorescence is the reason why the crop is known as “finger millet”. The shape of the head can be classified in to three as top curved, incurved and open (Phillips, 1995).

There are about nine species under the genus *Eleusine* Gaertn, six diploid (2n=16, 18 and 20) and three tetraploid taxa (2n=36 or 38). *Eleusine coracana* (L.) Gaertn, *Eleusine floccifolia* (Forssk.), *Eleusine indica*, *Eleusine intermedia, Eleusine jaegeri, Eleusine kigeziensis, Eleusine multiflora*, *Eleusine tristachya,* and *Eleusine africana*. All of the species are wild except *Eleusine coracana* which is cultivated. Two species, *Eleusine indica* and *Eleusine floccifolia,* are believed to be the genome donor to *Eleusine coracana* (Bisht and Mukai, 2001).

2.3. Economic importance of *Eleusine coracana*

Finger millet (*E. coracana*) is a cultivated species among the genus *Eleusine*. It is consumed by grinding the grains up for porridge, bread, and *'injera'* in Ethiopia by mixing it with other crops. In Ethiopia powerful distilled liquor called “*arake*” is made from finger millet by mixing it with other crops. Whole grain of finger millet is rich in protein and vitamins (National Research Council, 1996). The main protein fraction (eleusinine) has high biological value, with good amount of tryptophan, cystine, methionine, and total aromatic amino acids. All of these are vital for human health and growth and are deficient in most cereals. For this reason alone, finger millet is an important preventative food against malnutrition. Finger millet is rich in source of minerals. Whole grain contains 0.27 % calcium; 12-66 times more than most common cereals, also have high iron content. Finger millet diet may have therapeutic effect on

arteriosclerosis and coronary by lowering blood cholesterol level as the grain is normally consumed whole (National Research Council, 1996). Even though it is an important crop as a food security, production of the crop is inconsistent due to biotic and abiotic stresses. Among this, soil acidity is one of a major abiotic constraint to crop productivity especially in arid and semiarid regions.

2.4. Soil nutrient and their availability in acidic condition

In healthy soil, macro nutrients are more available and ready for uptake by plant. In acidic soils, aluminium and manganese can become more available and become more toxic to the plant, while calcium, phosphorous and magnesium are less available to the plant. The major impact of extreme pH on plant growth is related to bioavailability of plant nutrients or concentration of plant toxic minerals (McLaren and Cameron, 1996).

Table 1. Available forms of essential plant nutrients

Macronutrient Element	symbol	available form	Micronutrient Element	symbol	available form
Carbon	C	CO2, HCO^{3-}	Iron	Fe	Fe^{2+}
Hydrogen	H	H_2O	Manganese	Mn	Mn^{2+}
Oxygen	O	O_2, H_2O	Copper	Cu	Cu^{2+}
Nitrogen	N	NO^{3-}, NH^{4+}	Zinc	Zn	Zn^{2+}
Potassium	K	K^+	Molybedium	Mo	MoO_4^{2-}
Phosphorus	P	H_2PO^{4-}, HPO_4	Boron	B	$H_3BO_4^{2-}$
Sulphur	S	SO_2, SO_4^{2-}	Chloride	Cl	Cl^-
Calcium	Ca	Ca^{2+}			

Source: (McLaren and Cameron, 1996)

A nutrient that is necessary for healthy plants need to be dissolved before uptake by plants. Dissolution of these nutrients usually takes place in neutral to slightly acidic pH values. Soil pH affects the availability of nutrients and how the nutrients react with each

other. At a low pH, beneficial elements such as molybdenum (Mo), phosphorus (P), magnesium (Mg) and calcium (Ca) become less available to plants, while other elements such as aluminium (Al), iron (Fe) and manganese (Mn) may become more available and may reach levels that are toxic to plants (McLaren and Cameron 1996).

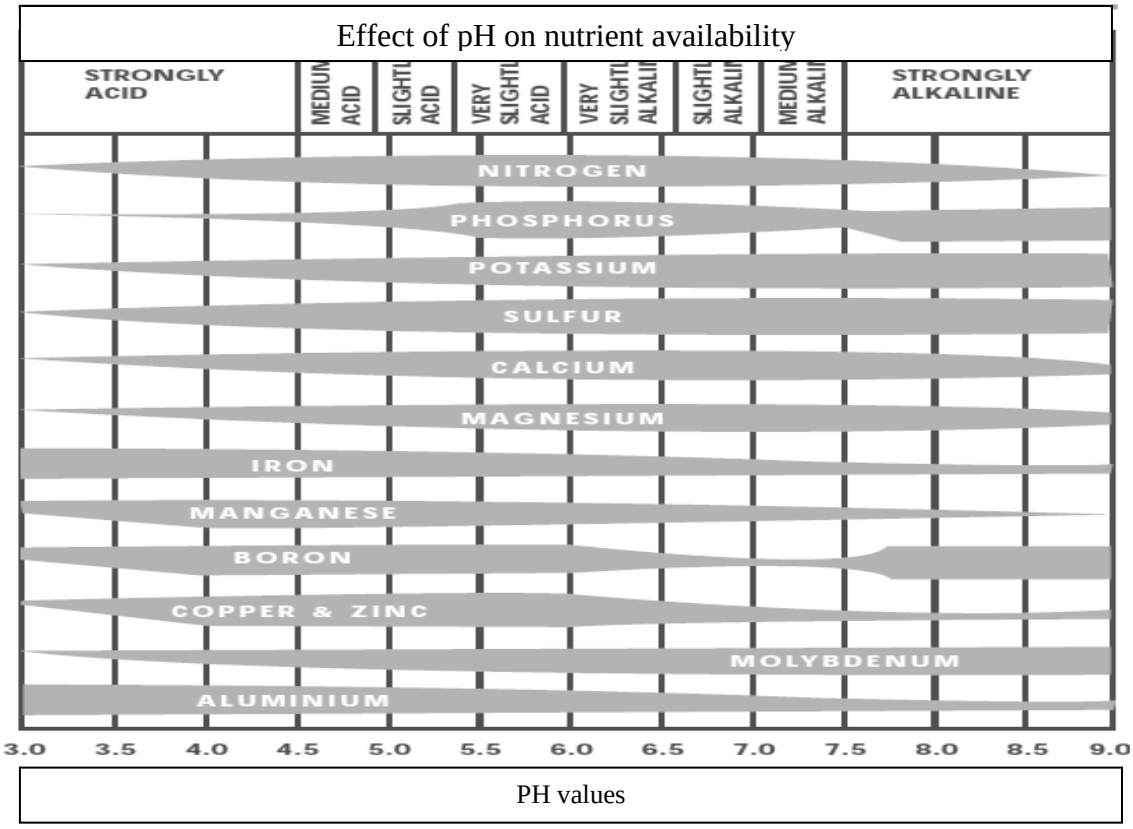


Figure 1. Effect of pH on the availability of different micro and macro nutrients (McLaren and Cameron 1996).

The impact of soil acidity on plant varies according to the tolerance of different species but can indicate stunted root growth, decreased yield, and increase susceptibility to disease. Some plants grow well over a wide pH range, while others are very sensitive to small variations in acidity or alkalinity. Soil pH values above or below the threshold may

result in less vigorous growth and nutrient deficiencies (Farm note 47/2002: Optimum soil pH for crop plants).

Table 2. Optimum pH requirement of some crop plants

Crop type	PH range	Crop type	PH range
Wheat	4.50 - 7.50	Cow peas	4.50 - 7.00
Barley	5.00 - 8.00	chick pea	6.00 - 9.00
Corn/maize/	5.00 - 7.00	peanut	5.50 - 9.00
Oats	4.50 - 7.00	soya bean	5.00 - 7.00
Triticale	4.00 - 6.50	field pea	5.30 - 8.00
Sorghum	5.00 - 7.00		
Rye	4.00 - 7.50		
rice	4.50 - 6.50		

Source: Farm note 47/2002: Optimum soil pH for crop plants. Department of agriculture Western Australia.

2.5. Soil acidity and aluminium toxicity

Acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production. It has been estimated that 15 % of the world´s soil is acidic and that over 50 % of the world's potentially arable lands are acidic (von Uexküll and Mutert, 1995). Aluminium (Al^{3+}) ranks third in abundance among the earth's crust elements, after oxygen and silicon, and is the most abundant metallic element. A large amount of Al is incorporated into aluminosilicate soil minerals and very small quantities appear in the soluble form, capable of influencing biological systems (May and Nordstrom, 1991). Aluminium bioavailability and, in consequence toxicity, is mainly restricted to this acidic environment.

When pH drops below 5.5, aluminosilicate clays and aluminium hydroxide minerals begin to dissolve, releasing aluminium-hydroxyl cations and Al^{3+} then it exchanges with other cations. The chemistry of Al^{3+} in soil solution is complicated by the fact that soluble inorganic (such as sulfate and fluoride) and organic ligands form complexes with Al^{3+}. Whether a ligand increases or decrease aluminium solubility depends on the particular aluminium-ligand complex and its tendency to remain in solution or precipitate. The mononuclear Al^{3+} species is considered as the most toxic form of aluminium (Kochian, 1995).

2.6. Effects of aluminium stress on plant growth and development

Aluminum phytotoxicity results in oxidative stress in plants (Zheng and Yang, 2005), resulting in peroxidation of the cellular membrane, and cell death, in addition to inhibiting root growth and development and consequently affecting both water and nutrient absorption (Doncheva *et al.*, 2005; Silva *et al.*, 2000). At high concentrations, Al can be a serious threat to agricultural production because it inhibits growth and lengthening of the roots through various mechanisms, including interactions inside the symplast, plasmatic membrane, and cell wall.

2.6.1. Aluminium induced root growth inhibition

Plant roots have close proximity with soil chemical components and can be easily hampered by toxic compounds like aluminium and manganese when the pH of the soil is decreasing. Root growth in plant is the combination of cell division and elongation. However, inhibition of cell division (decrease of S-phase cells) in the proximal meristem after 5 min Al^{3+} exposure and inhibition of root cell division in the apical meristem

within 10 or 30 minutes are mechanisms leading to root growth inhibition. Inhibited roots of plants have no potential of uptaking available nutients for their growth and development (Silva *et al.,* 2000). Aluminium can accumulate in the nuclei of cells in the meristematic region of the root tip within 30 minutes (Doncheva *et al.,* 2005; Silva *et al.,* 2000). The root growth inhibition and increase in root diameter observed in roots exposed to Al suggested that plant cytoskeleton could be a cellular target of Al phytotoxicity. Microtubules and actin microfilaments are altered in their stability, organization, and polymerization when plants are exposed to Al (Horst *et al.,* 1999).

2.6.2. Aluminium induced oxidative stress in plants

Oxidative stress occurs when any condition that disrupt the cellular redox homeostasis exist. Aluminium induced oxidative stresses have been suggested as the major factors leading to Al toxicity (Zheng and Yang, 2005). Aluminium exposure leads to oxidative stress due to aluminium ions form electrostatic bonds preferentially with oxygen donor ligands (e.g. carboxylate or phosphate groups), cell wall pectin and the outer surface of the plasma membrane seem to be major target sites of aluminium toxicity. Reactive oxygen species (ROS) includes superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2) produced as a result of Al toxicity. These ROS have the capacity to oxidize cellular components such as lipids, proteins, enzymes, and nucleic acids, blocking essential biomolecules and displacing essential metal ions like ca^{2+}, finally leading to cell death (Yamamoto *et al.,* 2001).

2.6.3. Aluminium accumulation and nutrient imbalances

Plant growth depends upon the availability and accumulation of essential nutrients in the soil. Accumulation of toxic elements like Al and manganese may disturb the nutritional balance of the plant. Aluminium accumulation is primarily and predominantly in the root apoplast (30–90% of the total absorbed Al) of peripheral cells and is only very slowly translocated to more central tissues (Schmohl and Horst, 2000). The primary binding of Al^{3+} in the apoplast is probably the pectin matrix and some also detected increase in Al contents in the same sensitive genotypes. Binding of Al to the pectin matrix and other cell wall constituents could alter cell wall characteristics and functions such as extensibility, porosity and enzyme activities thus leading to inhibition of root growth. Another mechanism for Al toxicity targeted to the apoplast invokes a rapid and irreversible displacement of Ca^{2+} from cell wall components by Al ions (Zheng and Yang, 2005). Moreover, aluminium can also interact strongly with the negatively charged plasma membrane and it can displace other cations (e.g. Ca^{2+}) that may form bridges between the phospholipids head groups of the membrane layer.

According to Blamey, (2001) the displacement of pectin-bound Ca^{2+} would inevitably alter physical properties of the cell wall, including extensibility, rigidity and permeability, which would be detrimental to cell extension as well as division. The blockage of Ca^{2+} permeable channels in the plasma membranes of plant cells could account for the Al-induced inhibition of Ca^{2+} influx into the plant cells.

2.6.4. Callose induction

Plans have different mechanism of tolerance against disease, wound, drought and pathogens. Induction of callose is one of the tolerance mechanisms. Callose is a

polysaccharide in the form of β-1, 3-glucan with some β-1, 6-branches and it exists in the cell walls of a wide variety of higher plants. Callose plays important roles during a variety of processes in plant development and in response to multiple biotic and abiotic stresses. Callose deposition can be induced by wounding, infection of pathogens, aluminum, abscisic acid, and other physiological stress. However, callose exerts a negative effect on plant defense against pathogen infection. This accumulation is reported to inhibit the symplastic transport and cell communication by blocking plasmadesmata, while avoiding Al induced lesions in the symplast (Sivaguru *et al.,* 2000). However, callose deposition in sensitive plant roots might cause uncontrolled rigidity of cell walls leading ultimately to protoplast degradation (Jones *et al.,* 2006).

2.7. Mechanisms of aluminium tolerance in plants

Plants have different mechanisms of tolerance for high aluminium concentrations in the soil. There is considerable variability in A1 tolerance within species and this has been useful to breeders in developing Al-tolerant cultivars of various crops. Generally, there are two main types of Al tolerance mechanisms: a) those that exclude Al from the root cells and b) those that allow Al to be tolerated once it has entered the plant cells (Barceló and Poschenrieder, 2002; Kochian *et al.,* 2005).

2.7.1. External tolerance mechanism (exclusion)

Plants have different mechanism of tolerating aluminium phytotoxicity. Aluminum tends to form strong complexes with oxygen donor ligands. The complexation of Al with chelating root exudates or binding to mucilage play a main role in the prevention of the accumulation of phototoxic Al in apoplast and symplast (Barcelo and poschenrieder,

2002). Organic acid anions such as citrate, malate and oxalate are able to form strong complexes with Al, where the Al-citrate complex bond is strongest, followed by the Al-oxalate, which are insoluble and are not available for plants. The transport of these organic acid anions from the radical cells is mediated by the anionic channel activity in the plasma membrane. This exudation is located in the radical apexes of some species, as this is a region which is very sensitive to Al toxicity due to constant cell division and elongation.

Although many types of organic acids are found in root cells, only one or two specific organic acids are secreted in response to Al treatment for any given species. Organic acid levels vary between species, cultivars and even between tissues of the same plant under identical growth conditions. In addition, organic acid anion biosynthesis and accumulation increase drastically in response to environmental stress (López-Bucio *et al.,* 2000). Tolerant genotypes exude a greater amount of organic acids than sensitive genotypes, which would support the notion that organic acid exudation is an Al tolerance mechanism.

Aluminium stimulated secretion of organic acid can occur in rapid response (pattern I) and delayed response (pattern II) as displayed in (Figure 2). In the rapid response, suggests that Al activates a pre-existing mechanism and that the induction of novel proteins is not required. In this case, Al might simply activate a transporter on the plasma membrane to initiate organic acid anion efflux. In contrast, the delay secretion might indicate that protein induction is required. These induced proteins could be involved in organic acid metabolism or in the transport of organic acid anions (Ma *et al.,* 2001).

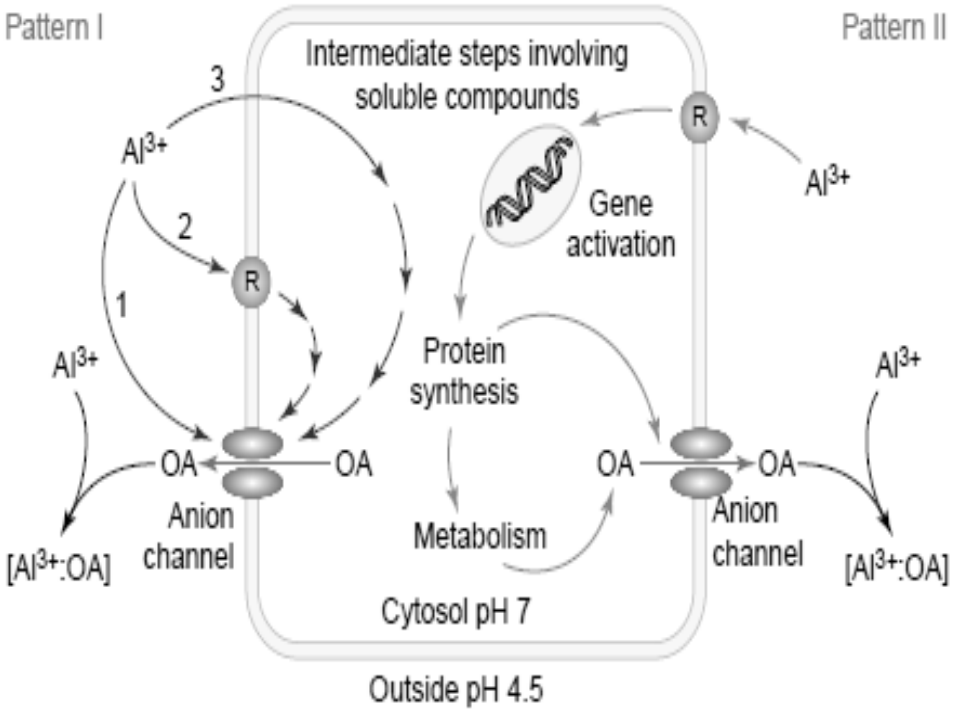


Figure 2. Models for the aluminium (Al)-stimulated secretion of organic acid anions (OA) from plant roots. In pattern I, Al activates an anion channel on the plasma membrane that is permeable to organic acid anions. In pattern II, Al interacts with the cell, perhaps via a receptor protein (R) on the plasma membrane, to activate the transcription of genes that encode proteins involved with the metabolism of organic acids or their transport across the plasma membrane (Ma et al., 2001).

2.7.2. Internal tolerance mechanism (inclusion)

Although Al exclusion from plant root tips and restriction of Al transport to upper plant parts seems to be the most important mechanism in Al resistance, there are numerous species that tolerate relatively high Al concentrations that is based on the complexation and detoxification of Al after its entry to the plant. Among the ligands that form stable complexes with Al are organic acid anions, phenolic substances and silicon may be implied in Al detoxification inside shoot tissues. This may indicate that organic anions

are able to protect plants by Al chelation in the cytosol. The metallic anion complex could then be transported around the plant for its storage. This mechanism immobilizes, compartmentalizes or detoxifies the Al from the symplast (Barceló and poschenrieder, 2002).

2.8. Status and distribution of acid soils in Ethiopia

In Ethiopia about 40.9 % is covered by strong to weak acid soils. From these 27.7 % moderate to weak acids with pH 5.5 - 6.7 and 13.2% covered by strong to moderate acidic soils with pH < 5.5. The western and southern parts of Ethiopia are dominantly covered by soils with pH < 5.5 (Schlede, 1989). Similarly, the soils in areas such as Nedjo, Diga, Gimibi and Bedi in Oromia, Chencha and Sodo in Southern Nation, Nationalities and Peoples of Ethiopia, Gozamin and Senan Woreda in eastern Gojam and Awi zone in West Amhara region have acidic problems in the soil. Evaluation of soil health in the direction from central (west Shoa) to western Ethiopia (west Wellega), the degree of soil acidity that is measured in terms of acid saturation percentage showed an increase. In Western and Eastern Wellega zones, the large proportion of exchangeable acidity was due to exchangeable aluminum while at west Shoa zone it was due to exchangeable hydrogen. According to Abdenna Deressa *et al.,* (2007) the acidity problem in East and West Wellega zones of Oromia region is becoming very critical and reached a level to affect crop productivity.

2.9. Screening methods of Al tolerance in plants

Genetic improvement of crops for acid soil tolerance has been accelerated by the availability of screening criteria for detecting Al tolerance in crop plants. 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.

2.9.1. Nutrient solution culture (Hydroponics)

Technically, hydroponic cultivation is a method of growing plants using mineral nutrient solutions, in water, without soil. Hydroponic culture is possibly the most intensive method of crop production in today's agricultural industry, mainly for ornamental plants which could be grown even in the absence of specialized spaces (gardens) restricted because of the demographic pressure (Haythem, 2012).

The advantages of hydroponic vegetable cultivation is a more efficient use of water and fertilizers, minimal use of land area, isolation of the crop from the underlying soil which may have problems associated with texture and nutrient availability, structure and drainage, disease, salinity and acidity, it provides easy access to root systems, tight control over nutrient availability and pH and non-destructive measurement of tolerance (Carvey and Ownby, 1995). There are two major criteria for evaluation of Al 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 Al stress is needed. Second, it is also suitable for identifying genotypes with superior alleles for Al tolerance (Hede *et al.*, 2002). On the other hand, hydroponic cultivation present limitations as it needs high cost and energy inputs. Hydroponic screening nutrient medium have been recommended for screening against aluminium stress in various crops,

in wheat (Delhaize and Ryan, 1995), in pigeon pea (Choudhary *et al.*, 2011), and rye (Gallego and Benito, 1997).

2.10. Genetic markers for diversity analysis

Genetic diversity refers to any variation in nucleotides, genes, chromosomes or whole genomes of organisms (Wang *et al.*, 2009a). A genetic marker is any visible character or otherwise assayable phenotype, for which alleles at individual loci segregate in a Mendelian manner. Genetic markers can be used to study the genetics of organisms, including trees, at the level of single genes (Spooner *et al.*, 2005).

There are different marker systems available for evaluating variability in plants. These are morphological, biochemical and molecular markers with different levels of applications and limitations.

2.10.1. Morphological markers

Traditionally diversity within and between populations was studied by assessing differences in the morphology, such as leaf area, plant height/width, seed number, seed size, flowering time, germination time, etc. which are observable characters of the individual and are the result of genetic differences in loci distributed throughout the genome (Kermali, 1994). Morphological markers reflect variation of expressed regions of genome. Morphological traits were among the traditional markers used in germplasm management and they are the strongest determinants of the agronomic value and taxonomic classification of plants. Especially, if the traits are highly heritable, morphological markers are one of the choices for diversity studies because the inheritance of the marker can be monitored visually (Yoseph, 2005).

However, morphological features have a number of limitations including low polymorphism, low heritability, late expression, vulnerability to environmental influences, time consuming, labour intensive, and the large populations of plants need large plots of land, which in turn limits their utility for assessing real genetic diversity (Muthusamy *et al.,* 2008).

2.10.2. Biochemical markers

To overcome the limitations of morphological traits, other markers have been developed at the protein level (Karp *et al.*, 1997a). These are also termed isozyme/allozyme markers or simply protein markers. Allozymes are allelic variants of enzymes encoded by structural genes. Enzymes are proteins consisting of amino acids, some of which are electrically charged, depending on the stretch of amino acids comprising the protein. When a mutation in the DNA results in an amino acid being replaced, the net electric charge of the protein may be modified, and the overall shape (conformation) of the molecule can change. Because changes in electric charge and conformation can affect the migration rate of proteins in an electric field, allelic variation can be detected by gel electrophoresis and subsequent enzyme-specific stains that contain substrate for the enzyme, cofactors and an oxidized salt (e.g. nitro-blue tetrazolium). Usually two, or sometimes even more loci can be distinguished for an enzyme and these are termed isoloci. Therefore, allozyme variation is often also referred to as isozyme variation (Kephart, 1990; May, 1992).

The technique is rapid, economical and co-dominant nature of allozyme data makes it useful for the characterization of genetic variation in plant species (Weising *et al.,* 2005).

Although protein markers circumvent environmental effects, the numbers of detectable markers are limited and they are typically tissue and developmental stage-specific and have no power to discriminate genetic diversity between closely related varieties (Park *et al.,* 2009). For this reason, most researchers began to focus on the use of DNA marker systems for genetic and ecological analyses of plant populations.

