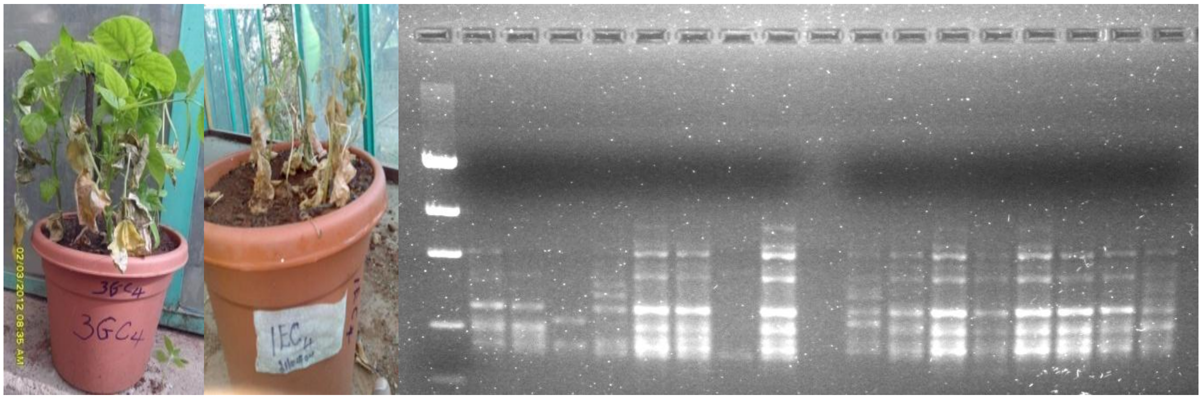
Addis Ababa University

School of Graduate Studies

Institute of Biotechnology

Genetic Diversity Study and Salt Tolerance Screening of Common Bean (*Phaseolus vulgaris* L.) Germplasm from Ethiopia



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ABSTRACT

Phaseolus vulgaris L. *(family Leguminosae), is a grain that provides protein. It is distributed in all parts of the world.* P. vulgaris *is a salt sensitive and one of a major export crop that can be grown in times of intermittent rainfall; also, it grows in the warm and low land areas of Ethiopia. Salinity as well as using less productive varaties make low production in Ethiopia. This research is aimed to investigate the salt tolerance and genetic diversity of P.* vulgaris *accessions from Ethiopia.The study was done on twelve accessions from Ethiopia. Shoot and Root Length (SL, RL) as well as Root Fresh and Dry Weight (RFW, RDW) were measured using 50, 100, 150, 200 mM NaCl salinity levels after germination.Tap water was used as a control. Studies were also carried out to estimate the genetic diversity among and within accessions of* P. vulgaris *using ISSR markers. Mostly, the growth of RL, SL, RDW and RFW were faster at 50 mM but they were significantly inhibited at high salt levels (150mM, 200mM). Accessions from Metekel (Dangure) and Harerge were found to be salt sensitive during shoot and root growth. However, accessions from Shinile and Metekel (Dibate) were relatively salt tolerant. The rest were intermediate. Besides, the highest RFW and RDW were recorded in West Wellega(F) variety at 200 mM NaCl. Seven selected ISSR primers yielded 69 reproducible bands from 96 individuals studied. All the loci were found to be polymorphic. The total genetic diversity (H) and Shannon's diversity information index (I) for entire populations showed, 0.35, and 0.53, respectively. Individuals from Shinile site showed the highest level of gene diversity (H = 0.29, I = 0.43); while the lowest variability was showen by accessions from Metekel (L) (H = 0.10, I = 0.15). Variation within accessions was higher (62.55%) as compared to that of inter accessions (37.45%) based on AMOVA. Accession from Harerge (K) was quite separated to the rest of other accessions in UPGMA analysis. The accession from Hadiya had a closer genetic similarity with that of West wellega (F) while the two germplasm resources from Metekel (G, L) were closely related to their geographical distribution. In PCO, majority individuals of Metekel (L) tended to form their own separate grouping. The result of the present study confirmed the presence of salt 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 P.* vulgaris.

Key words: Genetic diversity, Haricot bean, ISSR, *Phaseolus vulgaris,* Salinity

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LIST OF SYMBOLS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
CEC	Cation Exchange Capacity
cmoles/kg	cent moles of charge per kilogram
CTAB	Cetyltrimethyl Ammonium Bromide
dS/m	decisiemens per meter
EC	Electrical Conductivity
FAO	Food and Agricultural Organization
ISSR	Inter Simple Sequence Repeats
LSD	Least Significant Difference
MAS	Marker Assisted Selection
Meq/l	Milliequivalent per Liter
mmhos/cm	millimhos per centimeter
m.a.s.l	meter at sea level
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism

RFW	Root Fresh Weight
RDW	Root Dry Weight
ROS	Reactive Oxygen Species
SSR	Simple Sequence Repeat
SPSS	Statistical Package for Social Studies
SD	Standard Deviation
SE	Standard Error

1. INTRODUCTION

1.1. Background of the study

Common bean (*Phaseolus vulgaris* L.) also referred to as haricot bean, is a diploid (2n=22) annual leguminous plant that belongs to the genus *Phaseolus,* and it is characterized by pinnately compound trifoliate leaves. There are about 50 species under the genus *Phaseolus* (Heberge and Edwards, 1989). *P. vulgaris* was derived from wild ancestors distributed from Northern Mexico to Northwestern Argentina (Ibarra-Perez *et al*., 1997; Debouck, 1999).

The ancestors of *P. vulgaris* appear to have formed two distinct gene pools one in Mesoamerica (Mexico and Central America) and the other in the Southern Andes, a mountain range in South America, (Zeven, 1997; Gepts and Bliss, 1988; Blair *et al*., 2007; Burle *et al*., 2010). An ancestral wild form is still found at the boundary between temperate and subtropical dry climatic regions (Debouck, 1999). They were brought to Europe and Africa during the 16th century by returning Spanish and Portuguese explorers (Gepts and Debouck, 1991; Ibarra-Perez *et al*., 1997). Since that time, it has been developed into many different forms through selective breeding by local farmers. About 30% of the current world production comes from Mexico, Central and South America, and significant quantities are grown in Asia and Africa (Ibarra-Perez *et al*., 1997; Burle *et al*., 2010). In Ethiopia, common beans are concentrated in the dry and warmer parts of the country mainly along the Rift Valley (Alemu Demelash and Bekele Adam, 2005). They are adapted to the low and mid altitude areas at an altitudunal range of 900-2100 m.a.s.l. Besides, common beans grow in rainfall range of 200-600 mm per annum (IFPRI, 2010).

Common beans are largely a self-pollinated plant though cross pollination is possible (Ibarra-Perez *et al*., 1997). Their seeds are non-endospermic and vary greatly in size and color from the small black wild type to the large white, brown, red and spotted types (Cobley and Steele, 1976). It shows variation in growth habits from determinate bush to indeterminate extreme climbing types. The bushy type is the most predominant bean grown in Africa (Buruchara, 2007). Common bean is the main grain legume crop grown in low land and mid altitude area of Eastern and Southern Africa.

Nutritionally, common bean contains high protein content, a good source of energy and also provides folic acid, dietary fibre and complex carbohydrates (Edje *et al.*, 1980; Sousa and Delgado, 1993; Svetleva *et al.*, 2006). Its protein is high in lysine, which is relatively deficient in maize, cassava and rice making it a good complement to the diet made from these staples. Consumption of common bean is high because it is relatively inexpensive compared to meat (Pachico, 1993). For the poor, it plays a strategic role in alleviating malnutrition and for other health related functions. 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 salinity is one of a major abiotic constraint to crop productivity especially in arid and semiarid regions. Majority of the salinity problems occured from irrigated fields which are rich in NaCl (FAO, 2008; Ahmed, 2009).

Most of the water on earth contains about 30 g of sodium chloride (NaCl) per liter. This salt solution has affected, and continues to affect, the land on which crops are, or might be grown (Flowers, 2004). As a result, soil salinity is identified as one of the major environmental constraints limiting agricultural production all over the world (FAO, 2008; Ahmed, 2009). In addition, fresh water resources are becoming inadequate due to the competition for human consumption and industrial use rather than for agriculture. Therefore, use of low saline water is a subject of increasing interest. Salinity effect also seems to depend on other factors such as soil properties, climate, cultural practices and water management (Ahmed and Fallah, 2008).

The effect of soil salinity is observed as a cause of either loss of plant productivity or death (Yokoi *et al.,* 2002; Khan and Panda, 2008). Also, it exerts its undesirable effects through osmotic inhibition and ionic toxicity (Munns *et al*., 2006). Increased salinity caused a significant reduction in germination percentage, germination rate and root and shoots length and fresh root and shoots weights (Jamil *et al*., 2006). Although soil salinity existed long before humans began agriculture, the problem has been aggravated by agricultural practices such as irrigation (Mamo *et al*., 1996). According to Zhu (2001), about 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity.

Salt tolerance in plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes (Khan *et al*., 2002; Khan and Panda, 2008). According to Michael (1997), a continuous spectrum of plant tolerance to saline conditions is found ranging from glycophytes that are salt sensitive to halophytes that are salt tolerant. Among crop species, different threshold tolerances (EC, electrical conductivity) and different reduction rates of yield have been seen and this indicates that there is variation for salt tolerance mechanisms (Marler and Mickelbart, 1993; Gol, 2006; Mahmood *et al*., 2009). For example bean, eggplant and onion are very sensitive to low EC values. Tomato and rice are moderately sensitive to salt stress. Whereas, barley, cotton and wheat show tolerance to high EC values (Michael, 1997).

The semi-arid, arid and rift valley areas in Ethiopia have encountered a major problems of salinity and alkalinity. A study by Tamirie Hawando (2000) has revealed that 44 million ha (36%

of the country’s total land area) is potentially susceptible to salinity problems. Out of the 170,000 ha under irrigation in Awash Valley and in Central Rift-Valley lake area, almost 10% (11,000 ha) are feared to have been salinized and have already gone out of production.

This problem is expected to be severe in years to come; since under the current situation of the Ethiopia, there is a tendency to introduce and implement large scale irrigation agriculture so as to increase productivity (Mamo *et al*., 1996). In the absence of efficient ways of irrigation water management, salt build up is an expected problem. To alleviate the problem, screening germplasm for salt-tolerance to sustain a reasonable yield on salt affected soils has been adopted by scientists to overcome salinity problems (Marler and Mickelbart, 1993; El-Sayed and El-Sayed, 2011a; Mahmood *et al*., 2009).

Moreover, several biotechnological approaches are available to enhance biotic and abiotic stress tolerance in legumes, including marker assisted breeding, tissue culture and gene transformation (Coba *et al*., 2011). Numerous molecular marker techniques have also been used in legumes; such as, Random Amplified Polymorphism (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Inter Simple Sequence Repeat (ISSR) and their derivatives (Kassem *et al*., 2004).

The aim of this study is therefore, to carry out comparative evaluation of salt tolerance ability of different common beans cultivars and to analyze their responses to salinity stress with regard to growth, root and shoot characteristics. In addition, this study aims to investigate the genetic diversity of *P. vulgaris* populations collected from Ethiopia using ISSR markers. This will provide information on the overall genetic variability of *P. vulgaris* populations which may assist in the identification and selection of the genetic materials for conservation and use in salt salt affected regions of Ethiopia.

1.2. Significance of the study

According to Kinfemichael Geressu (2011), in Ethiopia salt affected soils are prevalent in the Rift Valley and the lowlands. The Awash Valley in general and the lower plains in particular are dominated by salt affected soils (Girma Taddese *et al*., 2002). Another study also showed that there is salinity problem in the irrigated fields in the Northern Highlands of Ethiopia (Fassil Kebede, 2009). Therefore, as *P. vulgaris* grows in most of the salinity prone regions of the country, this research will attempt to investigate its salt tolerance ability, an important abiotic stress that affects the productivity of many crops.

Besides, there was little or no genetic diversity study on Ethiopian *P. vulgaris*, particularly using molecular markers. In this study, we assess the genetic diversity of common bean accessions collected from different parts of Ethiopia where it is most grown as food and cash crop. The reason for selecting haricot bean is because the crop can be grown in times of intermittent rainfall, in low and irrigated areas and also it is one of the major export crops of the country. In general, previous reports on salt tolerance and genetic study of haricot bean are relatively few and this study attempts to shed some light on both aspects which could be important in selective improvement of some of the varieties for salt tolerance in the future.

2. LITERATURE REVIEW

2.1. Salinity and its stress on crops

Soil salinity is defined as a measurement of the total amount of soluble salt in soil. It can also be defined as a soil that has enough soluble salts to impair plant productivity (Flynn and Ulery, 2011). Salinity is one of the major limitations of plant growth which is observed as either loss of productivity or plant death and it extensively exists in arid and semiarid climate regions of the world **(**Yokoi *et al.,* 2002; Khan and Panda, 2008; Janmohammadi *et al.*, 2011**)**. Too much salt is unhealthy for plants. Mostly, in the root zone it interferes with the uptake of water and can cause death.

Salts in soil can originate from soil parent material, from irrigation water, from fertilizers, manures, composts, or other amendments. The predominant salts that accumulate in soils are salts of calcium, magnesium, sodium, potassium, sulfate, chloride, carbonate and bicarbonate. Any salt that accumulates in excessive amounts in soil can cause plant growth problems. Soils can be classified as saline when the EC is 4 dS/m or more (Foolad, 2004), which is equivalent to approximately 40mM NaCl. This definition of salinity derives from the EC that significantly reduces the yield of most crops.

The standard practice for determining how salty soils is to make a saturated paste of the soil with water, and then extract the water from the soil paste after 24 hours (Flynn and Ulery, 2011). It is the best for evaluating crop's response to soil salinity because it is related to the field conditions (Bashour and Sayegh, 2007).

2.2. Tests to quantify and qualify soil saltiness

Electrical conductivity is used to measure salt content of water based solutions (Flynn and Ulery, 2011). Pure water does not conduct electricity, but if the lowest possible amount of salt is present, electricity is conducted through the solution. A conductivity meter is used to measure how well electricity is conducted in a solution of water. This is referred to as" EC", which is short for electrical conductivity. Units of measure are either millimhos per centimeter (mmhos/cm) or decisiemens per meter (dS/m) which are synonymous (1dS/ m/ = 1mmhos /cm).

These are electrical units that describe how well electricity moves through solution. So, conductance measured over a known distance, and in soil extracts are reported as mmhos/cm. Readings of EC do not tell us what kind of salts is present in the soil but give us an idea of the amount of salt in the soil**(**Bashour and Sayegh**,** 2007**;** Horneck *et al*., 2007).

2.2.1. Cation-Exchange-Capacity (CEC) of the Soil

It is the capacity of a soil to adsorb and exchange cations. CEC is a measure of the net negative charge of a soil. Measured as the total quantity of cations that can be exchanged on a unit of soil material, expressed as milliequivalents per 100 grams of soil (meq/100 g), milli moles of charge per kilogram of soil (mmol [+ charge]/kg) or centimoles of charge per kilogram of soil (cmole [+ charge]/kg) (Bashour and Sayegh, 2007).

2.3. Effects of salt stress on plants

Salinity stress is a critical environmental constraint to crop productivity especially in arid and semiarid regions. Most of the crops are intolerable to high salinity conditions which results in decreased yield (Rahman *et al*., 2008). Generally, an effect of salt stress on plants is categorized as follows.