2.10.3. Molecular markers

Molecular markers, also called DNA markers, are thought of as signs along the DNA trail that pinpoint the location of desirable genetic traits or indicate specific genetic differences. Characterizations of plant genetic resources have been greatly facilitated by using a number of molecular marker systems due to their abundance in the genome. Molecular markers are not subject to environmental influences; so assessment can be carried out at any time during plant development (DeVicente and Fulton, 2003). They arise from different classes of DNA mutations such as substitution mutation, point mutation, rearrangements, insertion and deletion; or errors in replication of tandemely repeated DNA.

Due to the rapid developments, in the field of molecular genetics, a variety of different techniques have emerged to analyze genetic variation during the last few decades (Parker *et al.,* 1998). Some desirable properties of molecular marker 1) highly polymorphic nature: It must be polymorphic as it is polymorphism that is measured for genetic diversity studies; 2) Co-dominant inheritance: determination of homozygous and heterozygous states of diploid organisms; 3) Frequent occurrence in genome: a marker should be evenly and frequently distributed throughout the genome; 4) Selective neutral behaviors: the DNA sequences of any organism are neutral to environmental conditions

or management practices; 5) Easy access (availability): it should be easy, fast and cheap to detect; 6) High reproducibility; 7) Easy exchange of data between laboratories. It is extremely difficult to find a molecular marker, which would meet all the above criteria. Marker system can be identified that would fulfill at least a few of the above characteristics (Weising *et al.,* 2005). Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization-based markers and Polymerase Chain Reaction (PCR)-based markers.

2.10.3.1. Non PCR based technique

Eukaryotic genomes are very large and there was no simple way to observe genetic polymorphisms of individual genes or sequences. Genetic marker systems based on DNA-DNA hybridization were developed in the 1970s. The property of complementary base pairing allowed for methods to be developed whereby small pieces of DNA could be used as probes to reveal polymorphisms in just the sequences homologous to the probe. The genetic system derived using this approach is called restriction fragment length polymorphism (RFLP).

2.10.3.1.1. Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) has much greater power and was originally developed for mapping human genes than anything previously available (Botstein *et al.,* 1980). RFLP analysis is based on the ability of restriction enzymes (also called restriction endonucleases) to cleave DNA in to pieces (digested) at specific target nucleotide sequences consisting usually of a four or six nucleotide pairs. Variations in the characteristic pattern of a RFLP digest can be caused by base pair deletions, mutations,

inversions, translocations, transpositions and unequal crossing over which result in the loss or gain of a recognition site resulting in a fragment of different length and polymorphism (Schlotterer & Tautz, 1992). Only a single base pair difference in the recognition site will cause the restriction enzyme not to cut. If the base pair mutation is present in one chromosome but not the other, both fragment bands will be present on the gel, and the sample is said to heterozygous for the marker. The resulting restriction fragments are resolved by gel electrophoresis and then blotted (Southern, 1975) on to a nitrocellulose membrane and specific banding patterns are then visualized by hybridization with labeled probe.

The advantages of RFLPs are the co-dominant nature of the markers, their reliability, and specificity, while its limitation are the relatively large amount of high quality DNA required for restriction digestion, probes need to be developed, the technique is labor, time consuming, expensive (the requirement of radioactive isotope makes the analysis relatively expensive and hazardous) and require skilled person (Staub *et al.,* 1996). These limitations lead to the invention of PCR technique.

2.10.3.2. PCR based technique

The polymerase chain reaction enables the production of a large amount of a specific DNA sequence without cloning, starting with just a few molecules of the target sequence. One advantage of PCR-based marker methods over DNA-DNA hybridization marker methods is that the latter method requires isolation of large quantities of DNA.

The polymerase chain reaction has three basic steps: (1) Denaturing of the double stranded DNA template; (2) Annealing of a pair of primers to the region to be amplified; and (3) Amplification using a heat-resistant DNA polymerase called Taq polymerase.

2.10.3.2.1. Randomly Amplified Polymorphic DNA (RAPD)

RAPD was the first PCR based molecular marker technique developed and it is by far the simplest analysis (Williams *et al.,* 1990). RAPD analysis is based on the Short PCR primers (approximately 10 bases) are randomly and arbitrarily selected to amplify random DNA segments throughout the genome. These oligonucleotides serve as both forward and reverse primer, and are usually able to amplify fragments from 1–10 genomic sites simultaneously. Amplified fragments, usually within the 0.5–5 kb size range, are separated by agarose gel electrophoresis and polymorphisms are detected after ethidium bromide staining as the presence or absence of bands of particular sizes. These polymorphisms are considered to be primarily due to variation in the primer annealing sites, but they can also be generated by length differences in the amplified sequence between primer annealing sites.

The main advantage of RAPDs is that they are quick and easy to assay. Because PCR is involved, only low quantities of template DNA are required, usually 5–50 ng per reaction. Since random primers are commercially available, no sequence data for primer construction are needed. Moreover, RAPDs have a very high genomic abundance and are randomly distributed throughout the genome. The limitation of RAPDs is their low reproducibility (Schierwater and Ender 1993), and hence highly standardized experimental procedures are needed because of their sensitivity to the reaction conditions. RAPD analyses generally require purified DNA and precautions are needed to avoid contamination of DNA samples because short random primers are used that are able to amplify DNA fragments in a variety of organisms. RAPD have been successfully used to

estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of indigenous crop species, which includes in *Eleusine coracana* (Babu *et al*., 2006; Fakrudine *et al.,* 2004, and Kebere Bezaweletaw 2011).

2.10.3.2.2. Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism is a DNA fingerprinting technique, which detects DNA restriction fragments by means of PCR amplification. AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are viewed on denaturing polyacrylamide gels either through auto radiographic or fluorescence methodologies (Jones *et al.,* 1997a). AFLPs are DNA fragments (80–500 bp) obtained from digestion with restriction enzymes, followed by ligation of oligonucleotide adapters to the digestion products and selective amplification by the PCR. The PCR primers consist of a core sequence (part of the adapter), and a restriction enzyme specific sequence and 1–5 selective nucleotides (the higher the number of selective nucleotides, the lower the number of bands obtained per profile). The AFLP banding profiles are the result of variations in the restriction sites or in the intervening region. The AFLP technique simultaneously generates fragments from many genomic sites (usually 50–100 fragments per reaction) that are separated by polyacrylamide gel electrophoresis and scored as dominant markers.

The strengths of AFLPs lie in their high genomic abundance, considerable reproducibility, the generation of many informative bands per reaction, their wide range of applications, and the fact that no sequence data for primer construction are required. Its

limitations are the need for purified, high molecular weight DNA, the dominance nature of the markers.

2.10.3.2.3. Microsatellites marker

The term microsatellites was coined by Litt & Lutty (1989) and it is also known as Simple Sequence Repeats (SSRs). Microsatellites are sections of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species (Powell *et al.,* 1996). Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are favored in population studies and for the identification of closely related cultivars. If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR. Microsatellites and their flanking sequences can be identified by constructing a small-insert genomic library, screening the library with a synthetically labeled oligonucleotide repeat and sequencing the positive clones. Polymerase slippage during DNA replication, or slipped strand mispairing, is considered to be the main cause of variation in the number of repeat units of a microsatellite, resulting in length polymorphisms that can be detected by gel electrophoresis.

The strengths of microsatellites include the co-dominance of alleles, their high genomic abundance in eukaryotes and their random distribution throughout the genome, with preferential association in low-copy regions (Morgante and Olivieri 1993). Because the technique is PCR-based, only low quantities of template DNA (10–100 ng per reaction)

are required. Due to the use of long PCR primers, the reproducibility of microsatellites is high and analyses do not require high quality DNA.

2.10.3.2.4. Inter Simple Sequence Repeats (ISSR)

Inter Simple Sequence Repeat **(**ISSR) are DNA fragments of about 100–3000 bp located between adjacent, oppositely oriented microsatellite regions (Zietkiewicz *et al.*, 1994). Primers based on microsatellites are utilized to amplify inter-SSR DNA sequences. ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16–18 bp). About 10–60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size.

The main advantage of ISSRs is that no sequence data for primer construction are needed; only low quantities of template DNA are required (5–50 ng per reaction), but can have reproducibility problems. The sources of variation in ISSR markers could be mutations at the priming site (SSR), which could prevent amplification of a fragment and thus give a presence/absence polymorphism. Moreover, an insertion/deletion event within the SSR region or the amplified region would result in the absence of a product or length polymorphism depending on the amplifiability of the resulting fragment size. ISSR marker has a wide range of uses, including the characterization of genetic relatedness among populations, genetic fingerprinting, and detection of clonal variation, cultivar identification, phylogenetic analysis and assessment of hybridization (Wang *et al.*, 2009a). Inter Simple Sequence Repeats (ISSRs) have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of

indigenous crop species, which includes in Coffea arabica (Kassahun Tesfaye *et al.*, 2005) and in lentil (Edossa Fikru *et al.*, 2010).

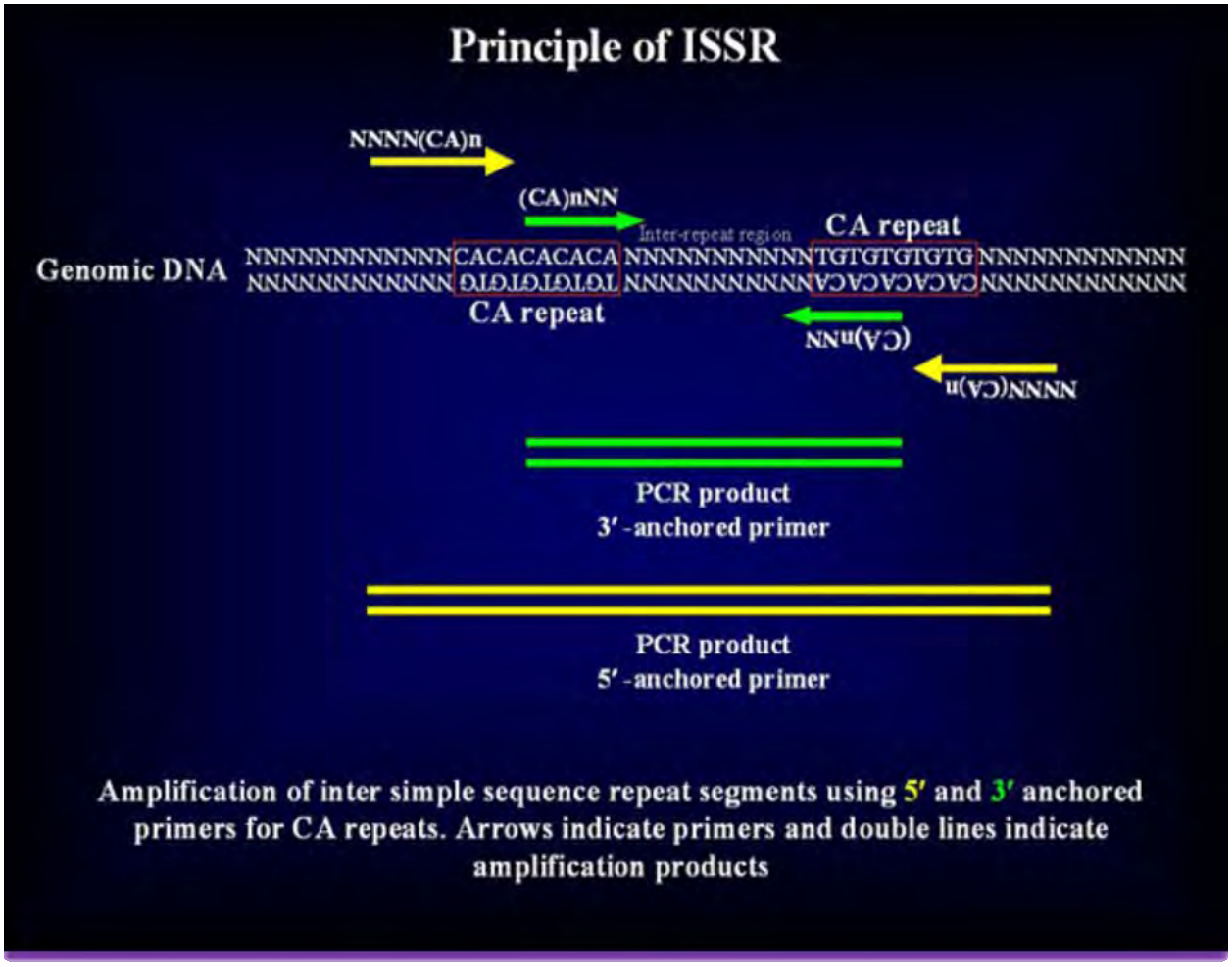


Figure 3. A schematic representation of ISSR-PC R with a single primer (AG)8, unanchored (a), 3'-anchored (b) and 5'-anchored (c) targeting a (TC)n repeat used to amplify inter simple sequence repeat region flanked by two inversely oriented (TC)n sequences.

3. OBJECTIVE OF THE STUDY

3.1. General objective

The main objective of this thesis research was;

- To investigate hydroponics-based Al-toxicity tolerance and extent of genetic diversity exist in Ethiopian (*E. coracana*) germplasms using ISSR marker.

3.2. Specific objectives

The specific objectives of this thesis research were:

- to identify the threshold concentration of Al tolerance on finger millet germplasm under hydroponics;
- to assess and characterize Al-tolerance of Ethiopian finger millet germplasm under hydroponics conditions;
- to assess the extent of genetic diversity of Ethiopian finger millet germplasm using ISSR markers.

4. MATERIALS AND METHODS

4.1. Aluminium tolerance study of Ethiopian finger millet

4.1.1. Plant Materials and Germination Conditions

Seeds of about 300 accessions and six improved cultivars of finger millet (*E. coracana*) were obtained from Ethiopian Institute of Biodiversity (EIB) and Bako Agricultural Research Center, respectively (Appendix 1). Twenty randomly selected and surface-sterilized seeds of each germplasms were wrapped and germinated in a tissue paper, moistened with distilled water, in Petri dishes for about 36 hours under dark conditions.

4.1.2. Hydroponics Experimental Setup

4.1.2.1. Equipment Setup

For this purpose, dense narrow holes were introduced into as many eppendorff tubes as required in such a way that the holes did not allow to pass finger millet seeds through but rather allowed in air bubbles for aerating the seedlings in the tube. "Rack" like plate to hold the perforated eppendorff were made from jar plastic plate by introducing wide holes capable of holding and submerging eppendorff tubes in the nutrient solution.

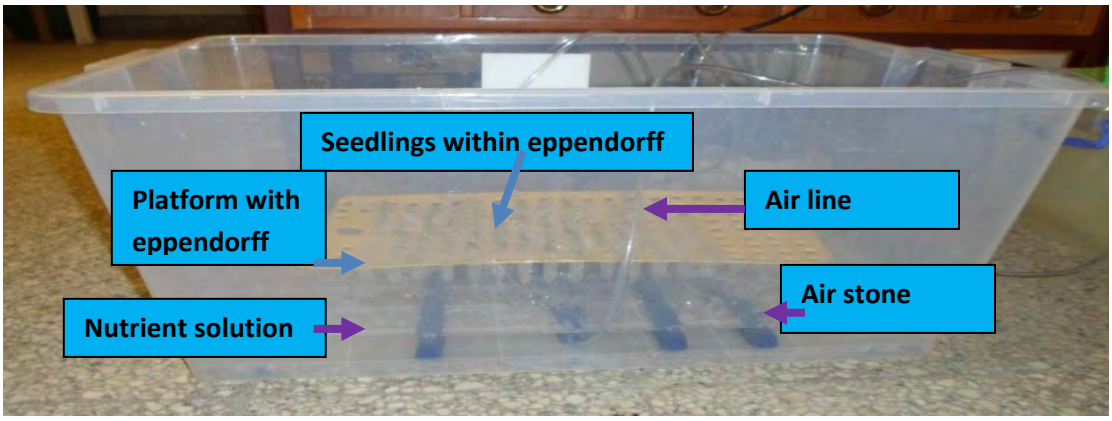


Figure 4. Schematic representation of hydroponic setup

4.1.2.2. Nutrient Solution Culture

Six in vitro germinated seedlings of approximately equal endosperm and equal root length from each germplasms were transferred into separate eppendorff tubes, which were then arranged on plastic plate and submerged into the nutrient solution. The experiment was laid down in RCBD (Randomized Complete Block Design) with three replications. Nutrient solution culture prepared according to Delhaize *et al*., (2004); was composed of 500 µM KNO_3, 500 µM $CaCl_2$, 500 µM NH_4NO_3, 150 µM $MgSO_4.7H_2O$, 10 µM KH_2PO_4, 2 µM $FeCl_3$ (III) and varying concentrations of $Al_2 (SO_4)_3.18H_2O$. The control experiment also involved all the above nutrients except $Al_2 (SO_4)_3.18H_2O$. The pH of the nutrient was adjusted to 4.3 by using 1M HCl and the solution was renewed every 24hrs.

4.1.2.3. Optimization of Al^{3+} concentration and number of days for seedling growth

The Al^{3+} ion concentrations used for investigating and identifying the optimum concentration for screening included 0, 5, 10, 25, 50, 100, 112.5, 150, and 200 µM of $Al_2 (SO_4)_3.18H_2O$. This initial optimization experiment showed 112.5 µM of $Al_2 (SO_4)_3.18H_2O$ to be an optimum Al^{3+} concentration and hence this was used for further preliminary screening and characterization experiments. Details of the optimization results are presented in (Table 5 and Figure 6).

Furthermore, the optimum number of days at which seedlings can be clearly distinguished into different classes of tolerance (tolerant, intermediate or susceptible) was determined by assessing the performance of seedlings of germplasm representing the three levels of tolerance for 5, 6, 8, and 10 days. This preliminary experiment showed

that seedlings grown for 8 days under Al-toxicity hydroponic conditions were better distinguished into three tolerance classes and hence it was selected as an optimum number of days for further screening and characterization activities.

4.1.3. Data recoding and statistical analysis

Root and shoot length of nine seedlings per accession were measured using a ruler while fresh weight of three seedlings of each accession was taken using a digital balance (version no. 339, capacity 210 AE Adam ® with 0.0001 precision).

Mean and analysis of variance (ANOVA) under each optimization, screening and characterization $Al_2 (SO_4)_3.18H_2O$ for each accession and variety grown under different batch was conducted using SPSS software version 20. Tukey Honesty Significance test was used to make pair wise mean comparison of each germplasm under control and Al-treated conditions. Relative Tolerance Index related to root length was estimated for germplasm categorization following method of Mendes, (1984).

$$RTI = \frac{\text{Root length undertreatment (Al)}}{\text{Root Length under control (without Al)}}$$

$$I = (1 - RTI) * 100\ \%$$

Where; RTI is Root tolerance index, RLI= Root length inhibition

4.2. Genetic diversity study in *Eleusine coracana*

4.2.1. Plant material and their germination condition

Based on their Al tolerance performance in hydroponics, 80 accessions from (Oromia, Amhara, Tigray, SNNP, National varieties and Exotic (Zimbabwe and India)) (Appendix 2) were selected and germinated in green house for about six weeks. Each population contained accessions that are from Al tolerant, susceptible and intermediate (Table 3).

Table 3. List and number of accessions of *E. coracana* used in the study

No	Region	Population	No. acc	No	Region	Population	No. acc
1	Oromia	Wellega	16	7	Tigray	Shire	5
2	Oromia	Ilu Ababora	5	8	SNNP	Omo	5
3	Amhara	Gondar	6	9	SNNP	Hadiya	7
4	Amhara	Gojam	9	10	India	India	5
5	Amhara	Awi	5	11	Zimbabwe	Zimbabwe	6
6	Tigray	Adwa	5	12		Nation Varieties	6

4.2.2. Extraction of genomic DNA in finger millet

The ISSR marker assay was conducted at Plant Genetics Laboratory of the Microbial, Cellular and Molecular Biology Department, Addis Ababa University. Total genomic DNA was extracted from approximately equal amount of (0.2g) three silica gel dried leaves per accession by using Cetyl Trimethyl Ammonium Bromide (2 % Cetyltrimethyl ammonium Bromide, 1 % polyvimylpyrolidone, 100 mM Tris: PH=8, 20 mM EDTA, 1.4 M NaCl) and 0.03 M beta-Mercapto-ethanol extraction protocol based on Borsch *et al., (*2003) with minor modifications appendix (3). Approximately equal amounts (0.2g) of

the dried leaf samples were bulked and ground with Mix and Mill grinding machine MM 400.

4.2.3. DNA quality and amount testing

The genomic DNA was tested using NanoDrop (NanoDrop™2000/2000c) spectrophotometer and 1 % agarose. Solution of 1 % agarose was prepared by boiling 0.5 g agarose in 50 ml of 1x TBE buffer in microwave oven until the solution was completely clear without any visible suspension. After allowing the solution to cool for a couple of minutes 2 µl of ethidium bromide was added and thoroughly mixed by swirling. The gel was poured slowly in to the gel tray that was well prepared with comb fixed properly. The gel was then allowed to solidify for at least 30 minutes and was placed in the electrophoresis tank after gently removing the comb. Tris Borate Ethylene diamine tetra acetic acid (TBE) buffer (1X) was poured in to the gel tank until the gel was completely submerged.

Genomic DNA of 2 µl from each sample along with 3 µl double distilled water and 1µl of loading dye (6x) was loaded on to the gel and electrophoresed at a constant voltage of 80V for 30-40 minutes. Gel picture was taken under UV transilluminator by Gel Doc system (Biosens SC750) with digital canon camera. From the genomic DNA test gel it was found that all the DNA samples were free of smear and of equivalent and intermediate band intensity. Based on this result, a working solution was made by diluting the genomic DNA in a 1:5 ratio, tested using the same procedure and diluted DNA stored at -20 ℃ until used.

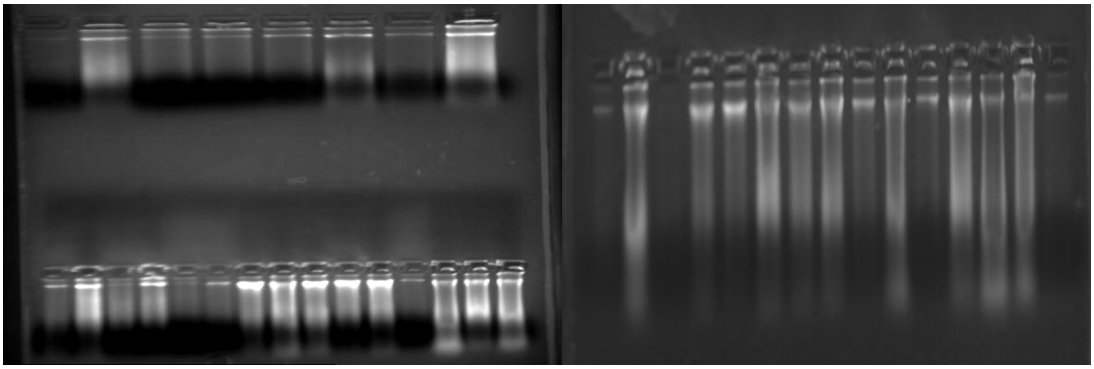


Figure 5. Genomic DNA test gel result

4.2.4. Primer selection and optimization

For the initial testing of primers variability and reproducibility seven primers were used. Three individuals were selected from each population with 1: 5 dilutions to screen the primers. A total of six polymorphic and reproducible ISSR primers were selected after testing and screenings. List of primers used and tested, their annealing temperature with respective sequences and their amplification pattern were shown in Table 4.