2.3.1. Osmotic effect

Excess salts in the root zone reduce the amount of water available to plants and cause the plant to expend more energy to exclude salts and take up pure water (Figure 1). Additionally, if salinity in the soil solution is great enough, water may be pulled out of the plant cell to the soil solution causing root cells to shrink and collapse (McCauley and Jones, 2005). The effect of these processes is ‘osmotic’ stress for the plant. Osmotic stress symptoms are very similar to those of drought stress, and include stunted growth, poor germination, leaf burn, wilting and possibly death (Ogle and John, 2010).

The visual symptoms that appear in salty soils are the same as those of moisture stress soils in dry conditions. Plants may be small in size, leaves may cup like and overall plant health and color are affected. In both cases the symptoms may progress to brown and brittle leaf tips, leaf margins, the overall leaf and finally the entire plant. These symptoms may occur within a few days of planting young seedlings or after several weeks. With older plants, water deficit may present as a rapid dying off or browning of leaves at the top of the plant (Ogle and John, 2010).

In general, the presence of salt in soil solution decreases the osmotic potential of soil which creates water stress and makes it difficult for the plant to absorb sufficient water for growth. This results in the immediate reduction in cell expansion in root tips and young leaves, and causes stomatal closure (Munns and Tester, 2008).

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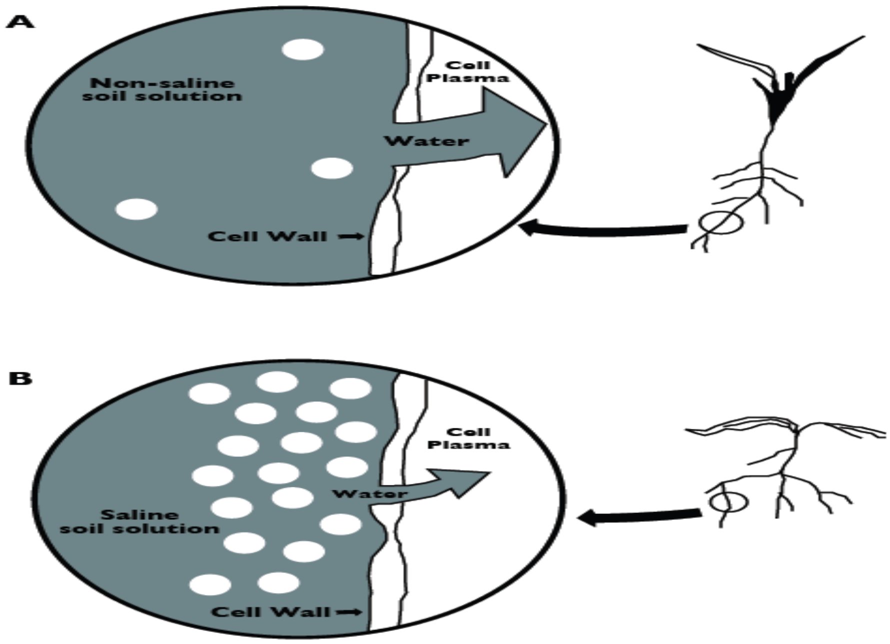

Figure1. Effect of salts on water uptake by plants. Water uptake by a plant in a non-saline soil (A), and uptake in a saline soil (B) (McCauley and Jones, 2005)

2.3.2. Nutrition and ion toxicity of salt

Excessive amount of soluble salts in the root environment causes osmotic stress, which may result in the disturbance of the plant water relations in the uptake and utilization of essential nutrients, and also toxic ion accumulation. As a result of these changes, the activities of various enzymes and the plant metabolism are affected (Nawaz *et al*., 2010). Specific ions such as sodium, chloride and boron have toxic effects on plants: reducing plant emergence and growth or causing damage to cells and membranes. Plants sensitive to these elements may be affected at relatively low salt levels if the soil contains enough of the toxic element (Joe, 2002). According to Munns and Tester (2008), the main site of Na^+ toxicity for most plants is the leaf blade, where Na^+ accumulates after being deposited in the transpiration stream rather than in the roots. A plant transpires 50 times more water than it retains in its leaves , so excluding Na^+ from the leaf blades is important, even more so for perennial than for annual species, because the leaves of perennials live and transpire for longer.

Moreover, ions such as Na^+ can influence soil chemistry and biology to such a degree as to limit plant nutrient availability by disrupting the uptake and utilization of other minerals needed by the plants (Nawaz *et al*., 2010). This can cause disorders in mineral nutrition. Because many salts are also plant nutrients, high salt levels in the soil can upset the nutrient balance in the plant or interfere with the uptake of some nutrients.

2. 3. 3. Structure and permeability problems of salts on soil

Certain ions negatively influence soil structure and permeability characteristics by retarding plant growth (Joe, 2002). For instance, too much sodium causes problems related to soil structure, as sodium percentage increases so does the risk of dispersion of soil aggregates (Horneck *et al*., 2007). Moreover, salt affected soils, like sodic and saline-sodic, show structural problems which are created by certain physical processes (slaking, swelling and dispersion of clay) and specific conditions (surface crusting and hard setting). Such problems may affect: water and air movement, plant water holding capacity, root penetration, seedling emergence, runoff and erosion, as well as tillage (Bohm *et al*., 2005).

2. 4. Mechanisms of salt tolerance in plants

Generally, there are two main types of salt tolerance mechanisms for the specific effects of salinity: those minimizing the entry of salt into the plant and those minimizing the concentration of salt in the cytoplasm (Nawaz *et al.*, 2010). Therefore, Plants have different mechanisms to tolerate high salt concentrations in the soil. This contains cellular homeostasis which includes ion homeostasis and osmotic adjustment, detoxification and growth regulation (Khan *et al*., 2002; Gol, 2006; Khan and Panda, 2008). These mechanisms are described in the following sections.

2.4.1. Homeostasis

The homeostasis of intracellular ion concentrations is fundamental to the physiology of living cells. Proper regulation of ion flux is necessary for cells to keep the concentrations of toxic ions low and to accumulate essential ions. Plant cells employ primary active transport, mediated by H^+-ATPases, and secondary transport, mediated by channels and co-transporters, to maintain characteristically high concentrations of K^+ and low concentrations of Na^+ in the cytosol. Intracellular K^+ and Na^+ homeostasis is important for the activities of many cytosolic enzymes, and for maintaining membrane potential and an appropriate osmotic potential for cell volume regulation (Zhu, 2003).

Mechanisms of ion homeostasis and water/osmotic homeostasis attempt to restore the cellular ion or water content levels similar to unstressed conditions (Verslues *et al*., 2006). One mechanism of coping with the ionic stress imposed by high salinity is avoidance of salt accumulation in plant parts where they are harmful. This can be done by increasing the Na^+ efflux at the plasma membrane, passive exclusion of ions from the cytoplasm by a permeable membrane or dilution of ions in the plant tissue. Another metabolic process for protection from ionic stress is the storage of Na^+ in the vacuoles. This is an ideal way of preventing Na^+ toxicity in the cytosol. Therefore, Na^+ provides osmotic adjustment to plant cells (Verslues *et al*., 2006). Halophytes, naturally salt tolerant plants, generally use this strategy (Flowers *et al*., 2010). Moreover, an increase of vacuolar volume under salt stress is an adaptive mechanism of plant cells to salinity.

The compartmentalization of Na^+ into the vacuoles is an important mechanism to prevent negative effects of Na^+ in the cytosol. It is also a cost-effective strategy since Na^+ contributes osmotic adjustment to plant cells. Positive turgor should be maintained for cell growth and stomatal opening. Under saline conditions, the water potential of the soil decreases because of osmotic stress and this causes turgor loss in cells. To take up water, plants have an osmotic adjustment mechanism which maintains turgor under osmotic stress. Generally, salt-sensitive plants mainly exclude the Na^+ ions out of the plasma membrane, however salt-tolerant plants use the Na^+ compartmentation strategy (Gol, 2006).