Table 4. List of different ISSR primers with annealing temperature, sequence and motives screened for amplification

No.	Primers	Ta	Sequence	Motif
1	810	45 ºC	5'-GAGAGAGAGAGAGAGAT-3'	Di-nucleotide
2	811	48 ºC	5'-GAGAGAGAGAGAGAGAC-3'	Di-nucleotide
3	848	48 ºC	5'-CACACACACACACACARG-3'	Di-nucleotide
4	866	55 ºC	5'-CTCCTCCTCCTCCTCCTC-3'	Tri-nucleotide
5	873	45 ºC	5'-GACAGACAGACAGACA-3'	Tetra-nucleotide
6	878	45 ºC	5'-GGATGGATGGATGGAT-3'	Tetra-nucleotide
7	880	45 ºC	5'-GG AGA GG AG AGG AGA-3'	Penta-nucleotide

Source: Primer kit 900 (UBC 900); R = Purines (A or G), Ta= annealing temperature

4.2.5. PCR and gel electrophoresis

Polymerase chain reaction (PCR) amplifications were performed in Biometra 2003 T3 Thermo cycler programmed to run the following temperature profile: a preheating and initial denaturation for 4 minutes at 94℃, then 15 seconds denaturation at 94℃, 1 minute primer annealing at 45℃/ 48/55℃ (based on primers used), 1.30 minutes extension at 72℃ for 40 cycles and the final extension for 7 minutes at 72℃ with holding temperature at 4℃. Each PCR reaction of ISSR markers had a final reaction volume of 25 µl, containing 4 mM dNTPs, 10x PCR buffer (100 mM Tris HCl (PH8.8), 500 mM KCl, 0.1% Tween 20 and 15 mM $MgCl_{2)}$, 3 mM $MgCl_2$, 8 pmol primer, 2 U Taq polymerase and 10-100 ng template DNA. A negative control, in which the template DNA was replaced by double distilled water, was also included in each round of reactions to check for absence/presence of contamination. The PCR products were stored at 4℃ until loading on the gel for electrophoresis. Amplification products were separated by electrophoresis in 1.67 % (w/v) agarose gels. A total of 8 µl PCR product of each sample and 6x loading dye (0.004 M Bromophenol blue, 4.1 Glycerol, 0.02 M Tris HCl) was loaded on to the ISSR gel. Fifteen wells comb was used for each ISSR gel slab. The first lane was loaded with 100 bp ladder by loading 2 µl (peq gold range mix) with loading dye in that well as a size standard and the last lane was control (without DNA template). The ISSR electrophoreses were done for about 3 hours at constant voltage of 80 V. After electrophoresis, the gels were stained in 50 µl (10 mg/ml) ethidium bromide mixed with 450 ml distilled water for 30 minutes and distained with distilled water for 30 minutes then banding patterns were visualized under UV light and photograph using canon

camera in the Gel Doc system (Biosens SC750) and documented for band scoring and analysis.

4.2.6. Data recording and statistical analysis

ISSR markers were treated as dominant markers and each locus was considered as a bi-allelic locus with one amplifiable and one null allele. Scoring was performed manually for each primer based on presence (1) and absence (0) or ambiguous (?), and each band was regarded as a locus. Only amplified bands that were clearly resolved were recorded, and a "0" and "1" data matrix was generated. Based on generated binary data (0 and 1) matrix different software's were used for analysis. POPGENE version1.32 software (Yehe *et al.,* 1999) was used to calculate genetic diversity for each population as number of polymorphic loci, percent polymorphism, gene diversity (h) and Shannon–Weaver diversity index (I). Analysis of molecular variance (AMOVA) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier *et al.,* 2006).

NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek *et al.,* 1999) software's were used to calculate Jaccard's similarity coefficient which is calculated with the formula:-

$$S_{ij} = \frac{a}{a+b+c}$$

Where,

'a' is the total number of bands shared between individuals i and j,

'b' is the total number of bands present in individual i but not in individual j and

'c' is the total number of bands present in individual j but not in individual i.

The unweighted pair group method with arithmetic average (UPGMA) (*S*neath and Sokal, 1973) was used in order to determine the genetic relationship among accessions and generates phenogram using NTSYS- pc version 2.02 (Rohlf, 2000). The neighbor joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988) was used to compare individual accessions and evaluate patterns of accession clustering using Free Tree 0.9.1.50 Software (Pavlicek *et al.*, 1999).

To further examine the patterns of variation among individual samples, a principal coordinated (PCOA) analysis was performed based on Jaccard's coefficient (Jaccard, 1908). The calculation of Jaccard's coefficient was made with PAST soft ware version 1.18 (Hammer *et al.*, 2001). The first three axes were later used to plot with STATISTICA version 6.0 software (Hammer *et al.*, 2001; Statistica soft, Inc.2001).

5. RESULTS

5.1. Screening finger millet genotypes for Al toxicity

5.1.1. Optimizing Al concentration and finger millet varietal characterization

The aluminium tolerance test showed that finger millet varieties grown at lower Al concentration had higher root growth or root length (RL) than those treated with relatively high level of Al concentration. Variety Necho had the longest root under control condition, but its root length kept decreasing with increasing Al^{3+} concentration until 200 μM where it showed the lowest root length. Degu, on the other hand, was a variety with the longest root at 112.5 μM Al^{3+} concentration and beyond has superior performance under strong acidic conditions (Table 5 and Figure 6).

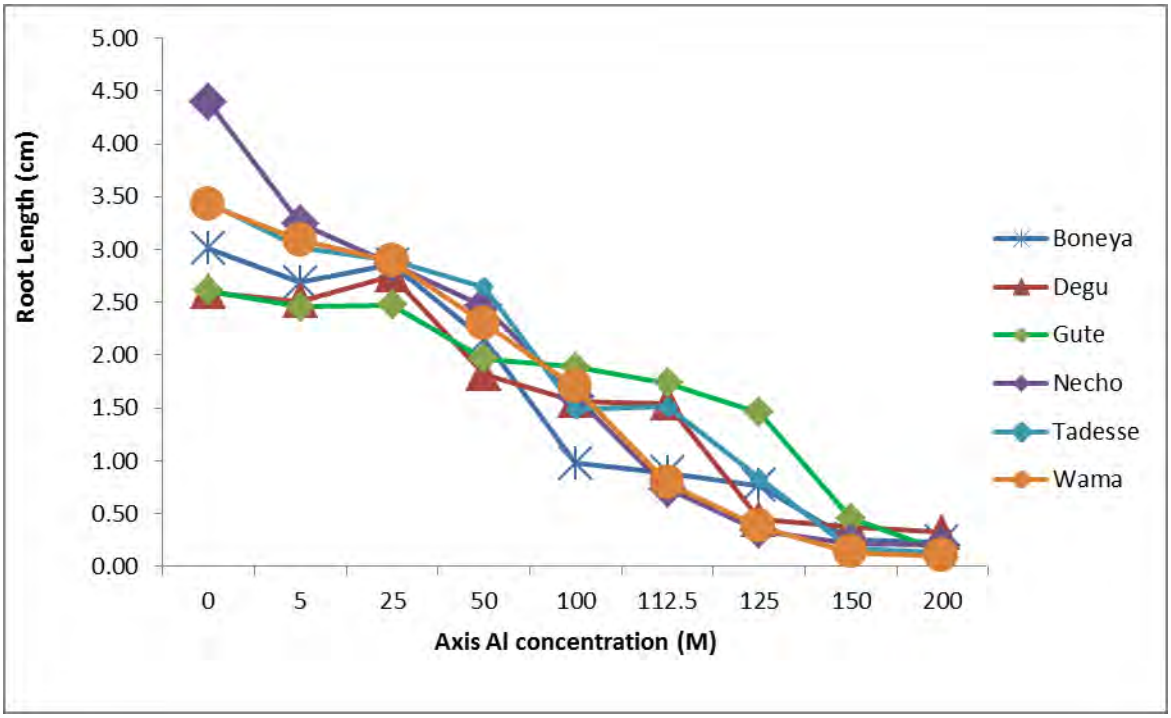


Figure 6. Diagram showing average root length of six finger millet varieties grown in nutrient solution culture for eight days with varying Al concentration starting from 0 µm to 200 µl

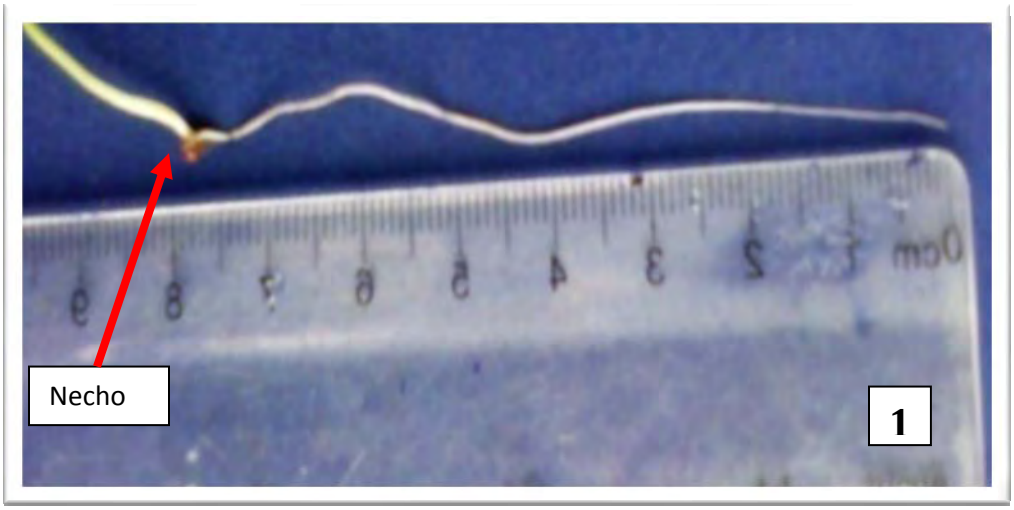


Figure 7. Sample pictures of 8-day old seedlings showing root length difference between different varieties grown at different Al^{3+} concentrations (1=control; 0µM and 2= treated; 112.5µM).

Table 5. Mean Root Length on six improved finger millet varieties grown under nutrient solution culture with varying Al concentration

Varieties	0 µM	25 µM	50 µM	100 µM	112.5 µM	125 µM	150 µM	200 µM
Boneya	3.01±0.19^{a}	2.24±0.11^{a}	2.14±0.29^{a}	0.98±0.29^{a}	0.89±0.16^{a}	0.77±0.14^{a}	0.26±0.08^{a}	0.23+0.08^{a}
Degu	2.60±0.13^{a}	2.31±0.15^{a}	1.82±0.25^{a}	1.57±0.22^{b}	1.54±0.31^{b}	1.46±0.32^{b}	0.38±0.09^{a}	0.32±0.08^{a}
Gute	1.88±0.15^{a}	2.03+0.08^{a}	1.97±0.17^{a}	1.89±0.18^{b}	1.53+0.33^{b}	0.44±0.16^{c}	0.16±0.01^{a}	0.14±0.02^{a}
Necho	4.18±0.33^{a}	2.82±0.12^{a}	2.47±0.19^{b}	1.61±0.16^{c}	0.73+0.29^{c}	0.34±0.10^{d}	0.22±0.05^{a}	0.20±0.05^{a}
Tadesse	3.44±0.18^{a}	2.67±0.12^{a}	2.64±0.25^{c}	1.49±0.07^{d}	1.51±0.29^{d}	0.83+0.16^{f}	0.17±0.02^{a}	0.13+0.01^{a}
Wama	3.42±0.27^{a}	2.69±0.22^{a}	2.30±0.30^{a}	1.70±0.12^{f}	0.79±0.25^{f}	0.38±0.17^{g}	0.13+0.01^{a}	0.10±0.00^{a}

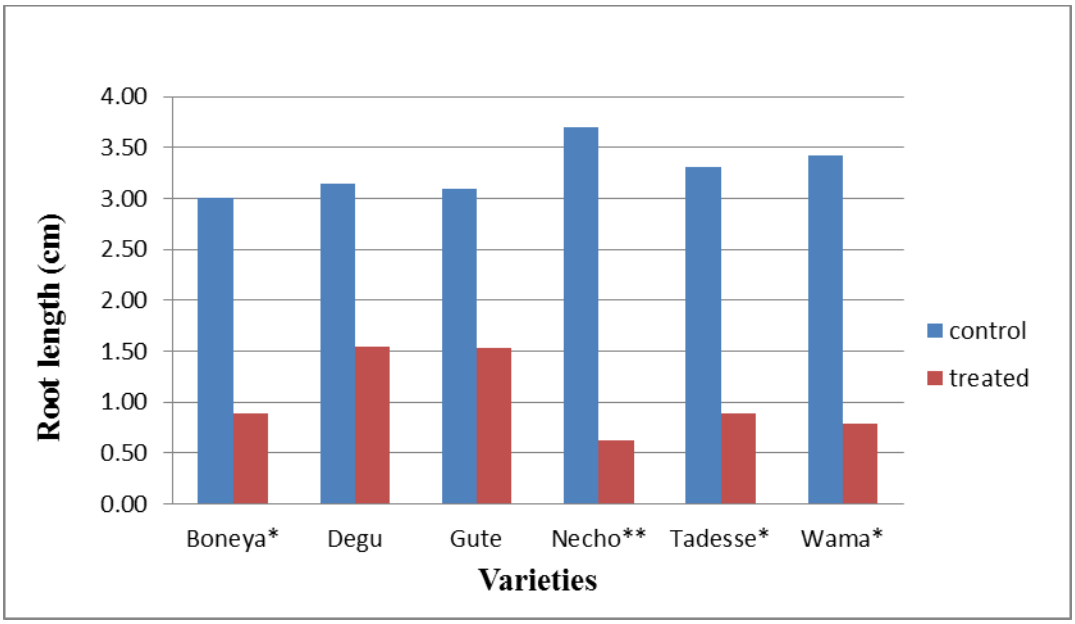
Different letters indicate significant differences among the treatments at 5 % level of significance based on the Tukey (HSD) test in each row. Means are followed by ± S.E. (standard error)

Analysis of variance revealed non-significant ($P < 0.05$) difference between finger millet varieties at 0, 5, 25, 150 and 200 μM of Al^{3+}concentration. However, 50, 100, 112.5 and 125 μM Al^{3+} concentration showed significant Al induced stress between the varieties, respectively, at $p < 0.05$ and $p < 0.01$ (Table 6). Therefore, 112.5 μM Al^{3+}concentration was selected as optimum concentration for extensive screening activities due to its multiple advantages. Firstly, it allows distinguishing of the various tolerance classes (susceptible, tolerant, and intermediate) at the highest accuracy level (i.e. $p < 0.01$ unlike the lower concentration levels). Secondly, its usage ensures high statistical accuracy with minimum cost as compared with the 150 μM Al^{3+}. At Al^{3+} concentration of 150 μM and above, the growth of roots of all the varieties were greatly hampered to the extent that there was nearly no difference among them.

Table 6. ANOVA on root length of six finger millet varieties grown in nutrient solution culture with varying Al^{3+} concentration

Al Concentrations	source of variation	SS	MS	df
0.00 µM	Between Groups	0.969	0.194^{NS}	5
	Within Groups	1.061	0.088	12
	Total	2.031		17
5.00 µM	Between Groups	0.693	0.139^{NS}	5
	Within Groups	0.936	0.078	12
	Total	1.629		17
25.00 µM	Between Groups	0.765	0.153^{NS}	5
	Within Groups	0.911	0.076	12
	Total	1.676		17
50.00 µM	Between Groups	1.407	0.281*	5
	Within Groups	1.042	0.087	12
	Total	2.449		17
100.00 µM	Between Groups	1.415	0.283*	5
	Within Groups	0.881	0.073	12
	Total	2.296		17
112.50 µM	Between Groups	2.34	0.468**	5
	Within Groups	1.004	0.084	12
	Total	3.343		17
125.00 µM	Between Groups	2.666	0.533**	5
	Within Groups	0.807	0.067	12
	Total	3.473		17
150.00 µM	Between Groups	0.122	0.024^{NS}	5
	Within Groups	0.181	0.015	12
	Total	0.303		17
200.00 µM	Between Groups	0.099	0.02^{NS}	5
	Within Groups	0.17	0.014	12
	Total	0.269		17

*NS=non-significant; * significant at p<0.05; ** significant at p<0.01*



** Significant at p<0.05; ** significant at p<0.01(using Tukey test), treated at 112.5 µM Al concentration and control= treatment without* Al^{3+}

Figure 8. Root Length of six finger millet varieties grown in nutrient solutions culture for eight days under 112.5 µM and 0 µM Al^{3+} concentration.

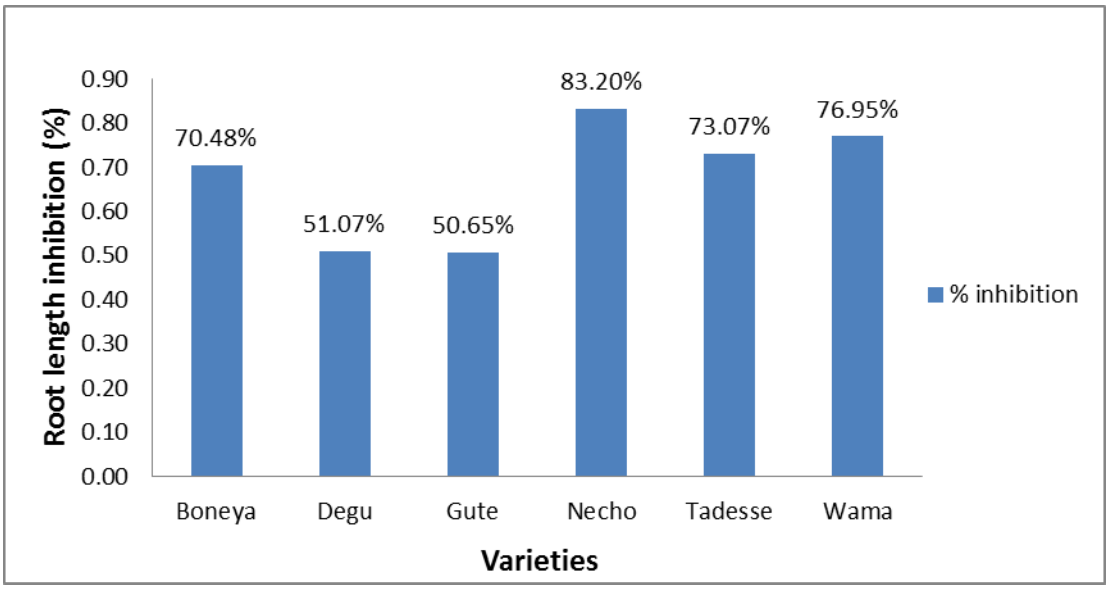


Figure 9. Root growth inhibition (%), of the six finger millet varieties grown in nutrient solution culture at 112.5 µM and 0 µM Al concentrations.

5.1.2. Screening of finger millet accession for Al^{3+} trait response

At this preliminary level, 288 landrace accessions were screened in six successive batches at the optimum Al^{3+} screening concentration identified in the study, 112.50 µM. Two varieties viz Gute as tolerant and Necho as susceptible were included in each batch of screening as standard checks. ANOVA of all the batches showed significant ($p < 0.001$) differences between the accessions evaluated (Table 7).

A total of 50 accessions were screened in each batches including the two standards. The average root length screened in Batch one varied from 0.20 cm to 2.30 cm, while the standards varieties Gute and Necho showed 2.11 cm and 1.54 cm, respectively. Furthermore, accessions screened in Batch two had an average root length ranging from 0.1 cm to 2.76 cm, while Gute and Necho had 1.92 cm and 0.12 cm, respectively. Similarly in Batch three it ranges from 0.11 cm to 2.61 cm, while Gute 2.30 cm and Necho 0.93 cm. Accessions screened in Batch four showed better average root growth as compared to the other batches and varied from 0.67 cm to 0.31 cm, while Gute and Necho produced 1.61cm and 0.31 cm, respectively. The root length in accessions of Batch five was between 0.32 cm to 3.00 cm, Gute and Necho were 1.94 cm and 1.16 cm, respectively. Likewise, the performance of accessions of batch six ranged between 0.17 cm to 0.27 cm, while Gute and Necho were 1.95 and 0.48, respectively details displayed in (Appendix 4 A- F).

From the screening result on 288 accessions along with the standard checks in six batches, only few of them were Al tolerant 75 (26.04 %), while 213 (73.95 %) of the accession showed medium to susceptible trait. From 288 accessions, 80 of them were

selected for further detail work of characterization under hydroponic and genetic diversity using ISSR molecular marker. Among the 80 accessions selected for in-depth analysis, 32 were from accessions which showed better root growth than the tolerant standard, 20 intermediate and 22 from susceptible category showed least RL than the susceptible standard.

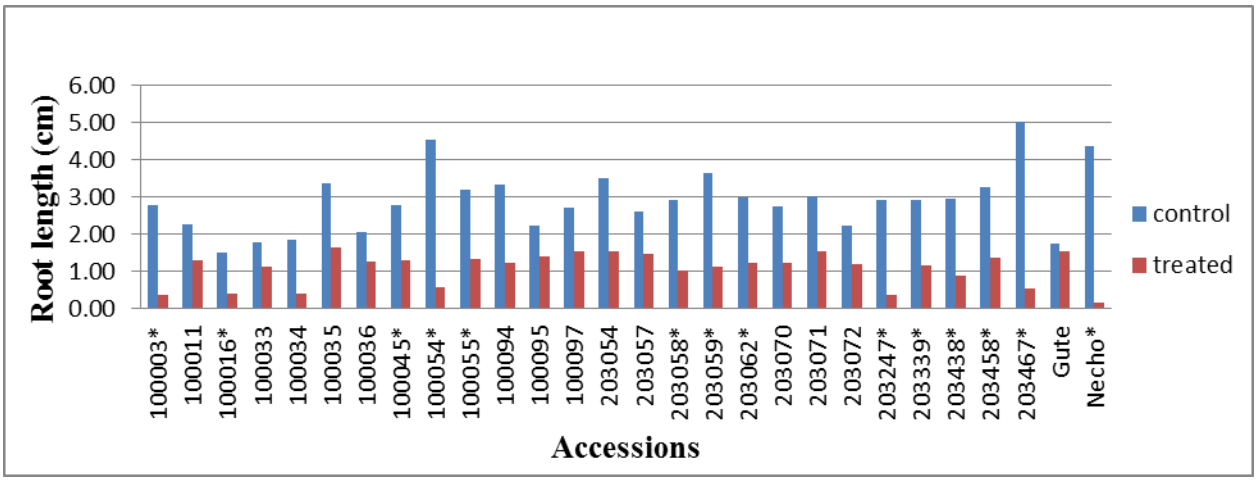
Table 7. ANOVA table of the six batches grown under hydroponics at 112.5 µM Al concentration

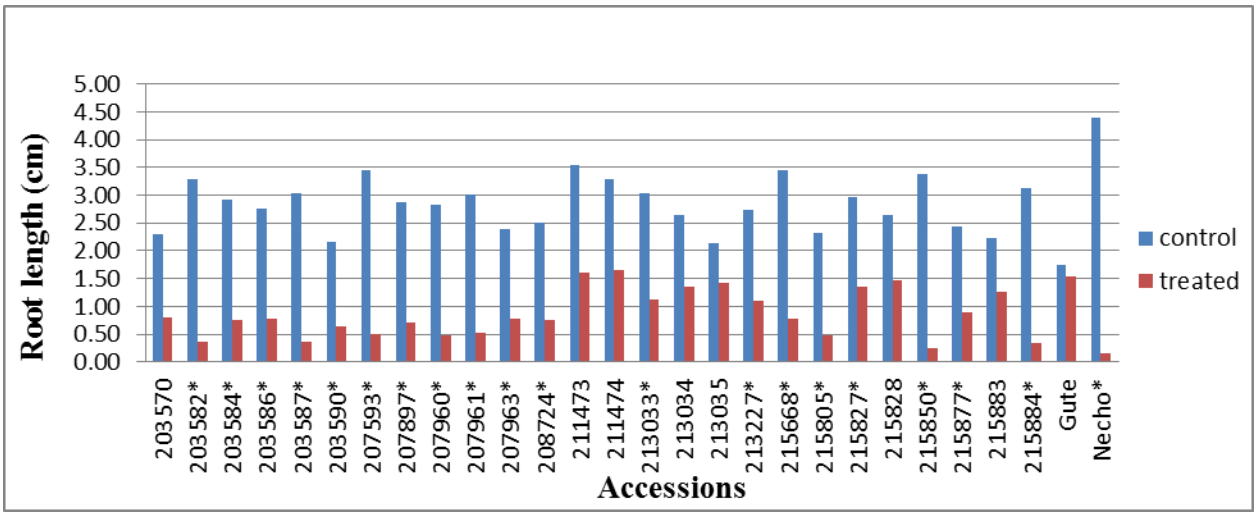
Batches	Sources of variation	SS	MS	d.f.
B1	Between Groups	51.301	1.047*	49
	Within Groups	44.204	0.442	100
	Total	95.505		149
B2	Between Groups	88.332	1.803*	49
	Within Groups	40.504	0.405	100
	Total	128.836		149
B3	Between Groups	62.177	1.269*	49
	Within Groups	19.081	0.191	100
	Total	81.258		149
B4	Between Groups	72.211	1.474*	49
	Within Groups	50.372	0.504	100
	Total	122.583		149
B5	Between Groups	68.062	1.389*	49
	Within Groups	27.235	0.272	100
	Total	95.297		149
B6	Between Groups	63.298	1.292*	49
	Within Groups	25.03	0.25	100
	Total	88.329		149

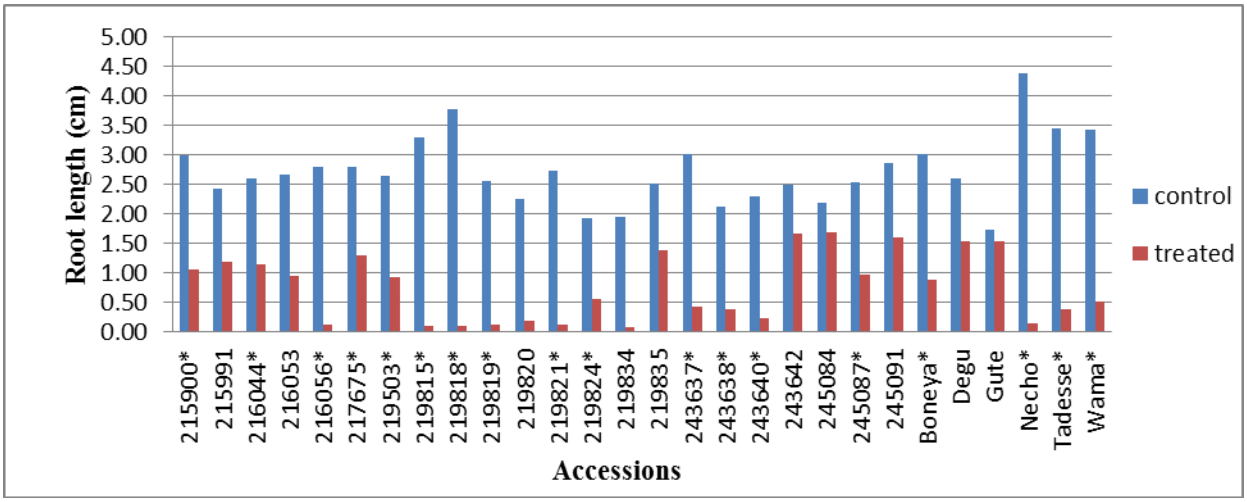
*Key: NS=non-significant; * significant at p<0.05; B1-B6= Batches 1 to 6, SS= Sum of Squares, MS= Mean squares, d.f. = Degree of freedom.*

5.1.3. Characterizing aluminium tolerance in Ethiopian finger millet

A total of 74 landrace accessions were selected based on their performance under the preliminary Al screening experiment along with six improved varieties for further evaluation with and without Al conditions. All accessions showed significant Al induced stress among accessions in root and fresh weight measurement (Figure 10 and 12). In root length 63 accessions (78.75 %) out of 80 accessions showed significant Al induced stress and 23 (28.75 %) in fresh weight, while no distinct and visible symptom of aluminum toxicity were observed in the shoot of finger millet genotypes (Figure 11).