2.4.2. Detoxification

Oxidative stress is an important aspect of salinity stress in plants in addition to water and ionic stress. This secondary effect appears as a consequence of hyper-osmolarity which is caused by imposing plants to drought or salt stress conditions (Dajic, 2006). The formation of Reactive Oxygen Species (ROS) such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($^1O2$) is a normal function of aerobic metabolism. The types of cellular activities that generate ROS include photorespiration, oxidation of fatty acids, and mitochondrial and chloroplast electron transport. However, ROS production increases under abiotic stresses including salinity (Xiong and Zhu, 2002). Under normal conditions the negative effects of ROS can be eliminated. However under stress, induced production of reactive oxygen species strikes a balance between oxidants and antioxidants. The excessive ROS can damage proteins, lipids and nucleic acids (Verslues *et al.*, 2006). Hence, the alleviation of this oxidative damage is important in defense against abiotic stresses. Antioxidant compounds (non enzymatic antioxidants) such as ascorbic acid, glutathione, thioredoxin, carotenoids and ROS scavenging enzymes such as superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), and glutathione peroxidase (GPX) are employed by plants to eliminate ROS. The activity and expression level of the genes that encode the ROS scavenging enzymes are increased under abiotic stresses.

2.4. 3. Growth regulation

Plants differ greatly in their tolerance to salinity as reflected in their different growth responses (Ghogdi *et al.*, 2012*)*. They respond by undergoing slow growth for stress, because plants use a limited resource for building blocks and energy during stress. However, others are sensitive and they continue growing, which can cause plant death. Thus, differences in percent biomass production in saline and control conditions can be used as an assessment of salt tolerance. For example, sugar beet as a salt tolerant species might show a 20% reduction in dry weight, cotton as a moderately salt tolerant species might show a 60% reduction and soybean as a sensitive species might be dead in 200mM NaCl (Gol, 2006). Furthermore, the concentrations of plant growth promoting hormones involved in cell elongation such as auxin, cytokinin, gibberellins and brassilonides are reduced (Gama *et al*., 2007).

Several studies such as genetic variability of cultivated *Phaseolus* bean cultivars exposed to salinity at germination stage, seedling stage and early vegetative growth have been conducted, and characters like yield, survival, vigor, leaf damage and plant height, have been the most commonly used criteria for identifying salinity tolerance. Other indices of tolerance have also been proposed that are based on specific physiological characteristics; for instance, accumulation of specific ions in shoots or leaves, or production of a specific metabolite (Gama *et al*., 2007).

2. 5. Salinity tolerance of common crops

In a wide variety of plants salt tolerance is seen depending on several factors such as the species of the plant, and they show a continuous spectrum of tolerance. This ranges from glycophytes that are salt sensitive to halophytes that are salt tolerant (Michael, 1997; Dajic, 2006). Many important crops are glycophytes and show susceptibility to soil salinity. Among this, different threshold tolerances (EC) and different reduction rates of yield have been seen and this indicates that there is variation for salt tolerance mechanisms (Marler and Mickelbart, 1993; Gol, 2006; Mahmood *et al*., 2009; Flynn and Ulery, 2011). There are great differences in the level of salt stress tolerance within both the halophytes and non-halophytes, which include sensitive, moderately tolerant and very tolerant species. Although halophytes represent only 2% of the terrestrial plant species, they are present in about half the higher plant families and exhibit a great diversity of plant forms (Dajic, 2006).

One of the most effective ways to overcome salinity problems is the use of salt tolerant species and varieties (Marler and Mickelbart, 1993; Mahmood *et al*., 2009; Ezin *et al*., 2010). The response of plants to increasing salt application may differ significantly among plant species as a function of their genetic tolerance. Plants generally respond to soil salinity with reduced growth and transpiration rates. However, not all plants respond the same to soil salinity because of numerous adaptations and biological processes that can improve plant tolerance to soil salinity.

The salinity level at which plants are first affected by salinity is referred to as the “threshold.” The threshold varies with crop tolerance and external environmental factors that affect the plant’s need for water. The rate at which the yield decreases as salinity increases is the “slope” (Marler and Mickelbart, 1993; Mahmood *et al*., 2009; Flynn and Ulery, 2011). The threshold and slope reflect how sensitive the plant is to salinity and such data can help us predict how well a plant will grow in that soil.

Therefore, salinity tolerance of common crops is summarized in Table 1 (Gol, 2006). The first three plants, bean, eggplant and onion, are very sensitive to low EC values whereas tomato and rice are moderately sensitive, but barley, cotton and wheat show tolerance to high EC values.

Table 1. Salinity tolerance of common crops (Gol, 2006)

Crop	Threshold salinity dSm/	Decreased in yield Slope % per dS/m
Bean (*Phaseolus vulgaris* L.)	1.0	19.0
Eggplant (*Solanum melongena* L.)	1.1	6.9
Onion (*Allium cepa* L.)	1.2	16.0
Pepper (*Capsicum annuum* L.)	1.5	14.0
Corn (*Zea mays* L.)	1.7	12.0
Sugarcane (*Saccharum officinarum* L.)	1.7	5.9
Potato (*Solanum tuberosum* L.)	1.7	12.0
Cabbage (*Brassica oleracea* var. *capitata* L.)	1.8	9.7
Tomato (*Lycopersicon esculentum* Mill.)	2.5	9.9
Rice, paddy (*Oriza sativa* L.)	3.0	12.0
Peanut (*Arachis hypogaea* L.)	3.2	29.0
Soybean [*Glycine max* (L.) Merr.]	5.0	20.0
Wheat (*Triticum aestivum* L.)	6.0	7.1
Sugar beet (*Beta vulgaris* L.)	7.0	5.9
Cotton (*Gossypium hirsutum* L.)	7.7	5.2
Barley (*Hordeum vulgare* L.)	8.0	5.0

2.6. Common bean production in Ethiopia

Common beans are an important traditional pulse in Ethiopia particularly in the Rift Valley, Hararghe highlands and Southern Region (Alemu Demelash and Bekele Adam, 2005; IFPRI, 2010). In these areas farmers grow white beans mainly for export purposes. In addition, common beans are not the only important cash crops but also the cheapest source of plant protein for farmers in the dry areas (Edje *et al.*, 1980; Kidane Georgis *et al*., 2009). Farmers in Ethiopia as well as in many other countries in East, Central and Southern Africa are cropping common bean as a basic agricultural practice and it is also their main diets source (CIAT, 2003).

In the Rift Valley of Ethiopia, more than 90% of beans are marketed whereas in the eastern zone the crop is grown both for food and cash (CIAT, 2003). Common bean production areas in Ethiopia can be broadly classified into four agro-ecological zones: the Central, Eastern, Southern and Western. This is based on altitudes, rainfall, soil, production systems and geographical locations. Production constraints, both biotic and abiotic are specific though some e. g. local varieties with low potential yield and susceptibility to pest and diseases are common to all zones (CIAT, 2003).

Generally, twelve pulse species are grown in the Ethiopia. Of these, faba bean (Vicia *faba* L.), field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens cultinaris* Medik.), grass pea (*Lathyrus sativus* L.), fenu greek (*Trigonella foenum-graecum* L.) and lupine (*Lupinus albus* L.) are categorized as highland pulses and grown in the cooler highlands. Conversely, common bean (*P. vulgaris* L.), soya bean (*Glycine max* L.), cowpea (*Vigna unguiculata* L.), pigeon pea (Cajanus cajan L.) and mung beans are predominantly grown in the warmer and low land parts of the country. Among the individual varieties, faba beans (broadly known as horse beans) accounts for the greatest portion of production, at 36 percent, followed by common bean (17 percent) and chickpeas (16 percent). Other pulses (e.g., lentils, peas, lupines, and mung beans) account for the remaining 32 percent (CSA, 2008; MOARD, 2008).