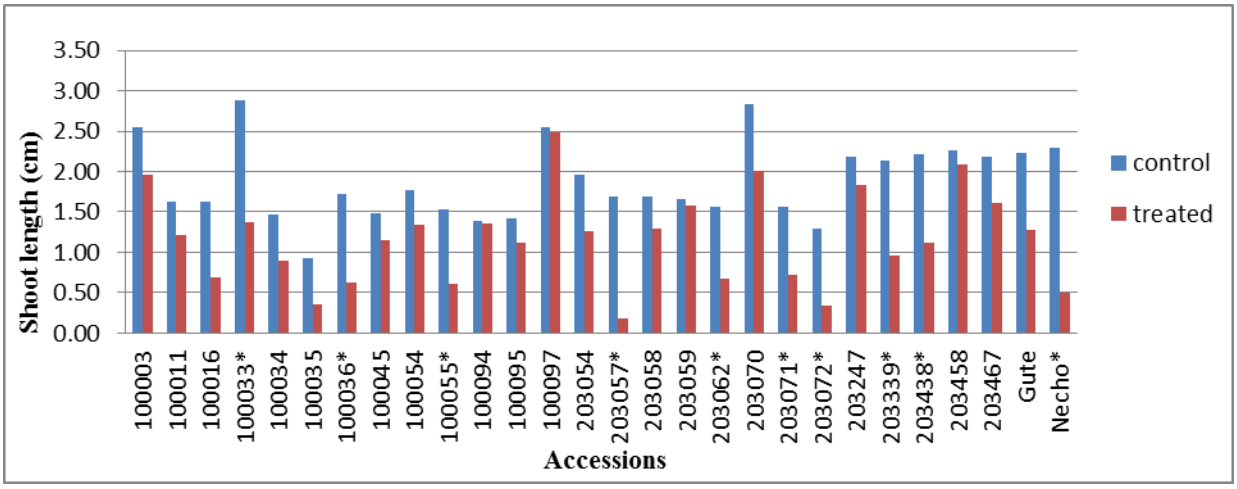


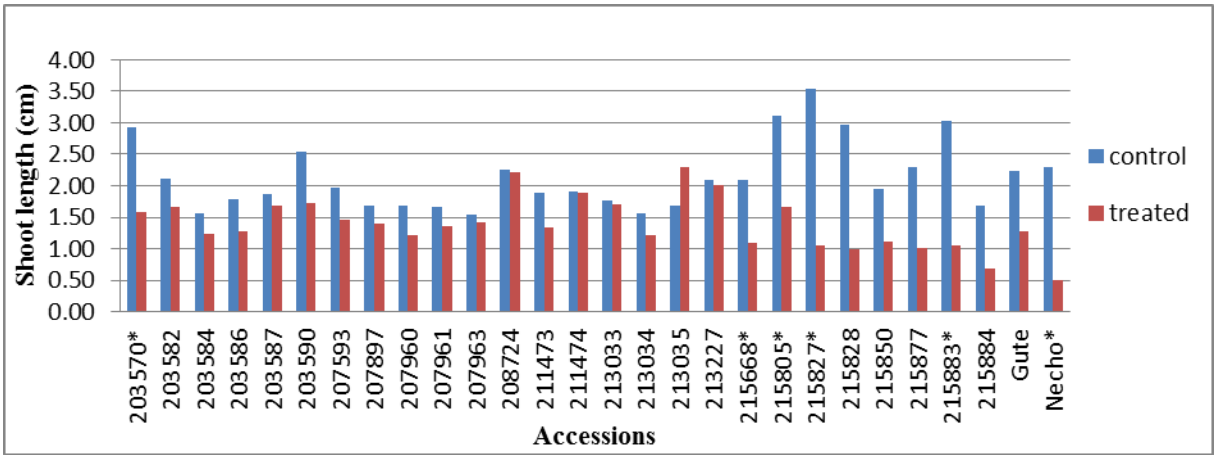


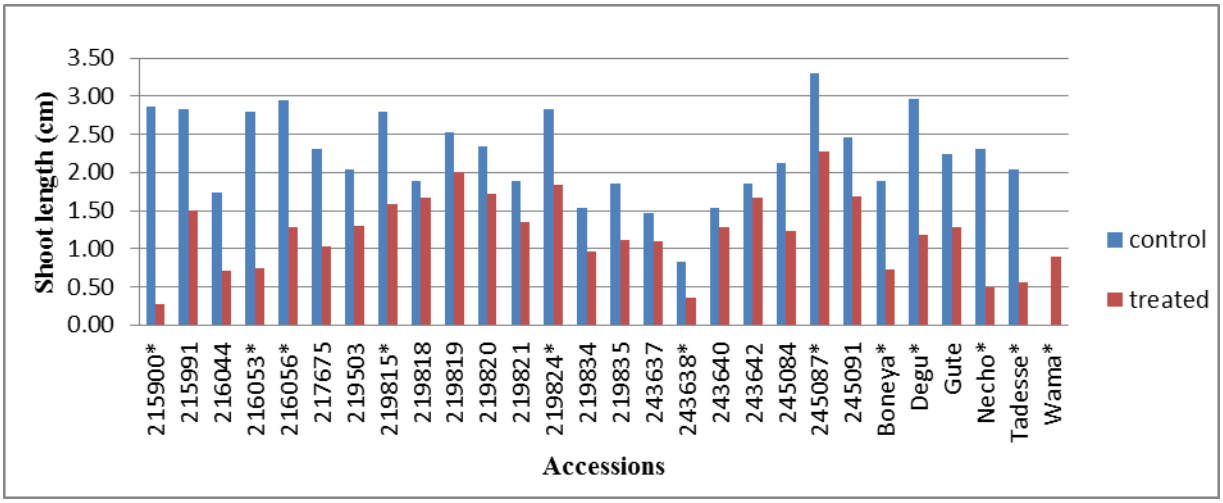


** Significant at p<0.05 Tekuy HSD test*

Figure 10. Effect of Al toxicity on root length (cm) on 80 accession grown under treated; 112.5µM and control; 0 µM Al3+ under hydroponics. *Key: (A= 1-2; B = 27-52; C= 53-80)*

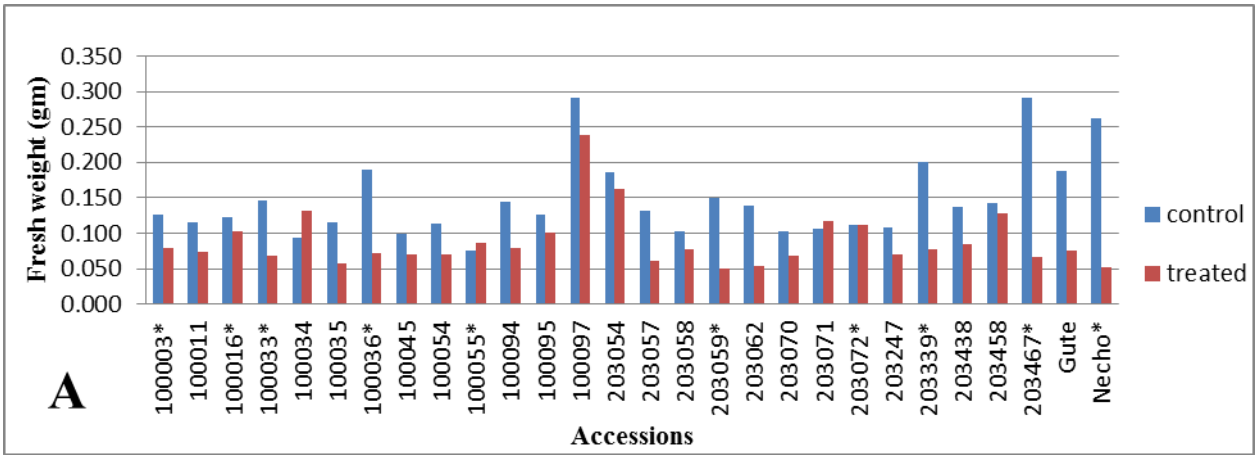

Shoot length (cm)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
100003
100011
100016
100033*
100034
100035
100036*
100045
100054
100055*
100094
100095
100097
203054
203057*
203058
203059
203062*
203070
203071*
203072*
203247
203339*
203438*
203458
203467
Gute
Necho*
Accessions
control
treated

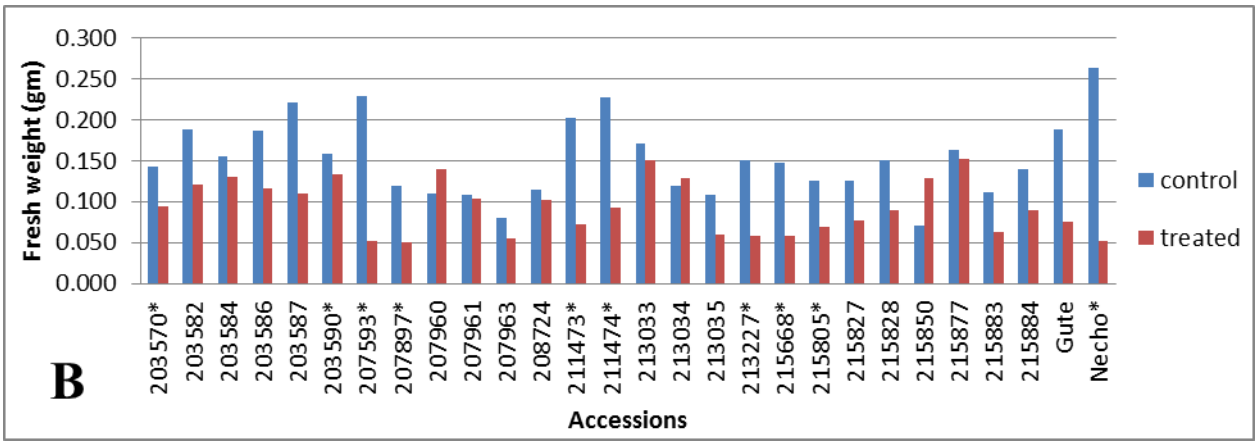

Shoot length (cm)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
203570*
203582
203584
203586
203587
203590
207593
207897
207960
207961
207963
208724
211473
211474
213033
213034
213035
213227
215668*
215805*
215827*
215828
215850
215877
215883*
215884
Gute
Necho*
Accessions
control
treated

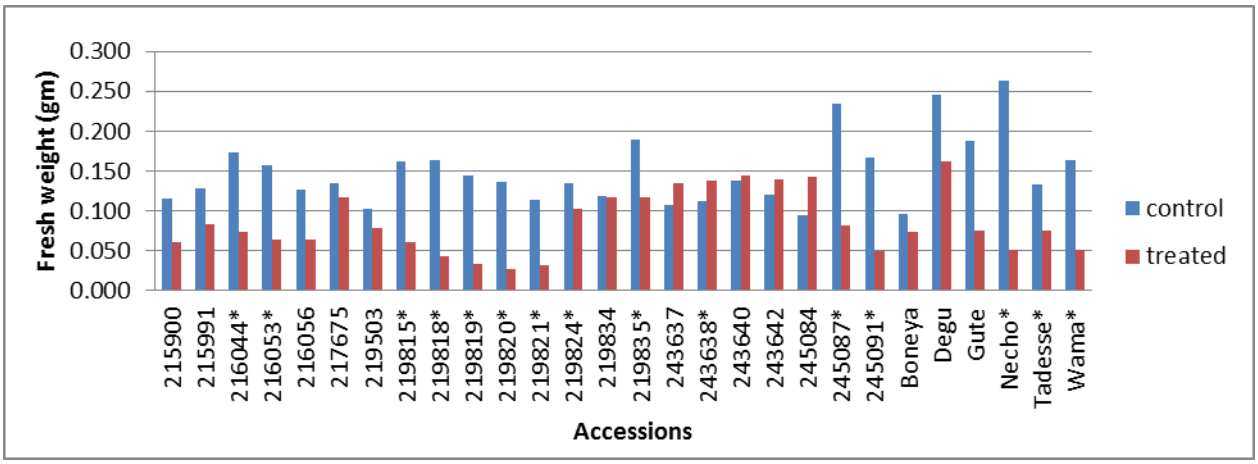


** Significant at p<0.05 Tekuy HSD test*

Figure 11. Effect of Al toxicity on shoot length (cm) on 80 accession grown under treated; 112.5µM and control; 0 µM Al3+ under hydroponics. *Key: (A= 1-2; B = 27-52; C= 53-80)*







** Significant at p<0.05 Tekuy HSD test*

Figure 12. Effect of Al toxicity on fresh weight (gm) on 80 accession grown under treated; 112.5µM and control; 0 µM Al3+ under hydroponics. *Key: (A= 1-2; B = 27-52; C= 53-80)*

5.1.4. Al tolerance in finger millet genotype as revealed by root tolerance index

Genotypes with tolerance indices larger than Gute variety considered as tolerant, whereas tolerance index less than Necho variety considered as very sensitive, while values between the Gute and Necho varieties were considered as intermediate. Accessions collected from Western Ethiopia, Gojam (100033and 213035), Awi, (100036and 243642) and Wellega (100095, 100097, and 245084) were best seven tolerant, while accessions (219815, 219818, 219819, 219820 and 219821) collected from Northern Ethiopia were showed least tolerance level. Accordingly, 21 genotypes classified as tolerant, 35 genotypes as susceptible and 24 as intermediate (Table 8).

Table 8. Root Tolerance Index (RTI) and classification of 80 accessions,

Acc.	0 µM	112.5M	RTI	class	Acc.	0 µM	112.5M	RTI	class	Acc.	0 µM	112.5 M	RTI	class
100003	2.978	0.357	0.12	S	203582	3.3	0.369	0.112	S	216044	2.6	1.144	0.44	I
100011	2.256	1.289	0.571	T	203584	2.933	0.744	0.254	S	216053	2.667	0.944	0.354	I
100016	1.522	0.389	0.255	S	203586	2.767	0.767	0.277	S	216056	2.8	0.133	0.048	S
100033	1.789	1.144	0.64	T	203587	3.044	0.367	0.12	S	217675	2.8	1.311	0.468	I
100034	2.867	0.411	0.143	S	203590	2.156	0.644	0.299	S	219503	2.644	0.922	0.349	I
100035	3.078	1.656	0.538	T	207593	3.456	0.511	0.148	S	219815	3.289	0.111	0.034	S
100036	2.067	1.267	0.613	T	207897	2.867	0.711	0.248	S	219818	3.978	0.104	0.026	S
100045	2.8	1.311	0.468	I	207960	2.833	0.468	0.165	S	219819	3.567	0.133	0.037	S
100054	4.556	0.578	0.127	S	207961	3.011	0.522	0.173	S	219820	3.556	0.189	0.053	S
100055	3.2	1.333	0.417	I	207963	2.389	0.767	0.321	I	219821	2.744	0.133	0.049	S
100094	3.333	1.222	0.367	S	208724	2.511	0.756	0.301	I	219824	3.933	0.567	0.144	S
100095	2.233	1.422	0.636	T	211473	3.556	1.6	0.45	I	219834	1.944	0.089	0.046	S
100097	2.222	1.544	0.693	T	211474	3.289	1.656	0.503	T	219835	2.522	1.389	0.551	T
203054	3.511	1.556	0.443	I	213033	3.044	1.133	0.372	I	243637	3.022	0.422	0.14	S
203057	2.611	1.467	0.562	T	213034	2.656	1.344	0.506	T	243638	2.333	0.389	0.167	S
203058	2.933	1.044	0.356	I	213035	2.133	1.422	0.667	T	243640	2.3	0.244	0.106	S
203059	3.633	1.144	0.315	I	213227	2.744	1.1	0.401	I	243642	2.5	1.667	0.667	T
203062	2.989	1.233	0.413	I	215668	3.456	0.767	0.222	S	245084	2.189	1.689	0.772	T
203070	2.744	1.222	0.445	I	215805	2.333	0.467	0.2	S	245087	2.533	0.978	0.386	I
203071	3.022	1.556	0.515	T	215827	2.967	1.344	0.453	I	245091	2.867	1.611	0.562	T
203072	2.222	1.2	0.54	T	215828	2.656	1.467	0.552	T	Boneya	3.011	0.878	0.292	I
203247	2.933	0.378	0.129	S	215850	3.378	0.256	0.076	S	Degu	2.6	1.54	0.592	T
203339	2.933	1.178	0.402	I	215877	2.444	0.889	0.364	I	Gute	2.744	1.533	0.559	T
203438	2.967	0.9	0.303	I	215883	2.233	1.267	0.567	T	Necho	4.389	0.156	0.035	S
203458	3.267	1.378	0.422	I	215884	3.122	0.344	0.11	S	Tadesse	3.444	0.4	0.116	S
203467	5.022	0.556	0.111	S	215900	2.989	1.056	0.353	I	Wama	3.422	0.522	0.153	S
203570	2.289	0.789	0.345	I	215991	2.422	1.2	0.495	I					

Key: RTI = root tolerance Index, S = susceptible, T = Tolerant, I = intermediate

5.2. Genetic diversity study

5.2.1. ISSR primers and their banding patterns

Among the seven primers tested initially, only six of them, with three di-nucleotides, one tri-nucleotide, one tetra-nucleotide and one penta-nucleotide repeat motif, which produce relatively clear, reproducible and polymorphic bands, were selected as informative markers. One primer 887 $(GGAT)_4$ failed to give amplification product and was excluded from the study. Number of scorable bands for individual primer ranged from 4 to 10 while the average number of bands per primers was 7.5. The highest number of bands was amplified with primer 880, while the lowest number was amplified with 811. The fragment size amplified with these primers was in the range of 200 to 1000 base pair (Table 9).

Table 9. List of ISSR primers used showing their repeat motifs, amplification pattern and number of scorable bands (NSB)

No.	Primers	Sequence	Ta	Repeat motif	Amplification pattern	NSB
1	810	(GA)8C	45	Di-nucleotide	Reproducible, polymorphic	7
2	811	(GA)8T	48	Di-nucleotide	Reproducible, polymorphic	4
3	848	(CA)6RG	48	Di-nucleotide	Reproducible, polymorphic	5
4	866	(CTC)6	55	Tri-nucleotide	Reproducible, polymorphic	10
5	873	(GACA)4	45	Tetra-nucleotide	Reproducible, polymorphic	9
6	880	(GGAGA)3	45	Penta-nucleotide	Reproducible, polymorphic	10

Single-letter abbreviations for mixed base positions: R = (A, G), NSB: number of scorable bands

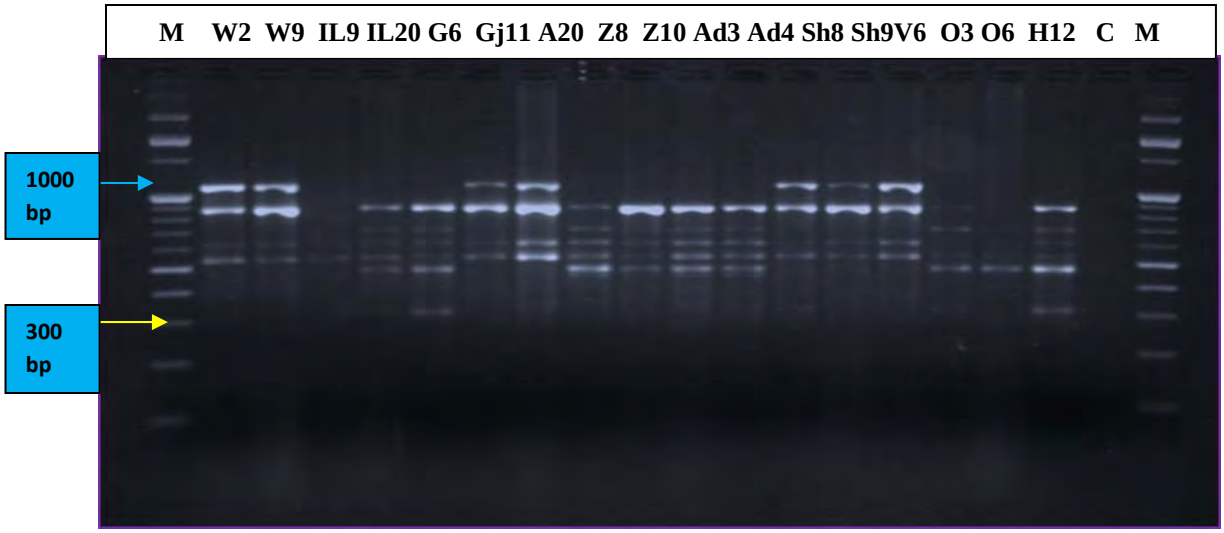


Figure 13. Banding pattern of primer 848 in finger millet accessions. M represents a 100 bp DNA ladder as a standard molecular marker; W = Wellega, IL= Ilu Ababora, G= Gondar, Gj= Gojam, A= Awi, Z= Zimbabwe, Ad= Adwa, Sh= Shire, O= Omo, H= Hadiya and V= Variety, while the numbers associated with these letters represent accessions, C represents control (a PCR reaction mix without template DNA).