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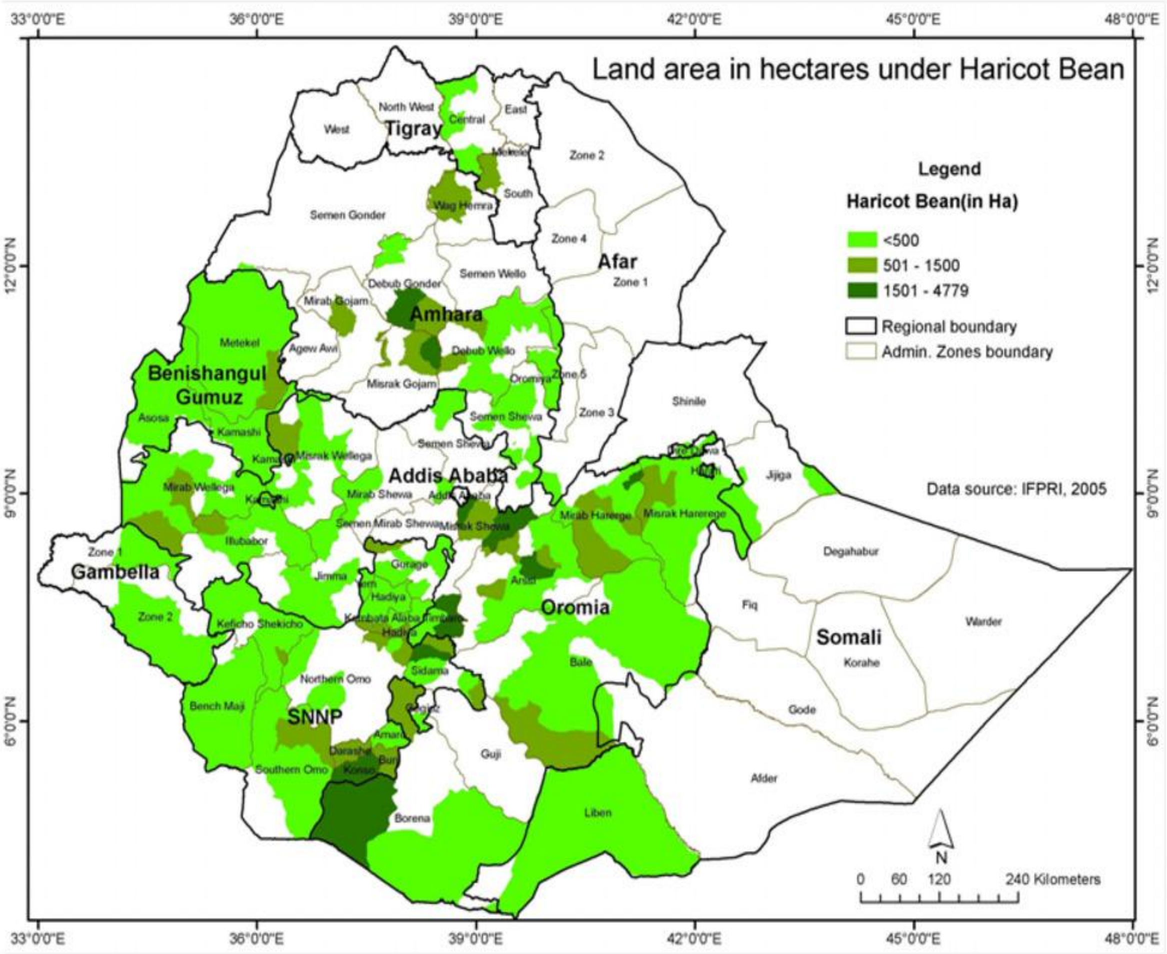

Figure 2. Geographic distribution of common beans in Ethiopia (IFPRI, 2010)

2.7. Salt affected areas in Ethiopia

Ethiopia is reported to possess over 11 million hectares of unproductive naturally salt affected wastelands (Tadelle Gebresellassie, 1993). The naturally salt affected areas are normally found in the arid and semiarid lowlands, in the Rift Valley and other areas that are characterized by higher evapo-transpiration rates in relation to precipitation (Leipzig, 1996; Tamirie Hawando, 2000). Soil salinity and low moisture are potential environmental problems and production constraints particularly in the arid zone. In general, this zone is liable to be converted into a desert if the current human activities continue intensely and the drought conditions continue (Leipzig, 1996; Mamo *et al*., 1996).

High salinity in the Ethiopian ground waters is apparent in some parts of the Rift because of the influence of saline geothermal waters. In the southern parts of the Rift, sodium and bicarbonate (high alkalinity) are the dominant dissolved constituents. High concentrations of dissolved salts in the ground waters from the sedimentary formations are also common as a result of reaction of abundant evaporate minerals. In these, high salinity may be manifested by high concentrations of sodium, chloride and/or sulphate in particular. Observed increased salinity in many ground waters from sediments in the south, southeast and northeastern parts of the country arises from the dissolution of evaporite minerals (the products of evaporation) in certain horizons of the sediments. Areas of the Rift affected by geothermal waters, as well as saline ground waters from the evaporate bearing sediments of eastern and northern Ethiopia may have increased concentrations of boron, although no data are available (MacDonald *et al*., 2001).

2. 8. Developing salt tolerant crops

To date, there is a growing need to produce salt tolerant crops as soil salinity is becoming one of the major constraints to agricultural productivity throughout the world. To achieve this, several methods for producing salt tolerant crops have been extensively tested. Two main approaches are employed to improve salt tolerance. One of them is the use of natural genetic variation by using direct selection in stressful environments or mapping quantitative trait loci and subsequent marker-assisted selection. Another approach is the generation of transgenic plants (Scianna and Logar, 2000; Coba *et al*., 2011).

There is limited success in attempts to improve salt tolerance of crops under salinity because of the necessity for complete understanding of the fundamental stress tolerance mechanism in plants. Thus, only a few examples of salt tolerant crops that have been developed exist in salinity research literature. The physiological and genetic complexity of the trait causes this difficulty (Scianna and Logar, 2007). Halophytes and glycophytes that show different salt tolerance levels are evidence that salt response is based on a genetic foundation (Marler and Mickelbart, 1993; El-Sayed and El-Sayed, 2011). Studies with barley, citrus, rice and tomato indicate that salt tolerance has the characteristics of a multigenic trait involving the functions of many genes (Flowers, 2004). Furthermore, the functions of these genes are influenced by several environmental factors.

Another important feature of salinity tolerance in plants is that it appears to be a developmentally regulated and stage specific phenomenon. Salinity tolerance varies according to the stage of plant development. There seems to be no correlation between tolerances of different ontogenic stages. For example, salt tolerance in tomato, barley, corn, rice and wheat increases with the age of the plant (Foolad, 2004). Different QTLs for salt tolerance at germination stage and at the early stage of growth were identified in barley (Mano and Takeda, 1997), in tomato (Foolad, 2004) and in Arabidopsis (Quesada, *et al*., 2002).

The response of plants to salt stress is controlled by many genes and the functions of these genes cause a wide variety of biochemical and physiological changes. Compartmentalization of toxic ions in the vacuole, activation of detoxifying enzymes, synthesis of late embryogenesis abundant proteins and accumulation of compatible solutes are examples of the roles of these genes (Bajaj *et al*., 1999; Serrano *et al.,* 1999). This polygenic feature of plant's response to salt stress makes it difficult to transfer individual genes using traditional plant breeding or MAS. Transgenic approaches have been employed to obtain genetically modified plants that are tolerant to salt stress. Over expression of genes involved in tolerance related physiological mechanisms is the main approach used in genetic engineering of this trait (Rontein *et al*., 2002).

Osmotic compatible solutes provide osmotic adjustment and these solutes are helpful in improving plant stress tolerance. The genes encoding enzymes that have roles in enhancing the synthesis of compatible solutes were engineered. For example, Thomas *et al*. (1995) worked on mannitol, Lilius *et al*. (1996) on glycine betaine, Zhu *et al*. (1997) on proline and Galston *et al*. (1997) on polyamines. Over expression of different vacuolar antiport proteins, which provide the exclusion of toxic ions from the cell cytosol were also studied and transgenic plants have been produced with enhanced stress tolerance (Zhang *et al*., 2001). Moreover, detoxifying enzymes, which decrease the harmful effects of oxidative stress, were studied and transgenic plants have been produced (Zhang and Blumwald, 2001).