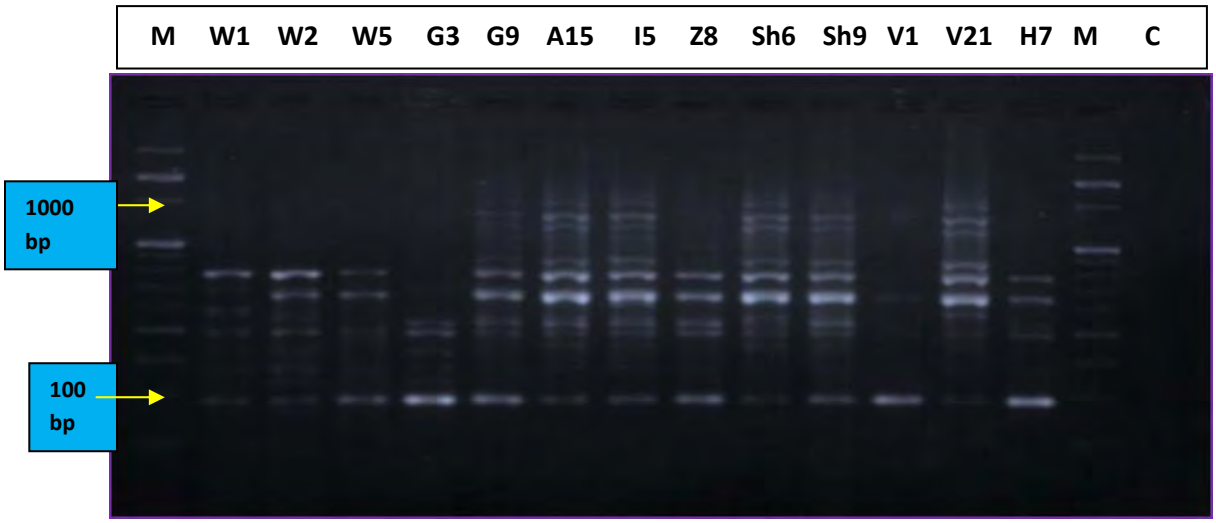


Figure 14. Banding pattern of primer 880 in finger millet accessions. M represents a 100 bp DNA ladder as a standard molecular marker; W = Wellega, IL= Ilu Ababora, G= Gondar, Gj= Gojam, A= Awi, Z= Zimbabwe, Ad= Adwa, Sh= Shire, O= Omo, H= Hadiya and V= Variety, while the numbers associated with these letters represent accessions, C represents control (a PCR reaction mix without template DNA).

5.2.2. ISSR based diversity in finger millet

PCR amplification with selected six ISSR-primers generated a total of 45 scorable bands and was detected two to nine number of polymorphic loci in 80 accessions of *E. coracana* species. Out of the 45 bands 35 fragments were polymorphic which accounts for 77.78 % and with 10 loci being monomorphic (Table 10). Among the polymorphic loci, maximum percentage of polymorphic loci (90 %) was generated by ISSR-880 primer and followed by ISSR-873 which accounts 88.89 %, while ISSR-primer 811 and 866 generated the least percentage of polymorphic loci which revealed 50 % and 70 %, respectively. Furthermore, penta-nucleotide primer (ISSR-880) also generated high number of percent polymorphism within population as compared to the di-, tri-, and tetra-nucleotide, showing that the penta-nucleotides interestingly contributed more polymorphism.

The overall gene diversity (h) by the six primers was 0.28, while Shannon's information index was 0.41. The highest gene diversity (h) 0.37 and Shannon's information index (0.54) were obtained from ISSR- primer 880, while ISSR-811 showed the least gene diversity of (0.17) and Shannon's information index of (0.25) (Table 10).

Table 10. Number of polymorphic loci (NPL), percentage of polymorphic loci (PPL), gene diversity (h) and Shannon information index (I) calculated for each primer

No.	Primer	NPL	PPL	H ± SD	I±SD
1	810	6	77.78 %	0.31 ± 0.18	0.46 ± 0.25
2	811	2	50.00 %	0.17 ± 0.22	0.25 ± 0.32
3	848	4	80.00 %	0.29 ± 0.18	0.43 ± 0.26
4	866	7	70.00 %	0.18 ± 0.19	0.29 ± 0.27
5	873	8	88.89 %	0.30 ± 0.17	0.46 ± 0.24
6	880	9	90.00 %	0.37 ± 0.16	0.54 ± 0.22
	Overall	35	77.78 %	0.28 ± 0.19	0.41 ± 0.26

NPL = Number of polymorphic Loci; PPL = Percent of polymorphic Loci; h = gene diversity; I = Shannon's information index and SD= standard deviation

Based on aggregated data from all the six ISSR-primers, the highest level of polymorphism was obtained from accessions of Gojam population, followed by Wellega, Awi, Zimbabwe, and Gondar, with 55.56 %, 46.67 %. 44.44 %, 44.44 %, and 40.00%, respectively, while the least percent polymorphism was detected in accessions collected from Omo, Ilu Ababora and Shire, with 13.33 %, 15.56 % and 17.78 %, respectively. In addition to this, when populations of finger millet were analyzed by grouping with their geographical region, various levels of polymorphism were detected. Accordingly, populations grouped under Amhara region showed highest percent of polymorphism, followed by exotic, Oromia, Tigray and SNNP, with 60.00 %, 51.11 %, 46.67 %, 28.89 % and 24.44 %, respectively (Table 11).

The gene diversity (h) indexes of the eleven populations with overall primers were calculated and the values ranged from 0.05 for Omo to 0.19 to Wellega, Gojam, Awi, and Zimbabwe population and with 0.28 in overall. Besides this, when populations were grouped in to geographical region gene diversity (h) was highest in Amhara (0.29) and least in SNNP (0.10). In addition, the same diversity patterns were also observed for Shannon's information index whereby the least was obtained from Omo (0.07), while highest diversity where showed by populations from Gojam (0.29) and 0.42 at species level (overall). In case of geographical region based analysis, Amhara showed the highest variability (0.35) while the least was obtained from SNNP (0.15) (Table 11).

Table 11. The Number of polymorphic loci (NPL), Percent polymorphic loci (PPL), gene diversity (h) and Shannon‘s information (I) Index, using data generated from all the six primers. The analysis was carried out at population level and grouping made based on geographic origin

Populations	**NPL**	**PPL**	**h±SD**	**I±SD**
Wellega	21	46.67 %	0.19 ± 0.21	0.28 ± 0.31
Ilu Ababora	7	15.56 %	0.06 ± 0.15	0.08 ± 0.21
Gondar	18	40.00 %	0.17 ± 0.21	0.24 ± 0.30
Gojam	25	55.56 %	0.19 ± 0.20	0.28 ± 0.29
Awi	20	44.44 %	0.19 ± 0.22	0.28 ± 0.31
Adwa	11	24.44 %	0.10 ± 0.19	0.15 ± 0.27
Shire	8	17.78 %	0.06 ± 0.14	0.09 ± 0.21
Omo	6	13.33 %	0.05 ± 0.14	0.07 ± 0.20
Hadiya	10	22.22 %	0.10 ± 0.19	0.14 ± 0.28
India	9	20.00 %	0.09 ± 0.18	0.12 ± 0.26
Zimbabwe	20	44.44 %	0.19 ± 0.23	0.28 ± 0.32
Overall	35	77.78 %	0.28 ± 0.19	0.41 ± 0.26
Groups				
Oromia	21	46.67 %	0.19 ± 0.21	0.28 ± 0.31
Amhara	27	60.00 %	0.24 ± 0.21	0.35 ± 0.30
Tigray	13	28.89 %	0.11 ± 0.19	0.17 ± 0.27
SNNP	11	24.44 %	0.10 ± 0.19	0.15 ± 0.28
Exotic	23	51.11 %	0.21 ± 0.22	0.30 ± 0.31
Overall	35	77.78 %	0.29 ± 0.19	0.43 ± 0.26

5.2.3. Analysis of Molecular Variance (AMOVA)

AMOVA was carried out using the overall 45-ISSR bands generated by *E. coracana* population with and without grouping (Table 12). AMOVA without grouping population revealed that higher percent of variation (58.54 %) is attributed to the within population variation while 41.45 % is due to the among population variation. On the other hand, of the total genetic diversity 5.88 % was attributed to populations within groups, 38.33 % to among groups and 55.79 % to differences within populations. The FST value showed a higher differentiation of (FST=0.44) among geographical regions than at population level (FST=0.41). There were highly significant genetic differences between the five groups as well as between the eleven populations of *E. coracana* (AMOVA; p<0.001). Both analytical approaches revealed higher within population variation as compared to among populations.

Table 12. Analysis of Molecular Variance (AMOVA) among and within eleven populations of *E. coracana*

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation	Fixation indices (FST)	P-value
Among populations	10	187.56	2.49	41.45	0.41	P<0.001
Within populations	63	204.28	3.52	58.54		P<0.001
total	73	391.84	6.01			
Among geographic groups	4	153.75	2.42	38.33		P<0.001
Among populations with in geographic groups	6	33.81	0.37	5.88	0.44	P<0.001
Within populations	63	204.28	3.52	55.79		P<0.001
total	73	391.84	6.31			

5.2.4. Clustering analysis

UPGMA and NJ analysis was carried out to construct dendrogram for the eleven population and 80 individuals based on 45 PCR bands amplified by six ISSR primers. UPGMA analysis of *E. coracana* populations revealed two major clusters (I and II) using Jaccard's similarity coefficient at 67 % similarity (Figure 15). Major cluster-I consisted of two sub-clusters (sub-cluster-I and sub- cluster-II) at 72 % similarity, major cluster-II also similarly consisted of two sub-clusters at 76 % similarity. Major cluster-I and Sub-cluster-I consisted of accessions collected from Wellega and Ilu Ababora with 89.1 % similarity, while Major cluster-I sub-cluster-II consisted of Awi, Gondar and Gojam accessions with 72 % similarity. Major cluster-II and sub-cluster-I consisted of accessions from India, Zimbabwe, Adwa and Shire and sub-cluster-II consisted of accessions from Hadiya and Omo at 88 % similarity.

The genetic similarity coefficients between pairs of populations indicated in (Table 13 and Figure 15) varied from 0.581 (between Hadiya and Gojam) to 0.905 (between Shire and Adwa). Besides this, individual based UPGMA clustering of an overall analysis showed strong clustering of individuals with respect to their population except few intermixed populations. The dendrogram constructed from UPGMA on individual based clearly identified five major clusters (I, II, III, IV and V) using Jaccard's similarity coefficient of around 81 % (Figure 16). The first cluster was composed of 13 accessions collected from Wellega and Ilu Ababora. The second major cluster consisted of 12 accessions which were collected from Wellega and Gondar. The third major cluster consisted of 8 accessions collected from Awi and Gojam. The forth major cluster consisted of 24 accessions collected from Adwa, Shire, India, Zimbabwe and improved

varieties and the last cluster consisted of 11 accessions from Omo and Hadiya (Figure 16). Most individuals from all populations tended to form their own cluster, while only few of the individuals were distributed all over the tree.

The dendrogram derived from NJ analysis of the whole ISSR data showed two distinct clusters (major cluster-I and major cluster-II). Major cluster I with two sub-clusters (sub-clusters I-I and I-II) and Major cluster II with two sub-clusters (sub-clusters II-I and II-II) (Figure 17). Major cluster II was dominated by accessions collected from Wellega, Ilu Ababora, and Gondar, while major cluster I consisted of mixed accessions.

Table 13. Pair wise Jaccard's similarity coefficient based comparisons among eleven populations of *E. coracana* collected from Ethiopia and exotic

Populations	Wellega	Ilu Ababora	Gonder	Gojam	Awi	India	Zimbabwe	Adwa	Shire	Omo	Hadiya
Wellega	1										
Ilu Ababora	0.891	1									
Gonder	0.703	0.763	1								
Gojam	0.718	0.716	0.718	1							
Awi	0.703	0.713	0.78	0.743	1						
India	0.661	0.658	0.715	0.682	0.723	1					
Zimbabwe	0.653	0.684	0.754	0.661	0.755	0.83	1				
Adwa	0.657	0.641	0.693	0.665	0.727	0.84	0.83	1			
Shire	0.672	0.67	0.707	0.695	0.725	0.85	0.82	0.905	1		
Omo	0.685	0.596	0.68	0.644	0.674	0.78	0.76	0.757	0.79	1	
Hadiya	0.598	0.591	0.682	0.581	0.663	0.78	0.76	0.745	0.749	0.88	1

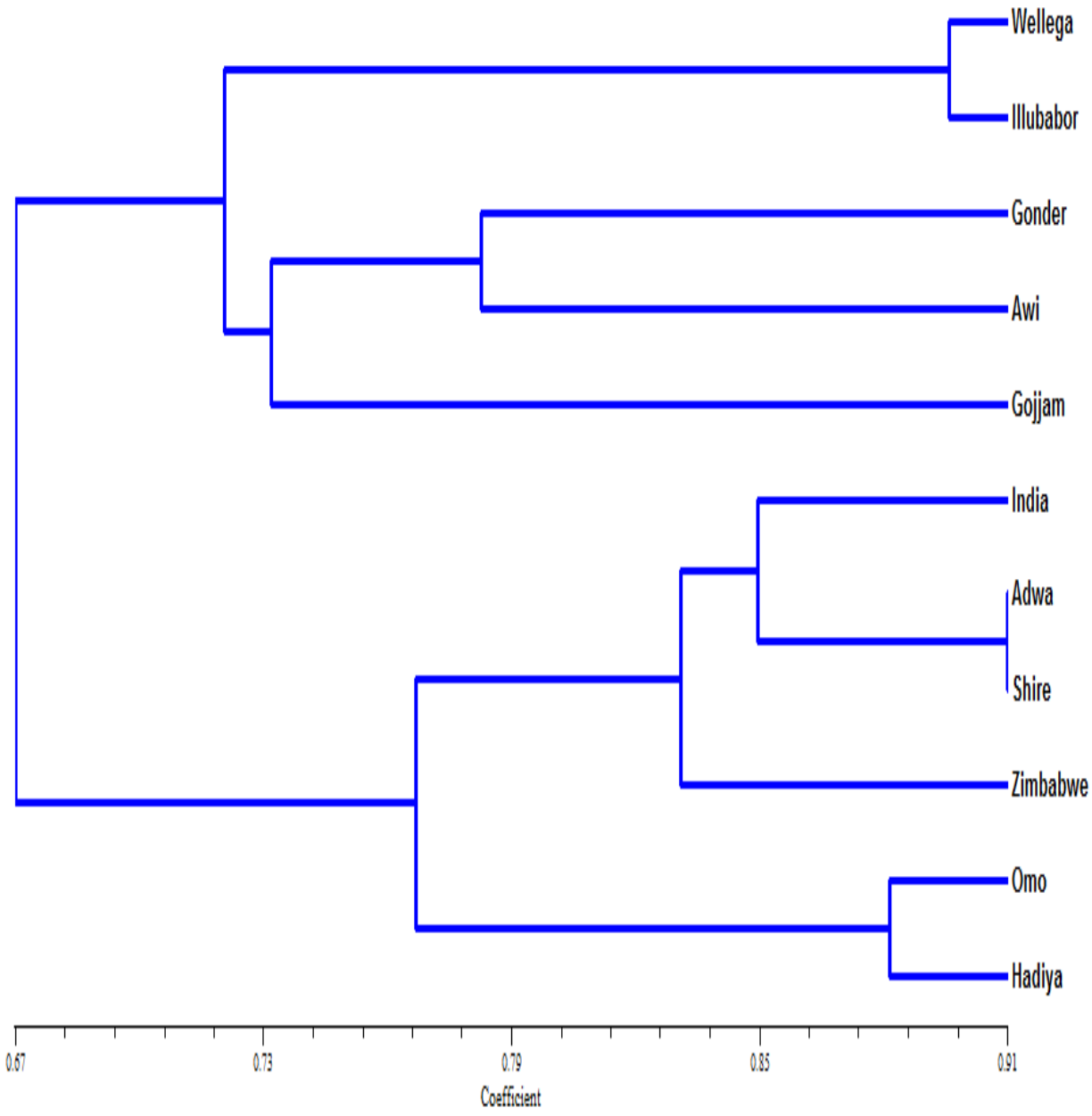
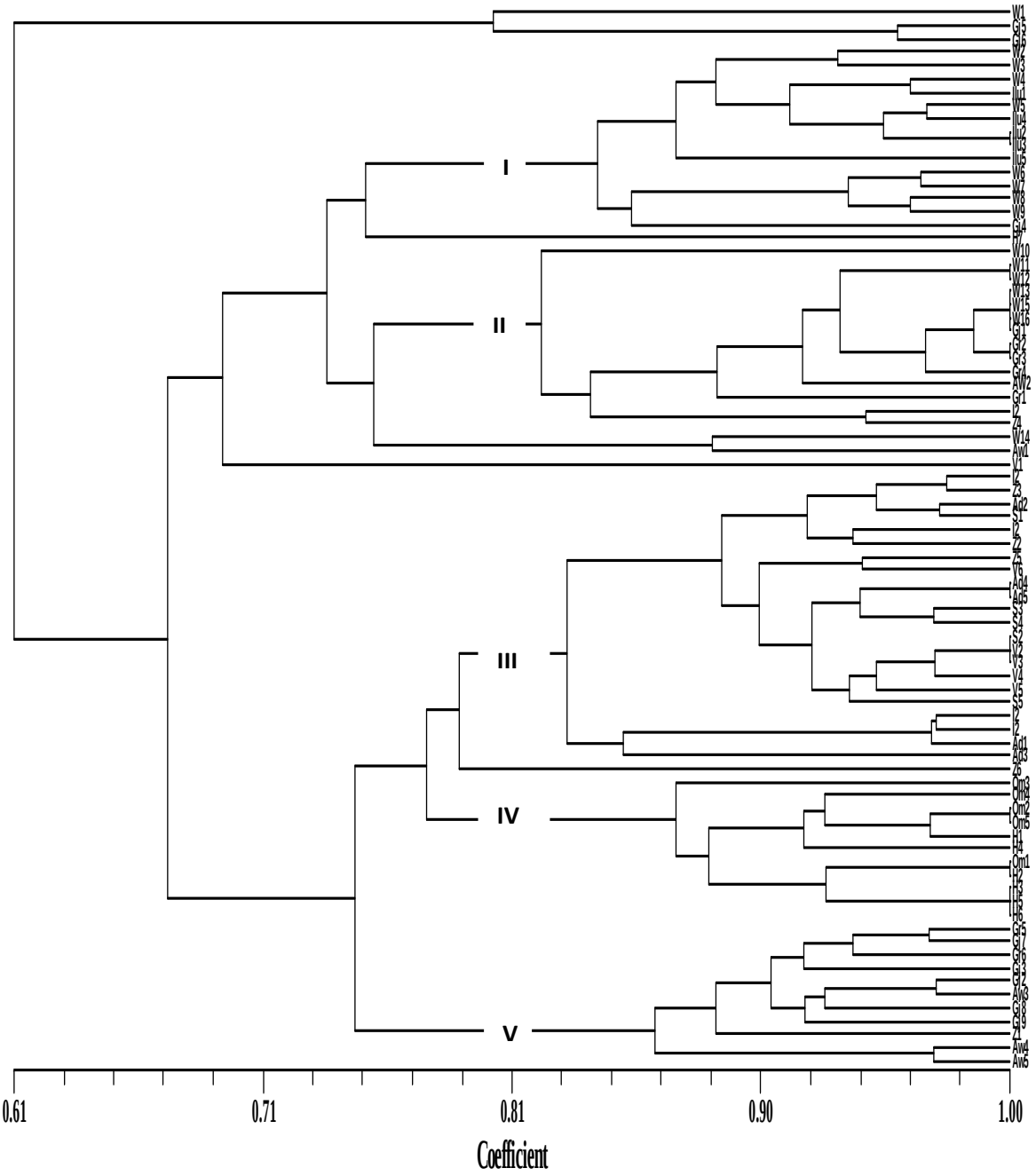


Figure 15. UPGMA based dendrogram for eleven populations of *E. coracana* collected from different regions of Ethiopia and exotic (India and Zimbabwe) using five ISSR primers.



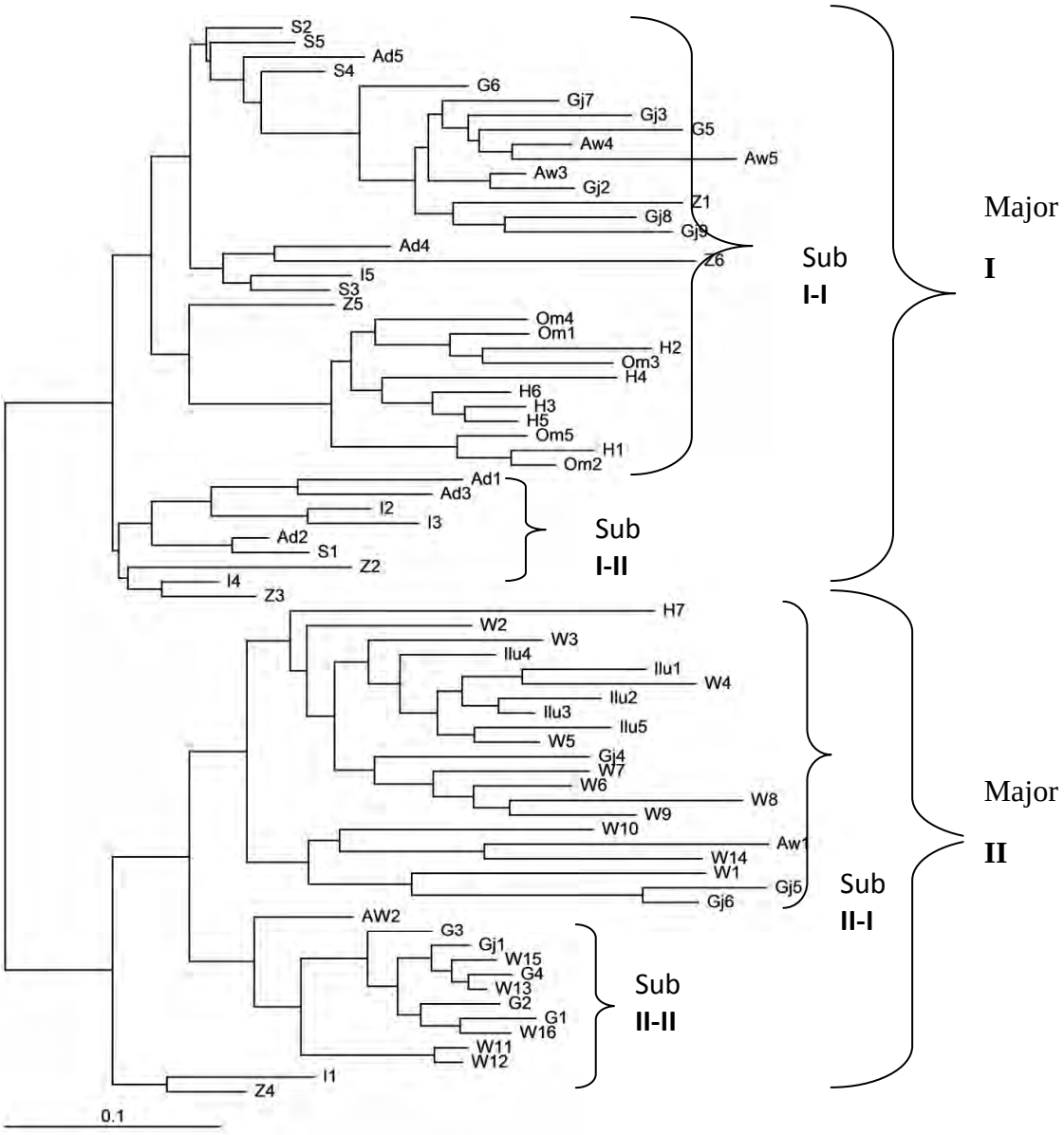
Key:

W1-16= Wellega Aw1-5=Awi S1-5= Shire Gj1-9= Gojam

Ilu1-5= Ilu Ababora I1-5= India O1-5= Omo Ad1-5=Adwa

G1-6= Gondar Z1-5= Zimbabwe H1-7= Hadiya

Figure 16. UPGMA based dendrogram for 80 individuals of *E. coracana* using six ISSR primers**.**



Key:

W1-16= Wellega	*Aw1-5=Awi*	*S1-5= Shire*	*Gj1-9= Gojam*
Ilu1-5= Ilu Ababora	*I1-5= India*	*O1-5= Omo*	*Ad1-5=Adwa*
G1-6= Gondar	*Z1-5= Zimbabwe*	*H1-7= Hadiya*	

Figure 17. Neighbor-joining analysis of 80 individuals based on 45 PCR bands amplified by five primers.

5.2.5. Principal coordinates analysis (PCOA)

Principal coordinate (PCO) analysis was carried out on all the ISSR data obtained from 80 accessions. The analysis was carried out using Past and Statistica soft ware's by employing Jaccard's coefficients of similarity. The first three coordinates of the PCO with Eigen values of 7.12, 4.52 and 3.38 and with percentage variation of 16.46 %, 10.45 % and 7.81 %, respectively, were used to show the grouping of all of the accessions using two and three coordinates (Figure 18 and 19). In the 3D plot most of the individual accessions that represent twelve different populations spread all over the plot and few individuals of Wellega and Gojam tended to form cluster. Using two coordinates (2D) (Figure 18) almost similar result was observed like that of UPGMA tree. Overall, no clear grouping was observed among accessions except some affinity of Wellega and Gojam which tended to make small sub-groups.

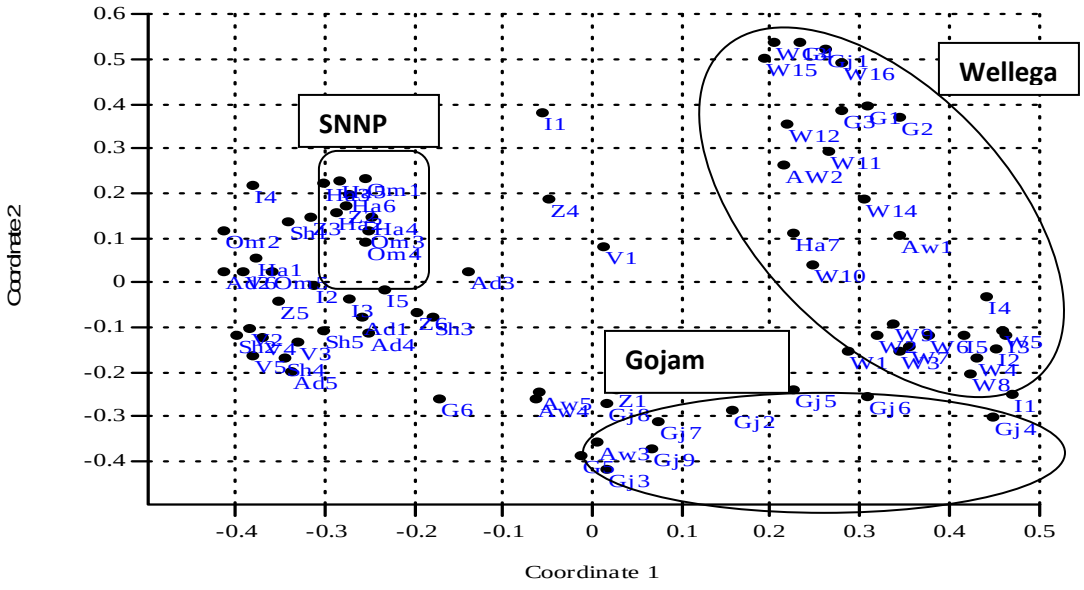


Figure 18. Two-dimensional plot obtained from principal coordinate analysis of 80 *E. coracana* accessions using six ISSR primers with Jaccard's coefficient similarity.

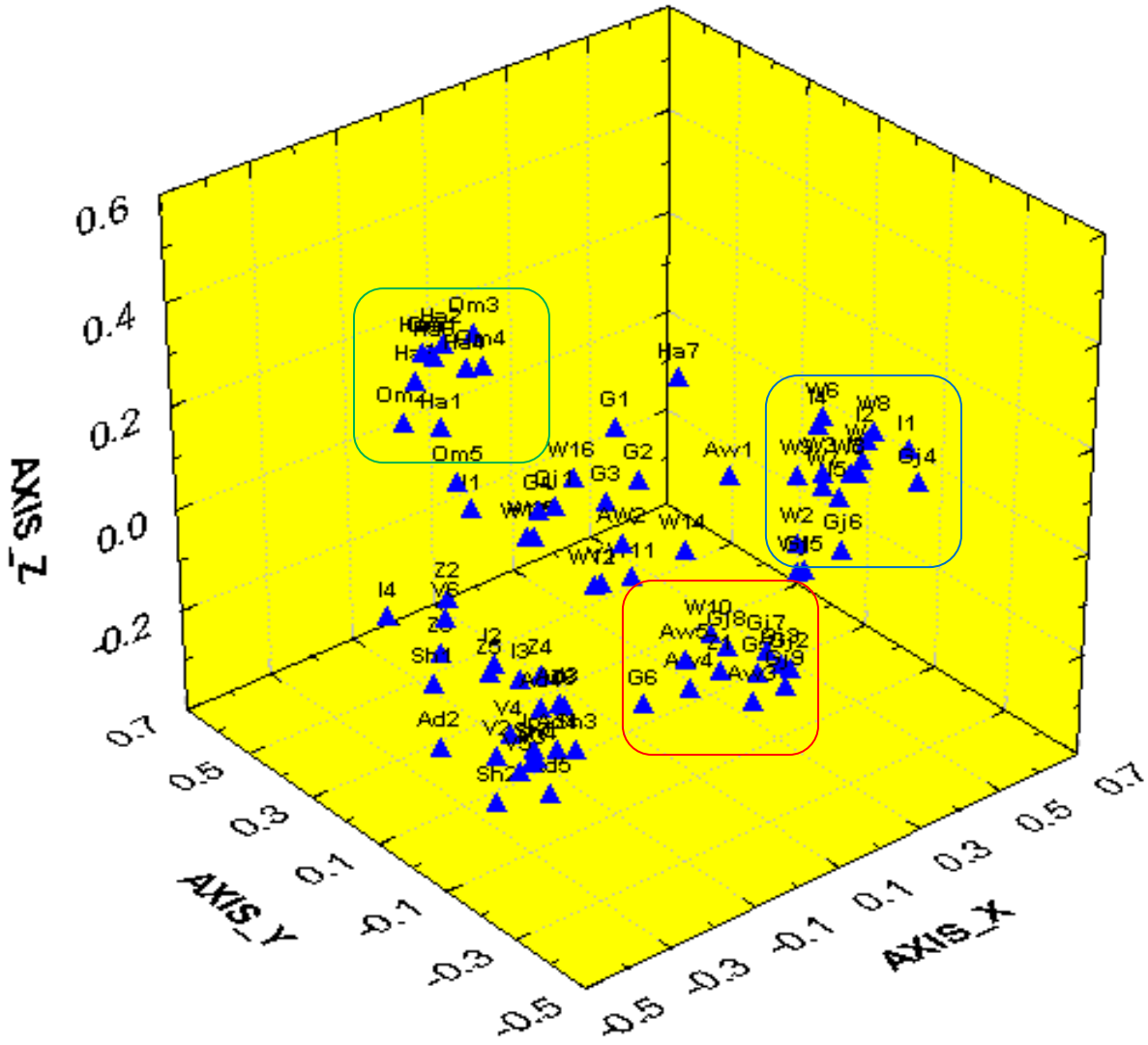


Figure 19. Three-dimensional (3D) plot obtained from principal coordinate analysis of 80 *E. coracana* accessions using six ISSR primers with Jaccard's coefficient similarity.

6. DISCUSSION

6.1. Aluminium tolerance among finger millet in Ethiopia

6.1.1. Variation among national finger millet variety in varying Al concentration

The present study employed nutrient solution culture to assess the optimum Al^{3+} concentration for screening and level of genotypes under optimum concentration among finger millet accessions from Ethiopia and few individuals from Zimbabwe and India. The different concentrations of Al^{3+} (5, 25, 50, 100, 112.5, 125, 150 and 200 µM) were used for optimization study in the six improved varieties viz Boneya, Degu, Gute, Necho, Tadesse and Wama. In low Al^{3+} concentration, proper discrimination of the finger millet germplasm cannot be done. The reason could be this low Al^{3+}concentration (less than 50 µM) was not strong enough to create stress condition at finger millet root. High Al^{3+} concentration at 125 µM, 150 µM and 200 µM make greater inhibition in all finger millet germplasms without discrimination. However, better discrimination was observed at 112.5 µM than other lower and greater concentrations and was selected and used as an optimum concentration level for screening purpose on their root length. The present aluminium tolerance study on finger millet germplasm at 112.5 µM tolerance level was higher as compared to the tolerance level of barley; 30 µM (Echart *et al.*, 2002), of maize; 20µM (Wagatsuma *et al*., 2005) and also of pigeon pea; 30 µM (Choudhary *et al.,* 2011). Crop specific aluminium concentration was able to discriminate genotypes into different classes in nutrient solution culture. The higher tolerance level in the present study might be due to finger millet germplasm were found Al tolerant than other crops.

Moreover, most of the accessions were collected from Western and Southern part of Ethiopia where soil acidity is predominant.

Gute and Degu were tolerant varieties; Boneya and Tadesse were intermediate varieties, while Necho and Wama varieties were found Al^{3+} susceptible. As compared to the control, varieties grouped under tolerant were showed significant Al^{3+} induced stress at 125 µM and above, while the intermediate and susceptible varieties showed significant stress symptom at 50 µM and 100 µM and above Al^{3+}concentrations. This result showed difference in varieties performance with different Al^{3+} concentrations similar with other crops, in pigeon pea (Choudhary *et al.,* 2011) and in barley (Echart *et al.,* 2002). Generally, the higher level of varietal difference found in the present study could be caused due to difference in genetic potential of genotypes. The complexation of Al^{3+} with chelating root exudates or binding to mucilage play a main role in the prevention of the accumulation Al in the apoplast and symplast (Barcelo and poschenrieder, 2002). Although many types of OA are found in root cells, only one or two specific organic acids are secreted in response to Al treatment for any given species. According to Lopez-Bucio *et al.,* (2000) organic acid level vary between species, cultivars and even between tissue of the same plant under identical growth condition that is why varietal variation found in the present study in finger millet.

6.1.2. Effect of Al-toxicity and variation among finger millet accession in Ethiopia

The present characterization study with (112.5 µM) and without (0 µM) Al^{3+} concentrations on 80 Ethiopian finger millet accessions from gene bank showed high root length inhibition on 63 (78.75%) accessions out of the total 80. High root length inhibition was reported in pigeon pea on 20 µM $AlCl_3$ (Choudhary *et al.,* 2011) and in

maize at 20 µM (Wagatsuma *et al.,* 2005), the present study also confirms the inhibition of root growth at 112.5 µM due to aluminium phytotoxicity. Root growth inhibition is considered to be the primary consequence of aluminum toxicity, resulting in a smaller volume of soil explored by the plant, consequently reducing its mineral nutrition and water absorption. Furthermore, it reduces cell membrane permeability and binds to the phosphate groups of the deoxyribonucleic acid decreasing replication and transcription activity and also cell division inhibition (Kochian *et al.,* 2005).

In the present study, no distinct and visible symptoms of aluminum toxicity were observed in the shoot of finger millet genotypes similar with the findings in pigeon pea on 20 µM $AlCl_3$ Choudhary *et al.,* (2011). Long term exposure may affect nutrient uptake, which can lead to nutritional deficiencies in shoots and leaves (Jiang *et al*., 2008). The overall effect of aluminium toxicity was expressed on the reduction of yield and its total biomass. Fresh weight reduction in 23 (28.75 %) accessions was also observed in this study. The decreased root growth could be the main cause for reduction in fresh weight.

From the RTI result, 20 accessions out of the total 80 (25 %) genotypes presented high aluminum tolerance, while the rest 60 accessions (75 %) showed low to medium tolerance. Accessions collected from Wellega, Ilu Ababora, Awi and Gojam were found Al^{3+} tolerant, while accessions collected from Northern part of Ethiopia were found Al^{3+} susceptible and those from SNNP were intermediate. According to Abdenna Deressa *et al.,* (2007), acidity affected soils are prevalent in these regions. Moreover, accessions collected from these areas were found Al^{3+} tolerant, this mainly being caused by their

enhanced tolerance against Al concentration that were developed due to long term exposure to soil acidity in this region.

6.2. Genetic diversity analysis of Ethiopian finger millet genotypes

6.2.1. ISSR marker in finger millet diversity study

In this study, ISSR markers were used to assess the level and pattern of genetic diversity among finger millet accessions from Ethiopia. The three di-nucleotide ISSR primers 810 (GA)8C, 811(GA)8T and 848 (CA)6RG, a tri-nucleotide ISSR primer 866 with (CTC)6, one tetra-nucleotide ISSR primer 873 with (GACA)4 and one penta-nucleotide ISSR primer 880 with (GGAGA)3 were used to study genetic diversity within and among populations. The number of scorable bands for individual primer ranged from 4 to 10, while the average number of bands per primer was 7.5.

Similarly, Fakrudin *et al*., (2004) have got 6.04 using 45 average numbers of bands per primer RAPD primers on 32 finger millet genotypes; Babu *et al*., (2006) reported 9.60 using 50 RAPD primers on 42 finger millet genotypes, and also with Kebere Bezaweletaw (2011) found 8.2 using 15 RAPD primers. Moreover, the choice of appropriate primer motives in ISSR fingerprint is critical to detect high polymorphism and reveal relationship within and among populations. The abundance and distribution of SSRs in the genomes of finger millet could be another factor that determines the levels of polymorphism.

The six di-, tri-, tetra-, and penta-nucleotide ISSR primers chosen for this study amplified large number of loci, displaying 77.78 % polymorphism with high gene diversity (h= 0.28) and Shannon's information index (I= 0.41). This is an evidence for the presence of high degree of variability among *E. coracana* accessions in Ethiopia. Among the eleven populations Gojam, Wellega, Awi, and Zimbabwe were found more diversified and genetically variable in percent polymorphism of 55.56 %, 46.67 %, 44.44 %, and 44.44 %, respectively, while accession collected from Omo showed the least in percent polymorphism with 13.33 %, gene diversity (h) and Shannon information index (I) of 0.05 and 0.07, respectively.

Kebere Bezaweletaw (2011) found 72.35 % percentage of polymorphism on 66 genotypes of finger millet from Ethiopia and Eritrea, while Fakrudin *et al.,* (2004) reported 85.82 % percentage of polymorphism on 32 germplasms from Indian. This study also found 77.78 % percentage of polymorphism on 80 genotypes of finger millet from Ethiopia, Zimbabwe and India using six ISSR primers. However, the present study is in contrast with Salimath *et al*., (1995) who found 26 % percentage of polymorphism on 17 genotypes of finger millet from Africa, Asia and Brazil. The high percent polymorphism observed in this study could be due to inclusion of large number of finger millet accessions from diverse ecological condition and large geographical range such as Ethiopia, Zimbabwe and India.

Analysis on the basis of geographical regions, Oromia, Amhara, Tigray, SNNP and Exotic showed a range of values of percent polymorphism, gene diversity and Shannon information's index. Among the five geographic groups, Amhara region accumulated more genetic diversity as compared to others. The population based analysis revealed

high and moderate intra-population molecular genetic diversity for Gojam and Awi populations, respectively; where both are located in Amhara region. However, populations from SNNP showed low level of genetic variability than other groups which could be explained by low level of gene flow with the other populations via market channels through human involvement and seed dispersal. The results suggested that high value of genetic diversity correlated with sample size and coverage of geographic area (20 samples of Amhara from Gojam and Awi, and 12 samples of SNNP from Hadiya and Omo (Table 3). The results appear to be in agreement with the fact that larger population size correlates with higher variability (Ravikanth *et al*., 2008). In this study; each genetic diversity parameters confirm that there is moderate to high gene diversity in population of finger millet collected from Ethiopia.

6.2.2. Genetic structure and classifying genetic diversity

The population genetic structure of a species reflects to the interactions among different processes during a long evolutionary history of species including shifts in distribution, habitat fragmentation and population isolation, mutation, genetic drift, reproductive biology, gene flow, and selection. Reproductive biology is likely to be particularly important in determining genetic structure of a given population (Hamrick and Godt, 1996).

At the present study analysis of molecular variance (AMOVA) using six primers on eleven finger millet populations showed among population differentiation and within-population variation of, 41.45 % and 58.54 %, respectively. High genetic variation within populations indicated that low genetic similarities among the individual plants sampled from a single

population. Higher within population genetic variation might be due to high genetic exchange or gene flows, which actually have a more homogenizing effect on the genetic variation among populations by the dispersal of the seeds and seed exchange via market channels.

6.2.3. Genetic relationship of finger millet genotypes

Cluster analysis of UPGMA and NJ analysis were used to construct a dendrogram to observe the genetic relationships among all individuals using Jaccard's similarity coefficient. UPGMA tree has showed a clear clustering of accessions on the basis of their regions of collection and respective populations (Figure 11 and 12). In addition to this, the range of genetic similarity among the total accessions was in the range of 60-100%. Two accessions of Ilu Ababora (Ilu 2 and 3), four accessions of Wellega (W5, 6, 11 & 12), two Varieties (Degu and Boneya) and three accessions of Hadiya (H3, 5 and 6) showed the highest level of genetic similarity (100 %). The separation and formation of a cluster by the eleven populations may suggest that these populations may have been divergent due to limited long distance gene flow but high gene flow with neighboring populations like in Wellega with Ilu Ababora, Gojam with Awi and Adwa with shire populations.

The present study was in agreement with the finding of Fakrudin *et al*. (2004) who found a clear apportionment of finger millet accessions in concordance with geographical origin using RAPD marker, while in contrast with Kebre Bezawletaw (2011) reported no clear-cut clustering of accessions to their geographic origin. Though the accessions assessed in this study mainly represented landraces from different geographical regions of Ethiopia, the analysis of UPGMA tree showed a clear-cut pattern of variation in relation to

geographical region, which could be due to the long history of domestication and cultivation of finger millet that might be resulted with development of local landraces limited in a particular location coupled with limited gene flow. The genetic structure observed in all the clustering analysis showed that there was moderate to low long distance gene flow but there is high short distance gene flow among populations of *E. coracana* from Ethiopia as observed in admixture of neighboring populations.

7. CONCLUSIONS

The current study showed that Root Length (RL) was affected more by Al toxicity than Shoot Length (SL). Lower Al toxicity levels less than 50 µM had no significant effect on the growth performance and germination in most finger millet varieties and accessions, while the growth of RL and SL showed a decline with increasing Al concentration and toxicity levels. The impact of Al toxicity on finger millet germplasm became intense upon toxicity level increments. The optimum Al^{3+} concentration for tolerance level could be 112.5 µM. Among national varieties, Necho and Wama were Al sensitive as revealed by root growth retardation compared to other varieties of finger millet. Thus, these varieties should not be recommended in area where soil acidity is predominant. However, Gute and Degu varieties were relatively Al tolerant as revealed by root growth performance and can be promoted in area where soil acidity is a challenge. This study is the first of its kind to evaluate the performance of Ethiopian finger millet to Al-toxicity. The study clearly showed the possibility of developing lines and genotypes that can tolerate acidity in Ethiopian context and support agricultural development in acidic soil area in the country.

In this study high genetic variability was also revealed among *Eleusine coracana* germplasms as revealed by ISSR marker. Few authors have published in the past about the genetic diversity and relationships of finger millet using molecular genetic markers like RAPD in *Eleusine coracana*. However, only very few are done on genetic diversity analysis and relationships on the *Eleusine coracana* using ISSR marker. The results of this work clearly demonstrate that ISSR markers can be successfully used for genetic

diversity and relationship among finger millet germplasms, although only limited numbers of accessions and primer combinations were analyzed.

The genetic diversity data generated by six ISSR primers revealed that high genetic diversity exists in finger millet germplasms. The assessed genetic diversity level varied among populations, which could be due to different environmental conditions in which they are growing, naturally distributed and human selection pressure. Moreover, Gojam and Wellega populations showed relatively high genetic diversity than others. The AMOVA analysis showed that, high genetic variation within populations than among populations shows the existence of high gene flow and low genetic differentiation among populations. The UPGMA cluster analysis supported the grouping of accessions to the defined geographical location and the respective population in the analysis of the total accessions. The findings of this study indicate that ISSR markers could be good tools to assess the genetic diversity and relationship at inter and intra population level of finger millet.

8. RECOMMENDATIONS

- This finding addresses the genetic diversity of *Eleusine coracana* accessions along with its aluminium tolerance. However, it only considered few accessions from different areas of Ethiopia. Hence, the genetic diversity and Al tolerance of Ethiopian accessions from different agro-ecology should be studied in detail.
- In this study the genetic diversity analysis of *Eleusine coracana* accessions was done using ISSR markers. Therefore, further study with different molecular markers such as SSRs as well may be needed to confirm the result of the present study as a single molecular marker cannot fulfill all of the desirable properties of molecular markers.
- For further selection of better aluminium tolerant finger millet germplasm, multi location field evaluation should be conducted for cultivar development.
- For further improvement of the crop and better aluminium tolerant germplasm, quantitative trait locus analysis should be considered.
- For future selection and evaluation, germplasms collected from Gojam and Wellega should be used for yield and performance stability for areas with acidic soil.