2. 9. Genetic markers for diversity analysis

Knowledge of genetic variation and genetic relationship among genotypes is an important consideration for classification, utilization of germplasm resources and breeding. Without determining the diversity reliably, it would not be possible to identify molecular markers or qualitative trait associations.

A genetic marker is any visible character or else assayable phenotype, for which alleles at individual loci segregate in a Mendelian manner (Weising *et al.*, 2005). These markers can be used to study the genetics of organisms at the level of genes (Spooner *et al.,* 2005). These biological markers could be of many different types but they can mainly be classified into morphological, biochemical and molecular types. The morphological (classical or visible, agronomic traits) markers are phenotypic traits, whereas; biochemical markers are based on gene product. The DNA (molecular) marker which reveals sites of variation in a DNA is a marker with a better resolution of diversity. Each type of marker system has its own merits and demerits. So, marker selection is dependent on the type of species, availability, cost and benefit analysis (Winter and Kahl, 1995; Semagn *et al.*, 2006).

2.9.1. Morphological marker

Morphological traits were among the earliest markers used in germplasm management (Stanton *et al*., 1994). They are usually visually described phenotypic characters such as flower color, seed shape, growth habits or pigmentation (Winter and Kahl, 1995).

Conventionally, classification of the various subgenera, species, and subspecies is based primarily on morphological attributes. However, these traits may not be significantly distinct and usually require growing plants to maturity prior to identification. Moreover, morphological characters may be vulnerable to environmental influences (Muthusamy *et al*., 2008). Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Despite the problems associated with this method, it continues to play a major role in studying and characterizing germplasm since it requires no complicated laboratory facilities and procedures.

2.9.2. Biochemical markers - allozymes (isozyme)

Isozymes are defined as structurally different molecular forms of an enzyme with, qualitatively, the same catalytic function. They originate through amino acid alterations, which cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules and also, their electrophoretic mobility. After specific staining the isozyme profile of individual samples can be visualized (Hadacova and Ondrej, 1972; Vallejos, 1983).

Allozymes are allelic variants of enzymes encoded by structural genes. Enzymes are proteins consisting of amino acids, some of which are electrically charged. 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, co-factors 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 iso loci. Therefore, allozyme variation is often also referred to as isozyme variation (Kephart, 1990 and May, 1992), isozymes have been proven to be reliable genetic markers in breeding and genetic studies of plant species (Heinz, 1987) due to their consistency in their expression, irrespective of environmental factors.

2.9. 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. Analogically, Jim (2001) describes the function of molecular markers as: just as a smoke rising into the sky makes it easier to locate a forest fire, a gene of interest is easier to locate when a researcher starts with a nearby marker.

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 (Paterson, 1996). Molecular markers are neutral because they are usually located in non-coding regions of DNA (Collard *et al*., 2005).

An ideal molecular marker must have some desirable properties such as: highly polymorphic nature; co-dominant inheritance; frequent occurrence in genome; selective neutral behaviors, that the DNA sequences of any organism are neutral to environmental conditions or management practices; easy access (availability); easy, fast and cheap to detect; easy and fast to assay; high reproducibility; and easy exchange of data between laboratories.

It is extremely difficult to find a molecular marker, which would meet all the above criteria. However, a wide range of molecular techniques are available that detects polymorphism at the DNA level. Depending on the type of study to carryout, a marker system can be identified that would fulfill at least a few of the above characteristics (Weising *et al*., 1995). 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.

2.9.3.1. Non PCR-based markers (hybridization-based markers)

In hybridization-based markers, DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA, to a labeled probe, which is a DNA fragment of known origin or sequence.

Restriction Fragment Length Polymorphism (RFLP): is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of particular restriction endonucleases, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another. This technique is mainly based on the special class of enzyme i.e. restriction endonucleases.

RFLPs are generally found to be moderately polymorphic. In addition to their high genomic abundance and their random distribution, they have the advantages of showing co-dominant alleles and having high reproducibility. In addition, RFLP markers were used for the first time in the construction of genetic maps by Botstein *et al*. (1980). On the other hand, the utility of RFLPs has been hampered because large quantities (1-10 µg) of purified, high molecular weight DNA are required for each DNA digestion and Southern blotting. Larger quantities are needed for species with larger genomes, and greater numbers of times are needed to probe each blot. The requirement of radioactive isotope makes the analysis relatively expensive and hazardous. The assay is time-consuming and labor-intensive and only one out of several markers may be polymorphic, which is highly inconvenient especially for crosses between closely related species. Their inability to detect single base changes restricts their use in detecting point mutations occurring within the regions at which they are detecting polymorphism.

RFLPs can be applied in diversity and phylogenetic studies ranging from individuals within populations or species, to closely related species. They have been widely used in gene mapping studies because of their high genomic abundance due to the plentiful availability of different restriction enzymes and random distribution throughout the genome (Neale and Williams, 1991). They also have been used to investigate relationships of closely related taxa (Miller and Tanksley, 1990), as fingerprinting tools (Fang *et al*., 1997), for diversity studies (Debreuil *et al.*, 1996) and for studies of hybridization and introgression, including studies of gene flow between crops.

2. 9. 3. 2. Polymerase Chain Reaction (PCR)-based markers

PCR based markers involve *in vitro* amplification of particular DNA sequences or loci, with the help of specifically or arbitrarily chosen oligo-nucleotide sequences (primers) and a thermos table DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods such as staining and autoradiography. PCR is a versatile technique invented during the mid-1980s (Saiki *et al*., 1985).

Random Amplified Polymorphic DNA (RAPD): is a PCR-based technology. The method is based on enzymatic amplification of target or random DNA segments with arbitrary primers. In 1991 Welsh and McClelland developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermo cyclic amplification. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (William *et al*., 1993). However, due to the stoichastic nature of DNA amplification with random sequence primers, it is important to optimize and maintain consistent reaction conditions for reproducible DNA amplification. RAPDs are DNA fragments amplified by the PCR using short synthetic primers (generally 10 bp) of random sequence. These oligo-nucleotides serve as both forward and reverse primer, and are usually able to amplify fragments from 1-10 genomic sites simultaneously. Amplified products (usually within the 0.5-5 kb size range) are separated on agarose gels in the presence of ethidium bromide and view under ultraviolet light (Jones *et al.*, 1997) and presence and absence of band will be observed. 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. Each product is derived from a region of the genome that contains two short segments in inverted orientation, on opposite strands that are complementary to the primer.

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 nano-gram (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. They are dominant markers and hence have limitations in their use as markers for mapping, which can be overcome to some extent by selecting those markers that are linked in coupling (Williams *et al.,* 1993).

The main drawback of RAPDs is their low reproducibility (Schierwater and Ender, 1993), and therefore highly standardized experimental procedures are needed because of their sensitivity to the reaction conditions. RAPD analyses generally require purified, high molecular weight 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.

The application of RAPDs and their related modified markers in variability analysis and individual-specific genotyping has largely been carried out, but is less popular due to problems such as poor reproducibility, faint or fuzzy products, and difficulty in scoring bands, which lead to inappropriate inferences. RAPDs have been used for many purposes, ranging from studies at the individual level (e.g. genetic identity) to studies involving closely related species.

Amplified Fragment Length Polymorphism (AFLP): it is essentially intermediate between RFLPs and PCR. AFLP is based on a selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) (Matthes *et al.*, 1998). The technique involves four steps: (1) restriction of DNA and ligation of oligo nucleotide adapters (2) preselective amplification (3) selective amplification, and (4) gel analysis of amplified fragments.

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. However, AFLP needs for purified, high molecular weight DNA, the dominance of alleles, and the possible non-homology of comigrating fragments belonging to different loci. In addition, due to the high number and different intensity of bands per primer combination, there is the need to adopt certain strict but subjectively determined criteria for acceptance of bands in the analysis. Special attention should be paid to the fact that AFLP bands are not always independent.