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No.	Acc	Region	Zone/woreda	Latitude	Longitude	Altitude

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1	9308	Tigray	Mehakelegnaw	14-08-33-N	38-47-56-E	2144
2	9309	Tigray	Mehakelegnaw	14-08-12-N	38-46-28-E	2112
3	9310	Tigray	Mehakelegnaw	14-07-29-N	38-45-32-E	2103
4	9311	Tigray	Mehakelegnaw	14-06-55-N	38-45-40-E	2091
5	9312	Tigray	Mehakelegnaw	14-06-19-N	38-46-36-E	2084
6	9313	Tigray	Mehakelegnaw	14-05-44-N	38-47-4-E	2086
7	9314	Tigray	Mehakelegnaw	14-05-58-N	38-47-28-E	2074
8	9315	Tigray	Mehakelegnaw	14-02-56-N	38-39-18-E	2093
9	9316	Tigray	Mehakelegnaw	14-02-54-N	38-39-14-E	2111
10	9317	Tigray	Mehakelegnaw	14-05-5-N	38-39-37-E	2055
11	9318	Tigray	Mehakelegnaw	14-05-20-N	38-39-32-E	2045
12	9319	Tigray	Mehakelegnaw	14-07-22-N	38-37-20-E	2147
13	9320	Tigray	Mehakelegnaw	14-07-30-N	38-34-52-E	2216
14	9321	Tigray	Mehakelegnaw	14-07-9-N	38-34-12-E	2183
15	9322	Tigray	Mehakelegnaw	14-07-19-N	38-32-18-E	2188
16	9323	Tigray	Mehakelegnaw	14-07-19-N	38-32-18-E	2188
17	9324	Tigray	Mirabawi	14-06-60-N	38-29-55-E	2041
18	9325	Tigray	Mirabawi	14-05-33-N	38-25-51-E	2059
19	9326	Tigray	Mirabawi	14-07-2-N	38-48-49-E	1928
20	9327	Tigray	Mirabawi	14-07-2-N	38-18-52-E	1928
21	9328	Tigray	Mirabawi	14-03-42-N	38-23-23-E	1938
22	9329	Tigray	Mirabawi	14-07-19-N	38-23-57-E	1970
23	9330	Tigray	Mirabawi	14-02-54-N	38-25-39-E	1975
24	9331	Tigray	Mirabawi	14-03-8-N	38-21-49-E	1967
25	9332	Tigray	Mirabawi	14-03-55-N	38-25-41-E	1975
26	9333	Tigray	Mirabawi	14-55-6-N	38-11-29-E	1806
27	9334	Tigray	Mirabawi	14-08-19-N	38-17-1-E	1910
28	9335	Tigray	Mirabawi	14-11-40-N	38-14-50-E	1869
29	9336	Tigray	Mirabawi	14-09-45-N	38-19-2-E	1935
30	9337	Tigray	Mirabawi	14-11-45-N	38-19-40-E	1952
31	9338	Tigray	Mirabawi	14-10-47-N	38-13-54-E	1872
32	9339	Tigray	Mirabawi	14-19-47-N	38-1017-E	1820
33	9340	Tigray	Mirabawi	14-23-30-N	38-10-43-E	1761
34	9341	Tigray	Mirabawi	14-24-58-N	38-08-24-E	1549
35	9342	Tigray	Mirabawi	14-25-5-N	38-04-14-E	1499
36	9344	Tigray	Mirabawi	14-26-26-N	37-57-15-E	1291
37	9345	Tigray	Mirabawi	14-14-56-N	37-38-15-E	1005
38	9347	Tigray	Mirabawi	14-05-26-N	37-26-57-E	1000
39	9348	Tigray	Mirabawi	14-09-33-N	39-21-4-E	1141
40	9349	Tigray	Mirabawi	14-03-6-N	37-10-31-E	893

No.	Acc	Region	Zone/woreda	Latitude	Longitude	Altitude

10. APPENDICES

Appendix 1. Plant material of *Eleusine coracana* for Al tolerance and genetic diversity

41	9350	Tigray	Mirabawi	12-57-2-N	37-19-18-E	1081
42	9351	Amara	Semen Gondar	12-24-7-N	37-18-8-E	1806
43	9352	Amara	Semen Gondar	12-26-11-N	37-18-21-E	1889
44	9354	Amara	Semen Gondar	12-22-11-N	37-16-21-E	1885
45	9355	Amara	Semen Gondar	12-21-9-N	37-11-49-E	1824
46	9356	Amara	Semen Gondar	12-21-59-N	37-10-15-E	1826
47	9357	Amara		12-16-42-N	37-06-7-E	1859
48	9358	Amara	Semen Gondar	12-11-36-N	37-02-17-E	1820
49	9359	Amara	Semen Gondar	12-18-12-N	37-05-41-E	1860
50	9360	Amara	Semen Gondar	12-18-11-N	37-05-14-E	1869
51	9361	Amara	Semen Gondar	12-20-20-N	37-03-54-E	1899
52	9362	Amara	Semen Gondar	12-23-36-N	37-01-7-E	2106
53	9363	Amara	Semen Gondar	12-33-57-N	37-05-12-E	2170
54	100001	Amara	Special			
55	100002	Amara	Semen Gondar			
56	100003	Amara	Semen Gondar			
57	100005	Amara	Semen Gondar			
58	100006	SNNP	Gurage			
59	100009	Oromiya	Mirab harerge			
60	100010	Oromiya	Mirab harerge			
61	100011	Amhara	Semen Gondar			
62	100012	Oromiya	Misrak Wellega			
63	100014	Amhara	Awi			
64	100016	Amhara	Mirab Gojam			
65	100017	Amhara	Special			
66	100018	Amhara	Mirab Gojam			
67	100019	Amhara	Debub Gondar			
68	100031	Amhara	Mirab Gojam			
69	100033	Amhara	Mirab Gojam			
70	100034	Amhara	Mirab Gojam			
71	100035	Amhara	Mirab Gojam			
72	100036	Amhara	Awi			
73	100045	Amhara	Awi			
74	100049	Bebshagul	Metekel			
75	100052	Amhara	Awi			
76	100054	SNNP	Debub omo			
77	100055	SNNP	Debub omo			
78	100056	SNNP	Debub omo			
79	100061	SNNP	Bench maji			
80	100063	Oromiya	Misrak Wellega			

No.	Acc	Region	Zone/Woreda	Latitude	Longitude	Altitude
81	100064	Oromiya	Misrak Wellega			
82	100065	Amhara	Misrak Wellega			
83	100076	Amhara	Special			
84	100086	Amhara	Debub Wello			
85	100088	Amhara	Mirab Gojam			
86	100089	SNNP	Debub Omo			
87	100091	Amhara	Semen Gondar			
88	100092	Oromiya	Misrak Wellega			
89	100094	Oromiya	Misrak Wellega			
90	100095	Oromiya	Misrak Wellega			
91	100096	Oromiya	Misrak Wellega			
92	100097	Oromiya	IIlu Ababora			
93	203054	Oromiya	Ilu Ababora			2000
94	203055	Oromiya	Ilu Ababora			1950
95	203056	Oromiya	Ilu Ababora			1850
96	203057	Oromiya	Ilu Ababora			1840
97	203058	Oromiya	Ilu Ababora			1810
98	203059	Oromiya	Ilu Ababora			2130
99	203062	Oromiya	Misrak Wellega			1870
100	203069	Oromiya	Misrak Wellega			1750
101	203070	Oromiya	Misrak Wellega			1780
102	203071	Oromiya	Misrak Wellega			1810
103	203072	Oromiya	Misrak Wellega			1487
104	203247	Zimbabwe		18-19-00-S	31-10-00-E	1487
105	203348	SNNP	Debub Omo		28-58-00-E	1000
106	203339	India		19-50-00-S	30-33-00-E	1100
107	203367	India		18-07-00-S	28-31-00-E	1100
108	203422			18-36-00-S	32-05-00-E	1320
109	203438	India		18-36-00-S	34-46-00-E	900
110	203458	India		17-34-00-S	29-56-00-E	900
111	203467			17-48-00-S	30-19-00-E	1350
112	203477			17-07-00-S	29-20-00-E	1100
113	203483	India		16-24-00-S	29-41-00-E	1100
114	203486			16-29-00-S	29-40-00-E	1200
115	203491			19-52-00-N	29-53-00-E	1100
116	203503			17-32-00-S	31-27-00-E	1300
117	203526			16-33-00-S	31-31-00-E	990
118	203531			16-16-00-S	30-28-00-E	400
119	203552			13-45-00-S	25-30-00-E	
120	203570			10-13-00-S	29-58-00-E	1550

No.	Acc	Region	Zone/Woreda	Latitude	Longitude	Altitude
121	203571			09-55-00-S	30-00-00-E	1500
122	203573			0945-00-S	29-10-0-E	1350
123	203575			10-00-00-S	28-4500-E	1400
124	203576			10-00-00-S	28-4500-E	1400
125	203577			10-00-00-S	28-4500-E	1400
126	203578			10-00-00-S	28-4500-E	1400
127	203579			09-45-00-S	28-50-00-E	
128	203581			09-30-00-S	30-05-00-E	1610
129	203582	Zimbabwe		09-30-00-S	30-05-00-E	1610
130	203583			09-00-00-S	30-10-00-E	1500
131	203584	Zimbabwe		09-00-00-S	30-10-00-E	1500
132	203586	Zimbabwe		09-55-00-S	30-00-00-E	1680
133	203587	Zimbabwe		12-15-00-S	26-00-00-E	
134	203588			19-34-00-S	31-17-00-E	1180
135	203589			20-25-00-S	31-03-00-E	800
136	203590	Zimbabwe		20-29-00-S	30-42-00-E	770
137	203591			19-46-00-S	30-58-00-E	1300
138	203592			18-41-00-S	29-19-00-E	1180
139	203593			18-54-00-S	31-02-00-E	1490
140	204749					2325
141	204750					2325
142	207451	Amhara	DEBUB GONDAR			
143	207593	Tigray	MEHAKELEGNAW			
144	207755	Tigray	MEHAKELEGNAW			
145	207756	Tigray	MEHAKELEGNAW			
146	207757	Tigray	MEHAKELEGNAW			
147	207897	Tigray	MEHAKELEGNAW	09-45-00-N	35-05-00-E	
148	207960	Oromiya	MIRAB WELLEGA	09-50-00-N	35-00-00-E	
149	207961	Oromiya	MIRAB WELLEGA	09-01-00-N	36-10-00-E	
150	207963	Oromiya	MISRAK WELLEGA	11-45-00-N	37-35-00-E	
151	208442	Amhara	DEBUB GONDAR			
152	208443	Amhara	DEBUB GONDAR	11-33-00-N	38-30-00-E	
153	208445	Amhara	DEBUB GONDAR			2290
154	208724	Oromiya	MISRAK WELLEGA			1880
155	208726	Oromiya	MIRAB WELLEGA			
156	211029	Tigray	MISRAKAWI	05-20-00-N	37-25-00-E	1700
157	211473	SNNP	HADIYA			1560
158	211474	SNNP	HADIYA			1700
159	212461	Oromiya	MIRAB HARERGE			1600
160	212462	Oromiya	MIRAB HARERGE	37-31-00-N	10-24-00-E	2340

No.	Acc	Region	Zone/woreda	Latitude	Longitude	Altitude
161	212692	Amhara	Mirab Gojam	37-42-00-N	12-02-00-E	1970
162	212693	Amhara	Debub Gondar	37-54-00-N	11-55-00-E	2380
163	212694	Amhara	Debub Gondar			
164	213032	SNNP	Hadiya			
165	213033	SNNP	Hadiya			2180
166	213034	SNNP	Hadiya			1380
167	213035	SNNP	Hadiya			
168	213227	Tigray	Mirabawi			
169	213835	SNNP	Hadiya			1650
170	214207	Amhara	Semen Gondar			1680
171	214208	Amhara	Semen Gondar			1630
172	214210	Amhara	Semen Gondar			
173	214987	Unknown	Unknown			
174	214988	Unknown	Unknown			
175	214989	Unknown	Unknown			
176	214990	Unknown	Unknown			
177	214995	Unknown	Unknown			
178	215668	Amhara	Semen Gondar			1651
179	215800	Oromiya	Mirab Wellega	08-32-00-N	34-40-00-E	1950
180	215801	Oromiya	Mirab Wellega	08-32-00-N	36-46-00-E	1950
181	215802	Oromiya	Mirab Wellega	08-32-00-N	36-46-00-E	1950
182	215803	Oromiya	Mirab Wellega	08-32-00-N	36-46-00-E	1950
183	215804	Oromiya	Mirab Wellega	08-32-00-N	36-46-00-E	1950
184	215805	Oromiya	Mirab Wellega	10-41-00-N	37-15-00-E	1920
185	215826	Amhara	Mirab Gojam	10-41-00-N	37-13-00-E	1940
186	215827	Amhara	Mirab Gojam	10-41-00-N	37-13-00-E	1940
187	215828	Amhara	Mirab Gojam	10-41-00-N	37-13-00-E	1990
188	215829	Amhara	Mirab Gojam	10-42-00-N	37-11-00-E	2000
189	215830	Amhara	Mirab Gojam	11-32-00-N	37-24-00-E	1800
190	215844	Amhara	Mirab Gojam	11-32-00-N	37-25-00-E	1800
191	215850	Amhara	Special	11-30-00-N	37-32-00-E	1720
192	215855	Amhara	Special	11-30-00-N	37-23-00-E	1850
193	215863	Amhara	Special	11-28-00-N	37-23-00-E	1880
194	215865	Amhara	Special	11-22-00-N	37-24-00-E	2350
195	215874	Amhara	Mirab Gojam	11-16-00-N	37-29-00-E	2230
196	215877	Amhara	Mirab Gojam	11-15-00-N	37-30-00-E	2320
197	215878	Amhara	Mirab Gojam	11-10-00-N	37-40-00-E	2380
198	215882	Amhara	Mirab Gojam	11-08-00-N	37-43-00-E	2400
199	215883	Amhara	Mirab Gojam	11-10-00-N	37-40-00-E	2330

No.	Acc	Region	Zone/woreda	Latitude	Longitude	Altitude
201	215884	Amhara	Mirab Gojam	10-34-00-N	36-56-00-E	2080
202	215900	Amhara	Mirab Gojam	10-43-00-N	37-4-00-E	2200
203	215907	Amhara	Mirab Gojam	11-10-00-N	36-52-00-E	2250
204	215909	Amhara	Awi	11-16-00-N	36-52-00-E	2150
205	215912	Amhara	Awi	11-20-00-N	36-56-00-E	2050
206	215917	Amhara	Mirab Gojam	11-39-00-N	36-57-00-E	2050
207	215921	Amhara	Mirab Gojam	11-36-00-N	37-26-00E	1940
208	215958	Amhara	Special	11-50-00-N	37-36-00-E	1880
209	215961	Amhara	Debub Gondar	12-39-00-N	37-29-00-E	2340
210	215969	Amhara	Semen Gondar	12-29-00-N	37-23-00-E	1980
211	215975	Amhara	Semen Gondar	12-17-00-N	37-4-00-E	1940
212	215985	Amhara	Semen Gondar	12-25-00-N	37-4-00-E	2210
213	215987	Amhara	Semen Gondar	12-6-00-N	37-46-00-E	1910
214	215991	Amhara	Debub Gondar	09-24-00-N	35-38-00-E	1950
215	216044	Oromiya	Mirab Wellega	09-35-00-N	35-25-00-E	1910
216	216053	Oromiya	Mirab Wellega	08-57-00-N	35-21-00-E	1650
217	216054	Oromiya	Mirab Wellega	08-57-00-N	35-15-00-E	1600
218	216056	Oromiya	Mirab Wellega			
219	217674	Unknown	Unknown			
220	217675	Unknown	Unknown			
221	217677	Unknown	Unknown			
222	219503	Amhara	Awi	14-09-00-N	38-44-00-E	1880
223	219807	Tigray	Mehakelegnaw	14-12-00-N	38-52-00-E	1880
224	219814	Tigray	Mehakelegnaw	14-08-00-N	38-51-00-E	1860
225	219815	Tigray	Mehakelegnaw	14-08-00-N	38-50-00-E	1870
226	219818	Tigray	Mehakelegnaw	14-05-00-N	38-53-00-E	2260
227	219819	Tigray	Mehakelegnaw	14-06-00-N	38-46-00-E	2080
228	219820	Tigray	Mehakelegnaw	14-08-00-N	38-48-00-E	2120
229	219821	Tigray	Mirabawi	14-10-00-N	38-16-00-E	1920
230	219824	Tigray	Mirabawi	14-08-00-N	38-16-00-E	1880
231	219834	Tigray	Mirabawi	14-04-00-N	38-16-00-E	1890
232	219835	Tigray	Mirabawi	14-10-00-N	38-50-00-E	2330
233	219838	Tigray	Mehakelegnaw		39-22-00-E	2634
234	220090	Tigray	Debubawi			2500
235	221697	Tigray	Debubawi	13-27-00-N	39-31-00-E	2200
236	221699	Tigray	Debubawi			
237	222975	Unknown	Unknown			
238	222978	Unknown	Unknown			
239	222980	Unknown	Unknown			
240	222990	Unknown	Unknown			

No.	Acc	Region	Zone/Woreda	Latitude	Longitude	Altitude
241	222991	Unknown	Unknown			
242	222992	Unknown	Unknown			
243	222994	Unknown	Unknown			
244	222999	Unknown	Unknown			
245	223001	Unknown	Unknown			
246	223002	Unknown	Unknown			
247	223003	Unknown	Unknown			
248	223004	Unknown	Unknown			
249	223005	Unknown	Unknown			
250	223006	Unknown	Unknown			
251	223007	Unknown	Unknown			
252	223008	Unknown	Unknown			
253	223009	Unknown	Unknown			
254	223011	Unknown	Unknown			
255	223013	Unknown	Unknown			
256	223014	Unknown	Unknown			
257	223016	Unknown	Unknown			
258	223017	Unknown	Unknown			
259	223018	Unknown	Unknown			
260	223019	Unknown	Unknown			
261	223024	Unknown	Unknown			
262	223025	Unknown	Unknown			
263	223026	Unknown	Unknown			
264	223034	Unknown	Unknown			
265	223035	Unknown	Unknown			
266	223036	Unknown	Unknown			
267	223037	Unknown	Unknown			
268	223038	Unknown	Unknown			
269	223039	Unknown	Unknown			
270	223144			12-19-00-N	37-33-00-E	2145
271	243634	Amhara	Semen Gondar	12-07-00-N	37-49-00-E	2070
272	243635	Amhara	Debub Gondar	11-38-00-N	37-20-00-E	1870
273	243636	Amhara	Mirab Gojam	11-38-00-N	37-20-00-E	1870
274	243637	Amhara	Mirab Gojam	11-38-00-N	37-20-00-E	1870

No.	Acc	Region	Zone/Woreda	Latitude	Longitude	Altitude
275	243638	Amhara	Semen Gondar			2050
276	243639	Amhara		11-19-00-N	36-47-00-E	1890
277	243640	Amhara	Awi	10-57-00-N	36-52-00-E	2200
278	243641	Amhara	Awi	10-57-00-N	36-47-00-E	1750
279	243642	Amhara	Awi			1750
280	243643	Amhara	Awi	10-59-00-N	36-38-00-E	1815
281	243644	Amhara	Awi			2169
282	244798	SNNP	AlabanaTemb			1915
283	245084	Oromiya	Misrak Wellega			1987
284	245085	Oromiya	Misrak Wellega			1927
285	245086	Oromiya	Misrak Wellega			1923
286	245087	Oromiya	Misrak Wellega			2060
287	245088	Oromiya	Misrak Wellega			2060
288	245091	Oromiya	Misrak Wellega			1954
289	245092	Oromiya	Illu Ababora			

Key: No. number, Acc. = Accessions, N = Northern, E = Eastern

Appendix 2. Plant material selected for characterization of Al tolerance and for molecular

No.	Acc.	Lab code	Region	No.	Acc.	Lab code	Region
1	100094	W1	Oromia	41	243642	AW5	Amhara
2	100095	W2	Oromia	42	207897	Ad1	Tigray
3	203070	W3	Oromia	43	219815	Ad2	Tigray
4	203071	W4	Oromia	44	219818	Ad3	Tigray
5	203072	W5	Oromia	45	219819	Ad4	Tigray
6	207960	W6	Oromia	46	219820	Ad5	Tigray
7	207961	W7	Oromia	47	213227	Sh1	Tigray
8	207963	W8	Oromia	48	219821	Sh2	Tigray
9	208724	W9	Oromia	49	219824	Sh3	Tigray
10	215805	W10	Oromia	50	219834	Sh4	Tigray
11	216044	W11	Oromia	51	219835	Sh5	Tigray
12	216053	W12	Oromia	52	100054	O1	SNNP
13	216056	W13	Oromia	53	100055	O2	SNNP
14	245084	W14	Oromia	54	100056	O3	SNNP
15	245087	W15	Oromia	55	100089	O4	SNNP
16	245091	W16	Oromia	56	203348	O5	SNNP
17	100097	IL1	Oromia	57	211473	H1	SNNP
18	203054	IL2	Oromia	58	211474	H2	SNNP
19	203057	IL3	Oromia	59	213032	H3	SNNP
20	203058	IL4	Oromia	60	213033	H4	SNNP
21	203059	IL5	Oromia	61	213034	H5	SNNP
22	100016	Gj1	Amhara	62	213035	H6	SNNP
23	100034	Gj2	Amhara	63	213835	H7	SNNP
24	100035	Gj3	Amhara	64	203339	I1	Exotic
25	215827	Gj4	Amhara	65	203438	I2	Exotic
26	215828	Gj5	Amhara	66	203458	I3	Exotic
27	215850	Gj6	Amhara	67	203467	I4	Exotic
28	215883	Gj7	Amhara	68	203483	I5	Exotic
29	215884	Gj8	Amhara	69	203247	Z1	Exotic
30	243637	Gj9	Amhara	70	203582	Z2	Exotic
31	100003	G1	Amhara	71	203584	Z3	Exotic
32	100011	G2	Amhara	72	203586	Z4	Exotic
33	215668	G3	Amhara	73	203587	Z5	Exotic
34	215900	G4	Amhara	74	203590	Z6	Exotic
35	215991	G5	Amhara	75	Boneya	V1	Varieties
36	243638	G6	Amhara	76	Degu	V2	Varieties
37	100036	AW1	Amhara	77	Gute	V3	Varieties
38	100045	AW2	Amhara	78	Necho	V4	Varieties
39	219503	AW3	Amhara	79	Tadesse	V5	Varieties
40	243640	AW4	Amhara	80	Wama	V6	Varieties

Appendix 3. DNA extraction protocol

1. Pour CTAB solution (700 µl per sample) in a 15ml-tube and add 0.2 vol % Mercapto-ethanol (use fume hood!). Mercapto-ethanol is stored at 4°C.

2. Aliquot CTAB in 1.5 ml eppendorf-caps and warm in water bath up to 65°C.

3. Weigh in 100 mg fresh leave material (50mg dry material) per sample. Pulverize thoroughly using a clean mortar and pestle. For fresh material add liquid nitrogen or quartz sand for dry material. First grind down slightly, then more powerful (cells have to be crashed). Use safety goggles!

4. Transfer the powder into an Eppendorf cap with warm CTAB solution immediately (use a new, clean spatula for each sample)

5. Add 700 µl of warm CTAB solution to the powdered sample (open the caps carefully), dissolve the powder and incubate the sample for 30 minutes at 65°C

6. Centrifuge for 5 minutes at 15000 rpm.

7. Transfer the supernatant (only clear liquid) in a new eppendorf-cap. Use blue pipette tips which are cut.

8. Add new CTAB solution (700 µl) to the tissue pellet and stir slightly with a new 1000 µl pipette tip, incubate 30 min at 65°C. Step 6 and 7 are repeated. The same is carried out for a third extraction. Each fraction proceeds with step 9 and is treated separately.

9. Add 600 µl chloroform to the cap with supernatant and shake carefully a few times upside down. This chloroform step should be carried out immediately.

10. Shake the samples thoroughly by turning inversing the eppendorf caps for approximately 5 minutes. (Longer incubation is possible)

11. Centrifuge for 5 min at 15000 rpm.

12. Transfer the supernatant (only clear liquid) in a new Eppendorf-cap. Use blue pipette tips which are cut. Work carefully; do not transfer suspended matter (normally the chloroform is covered by a thin layer of fine sediment material). Chloroform has to be disposed of in a special waste bottle.

13. Repeat the chloroform extraction (step 9-12) to make sure that all impurities are removed, and then proceed with step 14.

14. Add cooled iso-propanol (4°C), approximately 2/3 of the solution volume. Shake carefully by inversing the eppendorff cap. In most cases DNA becomes visible as white threads. Freeze for more than 2 h at -20°C. (BREAK POSSIBLE)

15. Centrifuge 10 min at 15000 rpm.

16. Aspirate liquid using yellow tips (without touching pellet!). If pellet is solid enough the larger part of the liquid may be poured out. (Alternatively add TE and proceed with qiagen kit)

17. Add 200 µl ethanol 70 % to the pellet. Rinse the inner cap surface by turning the cap.

18. Centrifuge for 10 min at 15000 rpm in a cooled centrifuge.

19. Aspirate ethanol using yellow tips. Dry the DNA-pellet at room temperature. (Usually 15 min are sufficient; after drying no liquid drops are to be seen)

20. Dissolve pellet in 100 µl TE (1x, p.a. grade) and store at 4°c. (BREAK POSSIBLE)

21. Add cooled 7.5 m nh4ac-solution (4°c, half of the solution volume). Mix carefully.

22. Add cool ethanol 100 % (double of the solution volume). Mix carefully. Freeze for more than 2 h at -20°c. (BREAK POSSIBLE)

23. Centrifuge 30 min at 15000 rpm. Aspirate fluid carefully.

24. Add 200 µl ethanol 70%. Rinse the inner cap surface by turning the cap.

25. Centrifuge 10 min. at 15000 rpm. Aspirate liquid and dry pellet at room temperature. Dissolve the pellet in 100 µl TE (1x, p.a. grade)

26. Repeat steps 21 to 24 with 3 M NaAC solution (4°c, half the volume) then proceed with step 27

27. Centrifuge 10 min. at 15000 rpm. Aspirate liquid and dry pellet at room temperature. Dissolve the pellet in 100 µl TE (1x, p.a. grade)

- cleaning the mortar and pestle:
  - ✓ rinse the mortar and pestle with water
  - ✓ clean the mortar and pestle in a 1:10 klorex-bath for 24 hours
  - ✓ rinse with ddH_2o
  - ✓ Autoclave the mortar and pestle wrapped in aluminum foil at 134°c

Appendix 4A. Mean root length, standard error (SE) and performance ranking under 112.5 µM Al on 48 accessions and two standard checks grown under Bach- I on nutrient solution culture concentration

SN	Acc.	Rip	M±SE	Ranking	SN	Acc.	Rip	M±SE	Ranking
1	Gute	3	1.111±0.02	9324	27	9338	3	2.067±0.24	9356
2	Necho	3	0.378±0.15	9321	28	9339	3	2.156±0.29	9341
3	9314	3	1.611±0.42	Necho	29	9340	3	1.367±0.37	9322
4	9315	3	0.556±0.04	9342	30	9341	3	1.522±0.43	9314
5	9316	3	0.922±0.52	9318	31	9342	3	0.399±0.12	9320
6	9317	3	0.933±0.13	9334	32	9344	3	1.322±0.48	9319
7	9318	3	0.466±0.18	9315	33	9345	3	1.255±0.33	9359
8	9319	3	1.700±0.30	9326	34	9347	3	0.867±0.27	9330
9	9320	3	1.611±0.19	9327	35	9348	3	1.878±0.29	9333
10	9321	3	0.311±0.06	9347	36	9349	3	0.956±0.29	9351
11	9322	3	1.544±0.16	9316	37	9350	3	0.944±0.47	9348
12	9323	3	1.400±0.48	9317	38	9351	3	1.789±0.50	9355
13	9324	3	0.200±0.01	9350	39	9352	3	1.411±0.70	9357
14	9325	3	0.956±0.39	9325	40	9354	3	2.267±0.21	9361
15	9326	3	0.611±0.34	9349	41	9355	3	1.889±0.56	9329
16	9327	3	0.778±0.18	9331	42	9356	3	1.455±0.20	9358
17	9328	3	1.233±0.58	9332	43	9357	3	1.933±0.35	9513
18	9329	3	1.989±0.31	9362	44	9358	3	2.022±0.55	9335
19	9330	3	1.700±0.43	Gute	45	9359	3	1.700±0.4	9338
20	9331	3	0.989±0.48	9328	46	9360	3	2.255±0.43	9339
21	9332	3	1.033±0.40	9345	47	9361	3	1.967±0.49	9336
22	9333	3	1.733±0.28	9344	48	9362	3	1.111±0.74	9360
23	9334	3	0.533±0.33	9337	49	9363	3	2.300±0.32	9354
24	9335	3	2.056±0.36	9340	50	9513	3	2.022±0.02	9363
25	9336	3	2.211±0.29	9323	51	Total	150	1.375±0.06	
26	9337	3	1.367±0.69	9352					

Appendix 4B. Mean root length, standard error (SE) and performance ranking on 48 accessions and two standard checks grown under Bach- II on nutrient solution culture under 112.5 µM Al concentration

SN.	Acc.	N	M±S.E	Rankin	SN.	Acc.	N	M±S.E	Ranking
1	Gute	3	2.200±0.21	100003	27	100056	3	1.289±0.22	203058
2	Necho	3	0.144±0.04	100005	28	100061	3	1.467±0.30	100056
3	100001	3	0.167±0.06	100016	29	100063	3	0.855±0.48	100052
4	100002	3	0.189±0.08	100010	30	100064	3	1.100±0.27	100061
5	100003	3	0.100±0.00	Necho	31	100065	3	0.811±0.36	100033
6	100005	3	0.100±0.00	100001	32	100076	3	1.989±0.32	203059
7	100006	3	0.911±0.30	100002	33	100086	3	0.833±0.35	203056
8	100009	3	0.355±0.137	100034	34	100088	3	1.189±0.89	100092
9	100010	3	0.133±0.01	100031	35	100089	3	2.578±0.20	203054
10	100011	3	0.889±0.79	100012	36	100091	3	1.992±0.08	100018
11	100012	3	0.344±0.14	100009	37	100092	3	1.589±0.21	100096
12	100014	3	0.500±0.40	100054	38	100094	3	0.656±0.43	203055
13	100016	3	0.122±0.02	100014	39	100095	3	2.344±0.28	100091
14	100017	3	0.656±0.28	100017	40	100096	3	1.889±0.50	100076
15	100018	3	1.889±0.17	100094	41	100097	3	2.089±0.26	100036
16	100019	3	1.055±0.49	100065	42	203054	3	1.733±0.48	100097
17	100031	3	0.289±0.09	203070	43	203055	3	1.922±0.49	100055
18	100033	3	1.478±0.16	100086	44	203056	3	1.544±0.38	203062
19	100034	3	0.255±0.07	100063	45	203057	3	2.333±0.32	Gute
20	100035	3	2.278±0.54	100011	46	203058	3	1.267±0.68	100035
21	100036	3	2.033±0.25	100006	47	203059	3	1.500±0.40	203057
22	100045	3	2.767±0.19	100019	48	203062	3	2.189±0.14	100095
23	100049	3	1.244±0.39	100064	49	203069	3	1.189±0.44	100089
24	100052	3	1.456±0.66	100088	50	203070	3	0.811±0.39	100045
25	100054	3	0.411±0.23	203069	51	Total	150	1.223±0.07	
26	100055	3	2.122±0.54	100049					

Appendix 4C. Mean root length, standard error (SE) and performance ranking on 48 accessions and two standard checks grown under Bach- III on nutrient solution culture under 112.5 µM Al concentration

SN.	Acc.	Rip	M±SE	Ranking	SN.	Acc.	Rip	M±SE	Ranking
1	Gute	3	2.300±0.32	203590	27	203578	3	1.311±0.19	203577
2	Necho	3	0.322±0.04	203591	28	203579	3	0.689±0.19	207961
3	203071	3	2.100±0.11	204751	29	203581	3	0.789±0.10	207493
4	203072	3	2.444±0.43	Necho	30	203582	3	0.511±0.10	203483
5	203247	3	2.322±0.12	203587	31	203583	3	0.455±0.187	203526
6	203339	3	2.067±0.40	203586	32	203584	3	0.833±0.20	203573
7	203348	3	2.044±0.19	203583	33	203586	3	0.433±0.15	203578
8	203367	3	1.811±0.13	207755	34	203587	3	0.378±0.12	203486
9	203422	3	1.733±0.45	207756	35	203588	3	0.778±0.35	203503
10	203438	3	1.467±0.811	203571	36	203589	3	1.711±0.38	203438
11	203458	3	2.611±0.27	203582	37	203590	3	0.111±0.1	203570
12	203467	3	1.533±0.13	203576	38	203591	3	0.255±0.04	203467
13	203477	3	0.711±0.20	207960	39	203592	3	0.644±0.34	203491
14	203483	3	1.022±0.18	207757	40	203593	3	0.678±0.23	203589
15	203486	3	1.422±0.14	203592	41	204749	3	0.933±0.13	203422
16	203491	3	1.567±0.35	203593	42	204750	3	0.789±0.11	203531
17	203503	3	1.422±0.02	203579	43	204751	3	0.311±0.09	203367
18	203526	3	1.133±0.26	207897	44	207493	3	1.022±0.27	203348
19	203531	3	1.767±0.25	203575	45	207755	3	0.455±0.13	203339
20	203552	3	0.855±0.21	203477	46	207756	3	0.489±0.10	203071
21	203570	3	1.478±0.23	203588	47	207757	3	0.589±0.18	Gute
22	203571	3	0.511±0.04	203581	48	207897	3	0.700±0.24	203247
23	203573	3	1.300±0.28	204750	49	207960	3	0.578±0.12	203072
24	203575	3	0.711±0.09	203584	50	207961	3	0.989±0.23	203458
25	203576	3	0.511±0.10	203552	51	Total	150	1.091±0.06	
26	203577	3	0.978±0.35	204749					

Appendix 4D. Mean root length, standard error (SE) and performance ranking on 48 accessions and two standard checks grown under Bach- IV on nutrient solution culture under 112.5 µM Al

SN.	Acc.	Rip	M±SE	Ranking	SN.	Acc.	Rip	M±SE	Ranking
1	Gute	3	1.611±0.19	214988	27	214988	3	0.167±0.03	214210
2	Necho	3	0.311±0.06	212692	28	214989	3	0.667±0.55	208726
3	207963	3	0.389±0.14	213033	29	214990	3	1.600±0.55	211473
4	208442	3	0.955±0.28	214207	30	214995	3	0.656±0.08	215874
5	208443	3	0.755±0.13	215829	31	215668	3	1.667±0.35	208442
6	208445	3	0.589±0.11	214208	32	215800	3	0.478±0.31	215827
7	208724	3	1.778±0.27	212693	33	215801	3	0.656±0.19	215804
8	208726	3	0.789±0.04	215878	34	215802	3	1.833±0.59	215803
9	211029	3	0.756±0.09	Necho	35	215803	3	1.500±0.86	215828
10	211473	3	0.844±0.13	214987	36	215804	3	1.455±0.17	214990
11	211474	3	0.755±0.05	207963	37	215805	3	1.678±0.72	Gute
12	212461	3	0.589±0.32	213835	38	215826	3	0.689±0.47	215865
13	212462	3	0.600±0.50	213032	39	215827	3	1.067±0.67	215668
14	212692	3	0.178±0.06	212694	40	215828	3	1.500±0.86	215805
15	212693	3	0.233±0.06	215800	41	215829	3	0.211±0.04	215850
16	212694	3	0.444±0.19	212461	42	215830	3	1.778±0.89	208724
17	213032	3	0.422±0.22	208445	43	215844	3	2.400±0.11	215830
18	213033	3	0.200±0.03	212462	44	215850	3	1.722±0.32	213227
19	213034	3	3.133±0.21	215801	45	215855	3	0.744±0.51	215802
20	213035	3	1.889±0.48	214995	46	215863	3	2.000±0.16	213035
21	213227	3	1.789±0.06	214989	47	215865	3	1.622±0.42	215863
22	213835	3	0.411±0.06	215826	48	215874	3	0.878±0.51	215877
23	214207	3	0.211±0.01	215855	49	215877	3	2.078±0.46	215844
24	214208	3	0.222±0.04	208443	50	215878	3	0.3±0.10	213034
25	214210	3	0.789±0.62	211029	51	Total	150	1.006±0.07	
26	214987	3	0.322±0.07	211474					

concentration

Appendix 4E. Mean root length, standard error (SE) and performance ranking on 48 accessions and two standard checks grown under Bach- V on nutrient solution culture under 112.5 µM Al concentration

SN.	Acc.	Rip	M±SE	Ranking	SN.	Acc.	Rip	M±SE	Ranking
1	Gute	3	1.944±0.23	215987	27	219503	3	0.767±0.30	219821
2	Necho	3	0.478±0.20	215921	28	219807	3	0.967±0.25	219834
3	215882	3	0.411±0.11	222975	29	219814	3	0.944±0.21	222992
4	215883	3	2.111±0.29	215882	30	219815	3	0.678±0.12	222999
5	215884	3	1.811±0.11	222978	31	219818	3	0.633±0.18	220090
6	215891	3	1.455±0.12	215975	32	219819	3	0.700±0.29	215891
7	215900	3	2.467±0.30	Necho	33	219820	3	1.889±0.27	222991
8	215907	3	1.511±0.67	215985	34	219821	3	1.244±0.08	217674
9	215909	3	1.167±0.03	215958	35	219824	3	0.733±0.08	219835
10	215912	3	1.567±0.33	223001	36	219834	3	1.278±0.31	215907
11	215917	3	0.822±0.56	219818	37	219835	3	1.478±0.26	215912
12	215921	3	0.367±0.18	219815	38	219838	3	1.678±0.14	219838
13	215958	3	0.533±0.11	219819	39	220090	3	1.400±0.15	221699
14	215961	3	0.789±0.06	219824	40	221697	3	1.044±0.37	215884
15	215969	3	0.778±0.54	219503	41	221699	3	1.678±0.21	219820
16	215975	3	0.444±0.29	215969	42	222975	3	0.389±0.06	Gute
17	215985	3	0.522±0.37	215961	43	222978	3	0.422±0.13	216044
18	215987	3	0.322±0.06	215917	44	222980	3	1.167±0.27	215883
19	215991	3	2.567±0.06	222990	45	222990	3	0.833±0.20	216056
20	216044	3	2.033±0.60	222994	46	222991	3	1.455±0.29	217675
21	216053	3	3.000±0.40	219814	47	222992	3	1.344±0.29	215900
22	216054	3	1.133±0.54	219807	48	222994	3	0.844±0.14	215991
23	216056	3	2.156±0.40	221697	49	222999	3	1.378±0.16	217677
24	217674	3	1.467±0.69	216054	50	223001	3	0.589±0.07	216053
25	217675	3	2.211±0.05	222980	51	Total	150	1.246±0.06	
26	217677	3	2.711±0.35	215909					

Appendix 4F. Mean root length, standard error (SE) and performance ranking on 48 accessions and two standard checks grown under Bach- VI on nutrient solution culture under 112.5 µM Al concentration

SN.	Acc.	Rip	M±S.E	Ranking	SN.	Acc.	Rip	M±S.E	Ranking
1	Gute	3	1.944±0.23	223026	27	223035	3	0.744±0.23	245088
2	Necho	3	0.478±0.20	223039	28	223036	3	0.444±0.23	223017
3	223002	3	1.689±0.27	223024	29	223037	3	0.911±0.51	243639
4	223003	3	0.844±0.22	223027	30	223038	3	0.344±0.12	223025
5	223004	3	0.755±0.48	223006	31	223039	3	0.200±0.01	223007
6	223005	3	1.956±0.29	223014	32	223144	3	1.556±0.39	244798
7	223006	3	0.244±0.14	223033	33	243634	3	1.789±0.16	245087
8	223007	3	1.167±0.36	223028	34	243635	3	0.422±0.17	243637
9	223008	3	0.733±0.33	223016	35	243636	3	2.133±0.06	223029
10	223009	3	0.822±0.24	223038	36	243637	3	1.211±0.68	243644
11	223011	3	0.533±0.20	243635	37	243638	3	2.700±0.35	223144
12	223013	3	0.667±0.27	223036	38	243639	3	1.122±0.42	243641
13	223014	3	0.289±0.07	Necho	39	243640	3	1.711±0.57	223002
14	223016	3	0.322±0.09	223019	40	243641	3	1.599±0.38	243640
15	223017	3	1.100±0.21	223011	41	243642	3	2.022±0.09	243634
16	223018	3	0.878±0.48	245085	42	243643	3	1.989±0.02	245086
17	223019	3	0.522±0.16	223034	43	243644	3	1.556±0.19	Gute
18	223024	3	0.222±0.06	223013	44	244798	3	1.167±0.46	223005
19	223025	3	1.144±0.04	223008	45	245084	3	2.056±0.12	223031
20	223026	3	0.167±0.01	223035	46	245085	3	0.633±0.28	243643
21	223027	3	0.222±0.12	223004	47	245086	3	1.833±0.15	243642
22	223028	3	0.322±0.20	223009	48	245087	3	1.211±0.23	245084
23	223029	3	1.478±0.52	223003	49	245088	3	1.067±0.30	243636
24	223031	3	1.978±0.09	223018	50	245091	3	1.044±0.24	243638
25	223033	3	0.289±0.13	223037	51	Total	150	1.057±0.06	
26	223034	3	0.633±0.20	245091					

SN = serial number, Rip = replication, M±S.E = Mean ± Standard Error, Acc. = Accessions

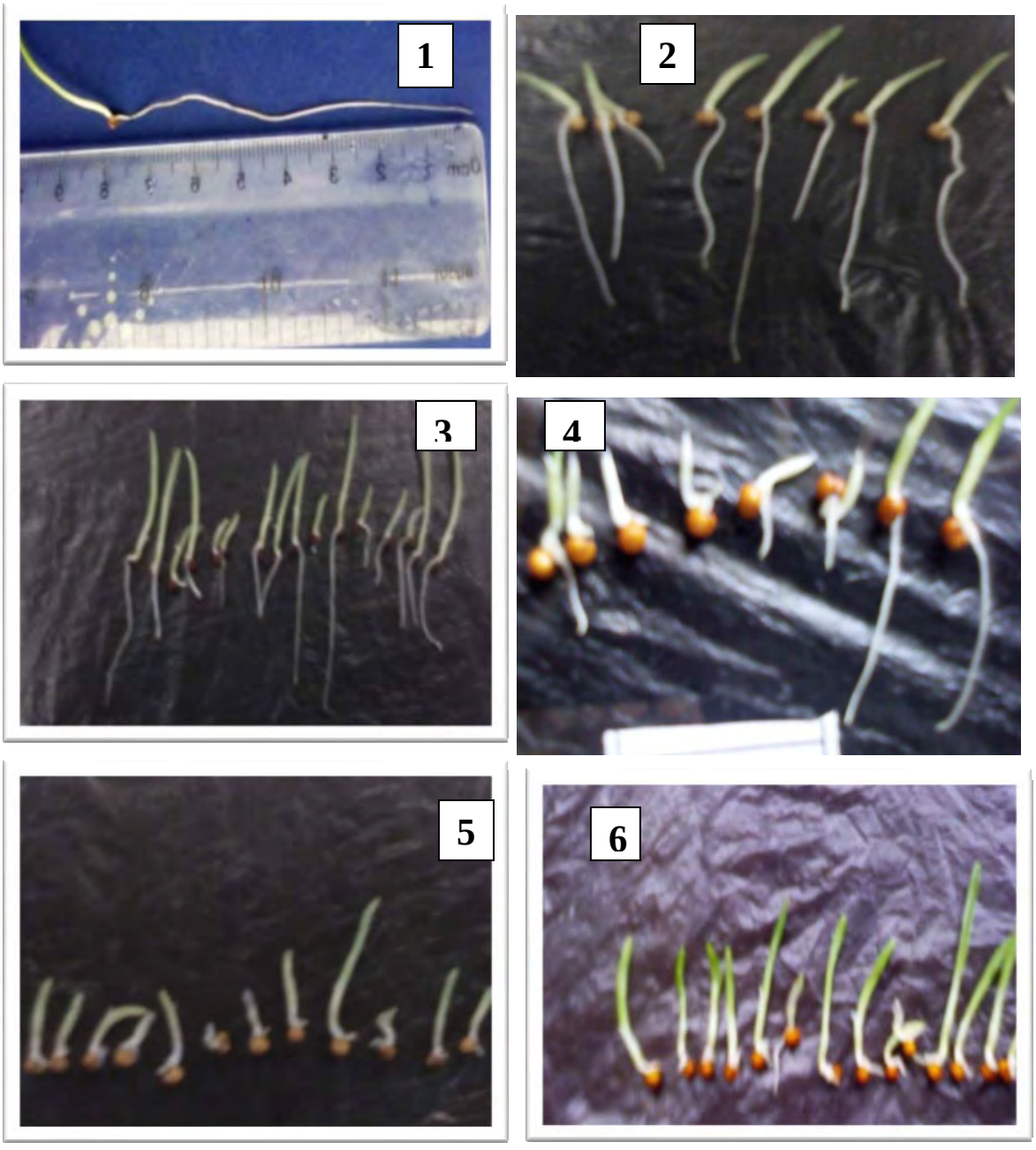
Appendix 5. Mean of Rl, SL and FW in characterization of Al tolerance 0 µM and 112.5 µM Al concentrations

	R.L		S.L		F.W	
Acc.	0 µM	112.5uM	0 µM	112.5µM	0 µM	112.5µM
100003	2.778	0.567	2.556	1.967	0.127	0.08
100011	2.256	1.289	1.633	1.211	0.116	0.075
100016	1.522	0.389	1.633	0.689	0.122	0.103
100033	1.789	1.144	2.889	1.378	0.147	0.068
100034	1.867	0.411	1.467	0.889	0.094	0.132
100035	3.378	1.656	0.933	0.36	0.116	0.057
100036	2.067	1.267	1.722	0.633	0.189	0.072
100045	2.8	1.311	1.489	1.156	0.099	0.071
100054	4.556	0.578	1.778	1.344	0.114	0.07
100055	3.2	1.333	1.533	0.611	0.076	0.087
100094	3.333	0.722	1.39	1.356	0.145	0.079
100095	2.233	1.222	1.422	1.111	0.127	0.1
100097	2.722	0.344	2.544	2.489	0.291	0.104
203054	3.511	1.556	1.967	1.267	0.186	0.062
203057	2.611	1.467	1.689	0.178	0.131	0.062
203058	2.933	1.044	1.689	1.3	0.103	0.077
203059	3.633	1.144	1.656	1.578	0.149	0.05
203062	2.989	1.233	1.556	0.667	0.139	0.054
203070	2.744	1.222	2.833	2.011	0.102	0.069
203071	3.022	1.556	1.556	0.722	0.106	0.117
203072	2.222	1.2	1.289	0.344	0.112	0.112
203247	2.933	0.378	2.178	1.833	0.108	0.07
203339	2.933	1.178	2.133	0.967	0.201	0.078
203438	2.967	0.9	2.211	1.111	0.137	0.084
203458	3.267	1.378	2.256	2.089	0.143	0.128
203467	5.022	0.956	2.189	1.611	0.291	0.067
203570	2.289	0.789	2.922	1.578	0.143	0.095
203582	3.3	0.689	2.111	1.667	0.188	0.121
203584	2.933	0.744	1.567	1.233	0.156	0.13
203586	2.767	0.767	1.789	1.278	0.187	0.116
203587	3.044	0.667	1.867	1.689	0.222	0.11
203590	2.156	0.644	2.544	1.733	0.159	0.134
207593	3.456	0.511	1.967	1.466	0.229	0.052
207897	2.867	0.711	1.689	1.4	0.119	0.051
207960	2.833	0.678	1.689	1.222	0.11	0.14
207961	3.011	0.522	1.656	1.367	0.108	0.104
207963	2.389	0.767	1.544	1.411	0.08	0.055

211473	3.556	1.6	1.889	1.333	0.202	0.073
211474	3.289	1.656	1.9	1.889	0.227	0.093

	R.L		S.L		F.W	
Acc.	0 µM	112.5uM	0 µM	112.5µM	0 µM	112.5µM
213034	2.656	1.344	1.567	1.222	0.12	0.13
213035	2.133	1.422	1.689	2.3	0.109	0.061
213227	2.744	1.1	2.1	2.011	0.151	0.059
215668	3.456	0.767	2.089	1.1	0.147	0.059
215805	2.333	0.467	3.111	1.667	0.126	0.069
215827	2.967	1.344	3.544	1.044	0.126	0.077
215828	2.656	1.467	2.967	1	0.15	0.09
215850	3.378	0.256	1.944	1.122	0.07	0.13
215877	2.444	0.889	2.3	1.011	0.163	0.153
215883	2.233	1.267	3.033	1.044	0.112	0.063
215884	3.122	0.344	1.689	0.678	0.14	0.089
215900	2.989	1.056	2.856	0.267	0.115	0.06
215991	2.422	1.6	2.833	1.5	0.129	0.084
216044	2.6	1.144	1.733	0.711	0.173	0.074
216053	2.667	0.944	2.8	0.744	0.158	0.064
216056	2.8	0.133	2.944	1.289	0.126	0.065
217675	2.8	1.311	2.3	1.033	0.134	0.118
219503	2.644	0.922	2.033	1.3	0.102	0.079
219815	3.289	1.911	2.8	1.578	0.162	0.06
219818	3.778	1.378	1.889	1.667	0.164	0.043
219819	2.567	1.333	2.533	2.011	0.144	0.033
219820	2.256	2.078	2.333	1.722	0.106	0.087
219821	2.744	1.533	1.889	1.344	0.114	0.132
219824	1.933	1.567	2.822	1.833	0.135	0.132
219834	1.944	1.089	1.533	0.967	0.119	0.117
219835	2.522	1.389	1.856	1.111	0.189	0.147
243637	3.022	0.422	1.467	1.089	0.108	0.136
243638	2.133	0.389	0.822	0.361	0.113	0.138
243640	2.3	0.244	1.533	1.278	0.138	0.144
243642	2.5	1.667	1.856	1.667	0.121	0.139
245084	2.189	1.689	2.122	1.233	0.095	0.144
245087	2.533	0.978	3.3	2.278	0.235	0.082
245091	2.867	1.611	2.456	1.689	0.167	0.05
Boneya	3.011	0.878	1.889	0.733	0.097	0.074
Degu	2.6	0.9	2.956	1.189	0.245	0.163
Gute	1.744	0.522	2.233	1.278	0.188	0.076
Necho	4.389	0.156	2.3	0.5	0.263	0.052
Tadesse	3.444	0.4	2.033	0.556	0.133	0.111
Wama	3.422	0.522	2.533	0.889	0.164	0.071

Appendix 6. Growth of finger millet under different Al concentrations



Key; 1 = plant growth under control, 2 = under 50 µM, 3 = under 100 µM and 4 =112.5 µM, 5= 150 µM and 6= 200 µM aluminium concentration.