AFLPs can be applied in studies involving genetic identity, parentage and identification of clones and cultivars, and phylogenetic studies of closely related species because of the highly informative fingerprinting profiles generally obtained. Their high genomic abundance and generally random distribution throughout the genome make AFLPs a widely valued technology for gene mapping studies (Vos *et al.*, 1995).

Inter Simple Sequence Repeats (ISSR): are DNA fragments of about 100-3000 base pairs (bp) located between adjacent, oppositely oriented microsatellite regions. They 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 ISSR is that no sequence data for primer construction are needed. Because the analytical procedures include PCR, only low quantities of template DNA are required (5-50 ng per reaction). Furthermore, ISSRs are randomly distributed throughout the genome, and the variation within unique regions of the genome may be found at several loci. However, ISSR is a multilocus technique and drawback includes possible non-homology of similar sized fragments.

Because of the multilocus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone and strain identification as well as taxonomic studies of closely related species. In addition, ISSRs are considered useful in gene mapping studies (Godwin *et al.,* 1997). ISSR markers also target divergence in regions containing dispersed repetitive DNA and can rapidly differentiate closely related individuals. It is especially useful in detecting clonal variation and fingerprinting of related individuals. It has great potential in the study of natural populations for addressing questions ranging from conservation biology to molecular ecology and systematics (Bornet and Branchard, 2001).

Microsatellites or Simple Sequence Repeat (SSR): SSRs 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 appropriate to distinguish closely related genotypes; since their high degree of variability favored in population studies and for the identification of closely related cultivars (Vosman *et al*., 1992). Microsatellites, like mini-satellites, represent tandem repeats, but their repeat motifs are shorter (1-6 base pairs). 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 oligo-nucleotide repeat and sequencing the positive clones.

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 *et al*., 2002). 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. However, high development costs are involved if adequate primer sequences for the species of interest are unavailable makinging it difficult to apply to unstudied species and this is one of the main drawbacks of microsatellites.

In general, microsatellites show a high level of polymorphism. As a consequence, they are very informative markers that can be used for many population genetics studies, ranging from the individual level (e.g. clone and strain identification) to that of closely related species. On the other hand, their high mutation rate makes them unsuitable for studies involving higher taxonomic levels. Microsatellites are also considered ideal markers in gene mapping studies (Hearne *et al.*, 1992).

3. OBJECTIVE THE STUDY

3.1. General objective

This research aims to investigate the salt tolerance of common bean (*P. vulgaris*) accessions from Ethiopia at different salt concentrations and also assess their genetic diversity using ISSR markers.

3.2. Specific objectives

- To determine the effects of salt concentration on root biomass and also the root and shoot height of different common beans;
- To investigate important physicochemical properties of the soil that help to determine the salt content of the soil;
- To investigate the genetic diversity within and among common bean populations based on ISSR markers;
- To recommend salt tolerant genotypes of *P. vulgaris* for future breeding effort.

4. MATERIALS AND METHODS

4.1. Plant materials

This study was conducted from September 2011 to August 2012 at Addis Ababa University, Ethiopia Seeds of twelve common bean (*P. vulgaris*) populations were obtained from the Institute of Biodiversity and Conservation (IBC), Addis Ababa, Ethiopia. They were collected from different low land areas of Ethiopia (Table 2).

Table 2. Passport data of *P. vulgaris* accessions and their respective codes (IBC)

No.	Acc. name	Regional state	Zone (woreda)	Ass. Code	Latitude	Longitude	Altitude
1.	241739	SNNP	North Omo	A	06-52-59-N	37-47-56-E	1715.00
2.	241737	SNNP	Hadiya	B	07-31-51-N	38-01-94-E	1740.00
3.	211362	Benishangul	Metekel (Dangure)	C	11-41-15-N	35-37-19-E	1300.00
4.	211331	Somali	Shinile	D	09-28-33-N	41-01-49-E	1850.00
5.	211327	Oromiya	Harerge	E	09-05-00-N	40-45-00-E	1850.00
6.	208703	Oromiya	W. Wellega*	F	08-32-26-N	34-49-18-E	1846.00
7.	211356	Benishangul	Metekel (Dibate)	G	10-21-32-N	36-19-30-E	1250.00
8.	208702	Oromiya	W. Wellega	H	08-31-17-N	34-44-09-E	1880.00
9.	208698	Oromiya	W.Wellega	I	08-30-59-N	34-46-28-E	1880.00
10.	208695	Oromiya	W.Wellega	J	08-32-00-N	34-46-52-E	1880.00
11.	211329	Oromiya	Harerge	K	09-02-00-N	40-44-00-E	1870.00
12.	211361	Benishangul	Metekel (Dangure)	L	11-32-59-N	36-37-19-E	1300.00

** W. Wellega =West Wellega*

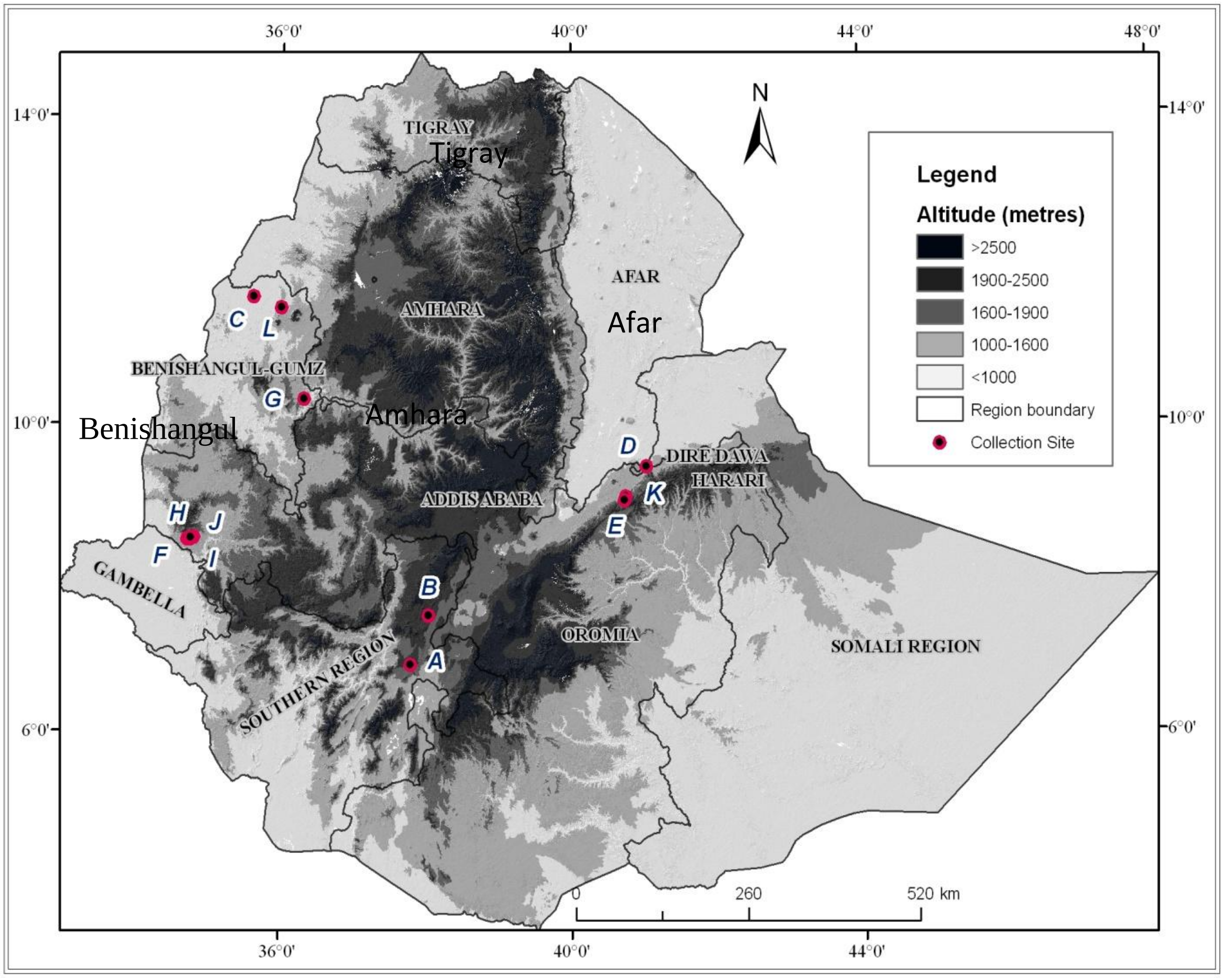

Figure 3. Map of Ethiopia showing sites where population of *P. vulgaris* were collected.

A = North Omo, B = Hadiya, C = Metekel (Dangure), D = Shinile, E = Harerge,
F=West Wellega, G= Metekel (Dibate), H=West Wellega, I= West Wellega, J =West Wellega K= Harerge, L= Metekel (Dangure)

4.2. Salinity tolerance study

4.2.1. Soil analysis

A soil sample that was collected near Addis Ababa, Debre Zeit, was subjected to various soil chemical analyses (pH, electrical conductivity, soluble cations, texture, and percent water saturation) at Jije Analytical Service Laboratory (JASL) Addis Ababa, Ethiopia. Important chemical properties of the soil were determined using the following standard methods and procedures. EC and pH of the soil samples were determined by using saturated paste extract with conduct and pH meter, respectively. Electrical conductivity (EC) and soluble cations were measured in the saturation extracts (USDA-NRCS, 1996). Soluble cations in saturated paste extract were determined with the following equipment: soluble Ca and Mg by Atomic Absorption Spectrophotometer (RDP, 1986), and Na and K by Flame Photometer (FAO, 1984). The cation exchange capacity (CEC) and exchangeable base (Ca, Mg) were extracted using 1:1 alcohol and Ammonium Acetate extraction method after Bower *et al*. (1952). Particle size analysis was determined after samples were dispersed in sodium hexameta-phosphate solution and shaken on a horizontal reciprocating shaker for 12 hrs using hydrometer method. Percent water saturation was done based on mass (gravimetric) basis.

4.2.2. Pot experiment

Common beans (*P. vulgaris*) were sown in the glass house within small round plastic pots at the College of Natural Sciences, Addis Ababa University. To reduce the compaction and improve the drainage system, the pots were filled with 7kg of sandy clay loam soil and each seed were sown at 30mm depth in three replicates. The pots were laid out using a randomized block design. Two weeks after sowing, the plants were thinned out to six per pot (Ahmed, and Fallah, 2008). Then based on methods of El-Sayed and El-Sayed (2011), salt treatment started when seedlings became well established (i.e. after 15 days). This was done with the application of NaCl to produce a concentration range of 50, 100, 150 and 200 mM. Tap water was used as a control. The treatments were applied twice a week by alternating equal amounts of water to compensate for the evapo-transpiration and to avoid excessive salt accumulation in the plant. After approximately two to three months of treatment, the plants were analyzed for various growth parameters following the method of Mensah and Ihenyen (2009). Moreover, salinity levels were obtained by dissolving 2.92, 5.84, 8.76 and 11.68 g NaCl in one liter of tap water to get 50,100, 150 and 200 mM, respectively.

The data obtained from the experiments were subjected to statistical analysis using appropriate software (SPSS version 16) and were presented accordingly. ANOVA was used to evaluate the extent of variation induced by the different salt concentrations among the different varieties used for the study.

4.3. Analysis of genetic diversity using ISSR markers

4. 3.1. DNA extraction

Young leaves of common beans in the greenhouse were collected separately from randomly selected eight individuals from each of 12 accessions (Figure 4). Total genomic DNA was isolated from about 0.3 g of the fine grounded leaf sample using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) based on Borsch *et al*. (2003) extraction protocol with slight modifications in the extracyion fraction,where by the second extractions were exclusively used for ISSR fingerprint (Appendix 1). Then, the genomic DNA samples were diluted with sterile double distilled water in 1:20 ratio, for PCR amplification. For instance, 5µl of genomic DNA was diluted with 100 µl of sterile double distilled water.

Figure 4. Young leaves of *P. vulgaris*.

4. 3. 2. Primer selection and optimization

The ISSR marker analysis was carried out at the plant molecular biology laboratory of the Institute of Biotechnology, College of Natural Sciences, Addis Ababa University. A total of 19 primers were chosen to select the primers that can produce reproducible and polymorphic bands. Then from each accession, two individuals were selected to screen the primers. Based on amplified band profiles and polymorphic patterns of DNA fingerprinting, seven primers (825,827,834,836,841,866 and CR-2) were selected for further PCR reaction. Table 3 shows the list of primers used and tested, their annealing temperature with respective sequences and other properties.

Table 3. List of primers, primer sequence, annealing temperature, amplification pattern and repeat motives used for optimization

primer code	**Primer Sequence**	**Annealing temperature**	**Amplification pattern**	**Repeat motives**
CR 2	CACACACACACACACAAG	49	polymorphic, reproducible	Dinucleotide
809	AGAGAGAGAGAGAGAGG	49	Not polymorphic	Dinucleotide
810	GAGAGAGAGAGAGAGAT	45	polymorphic, reproducible	Dinucleotide
812	GAGAGAGAGAGAGAGAA	45	Not polymorphic	Dinucleotide
818	CACACACACACACACAAG	45	Not polymorphic	Dinucleotide
824	TCTCTCTCTCTCTCTCG	45	polymorphic, reproducible	Dinucleotide
825	ACACACACACACACACT	49	polymorphic, reproducible	Dinucleotide
827	ACACACACACACACACG	49	polymorphic, reproducible	Dinucleotide
834	AGAGAGAGAGAGAGAGYT	49	polymorphic, reproducible	Dinucleotide
836	AGAGAGAGAGAGAGAGYA	49	polymorphic, reproducible	Dinucleotide
841	GAGAGAGAGAGAGAGAYC	49	polymorphic, reproducible	Dinucleotide
857	ACACACACACACACACYG	49	Not reproducible	Dinucleotide
860	TGTGTGTGTGTGTGTGRA	49	Not polymorphic	Dinucleotide
866	CTCCTCCTCCTCCTCCTC	49	polymorphic, reproducible	Trinucleotide
872	GATAGATAGATAGATA	45	Not polymorphic	Tetranucleotide
873	GACAGACAGACAGACA	45	polymorphic, reproducible	Tetranucleotide
878	GGATGGATGGATGGAT	45	Not polymorphic	Tetranucleotide
880	GGAGAGGAGAGGAGA	45	polymorphic, reproducible	Pentanucleotide
881	GGGTGGGGTGGGGTG	45	Not polymorphic	Pentanucleotide

Single-letter abbreviations for mixed base positions: R = (A, G) Y = (C, T)

4. 3. 3. PCR and gel electrophoresis

The polymerase chain reaction (PCR) was conducted using TC-412, version-34.11 and block 96 x 0.2ml thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 2µl template DNA, 17.07µl H_20, 0.2µl dNTPs (100mM), 2.5µl PCR buffer (1X buffer B), 2.5µl $MgCl_2$ (25mM), 0.5µl primer (20pmol/µl) and 0.2µl Taq Polymerase (5u/µl). PCR amplification conditions were set at: initial denaturation at 94°C for 5 minutes followed by 40 cycles of 94 °C for 30 seconds, 1minute primer annealing at (49°C or 45°C) depending on primers used, 2 minutes extension at 72°C and with a final extension of 7 minutes at 72°C. The amplified PCR products were stored at 4°C until the time of electrophoresis. The amplification products were resolved by electrophoresis using 1.7% agarose gel with 1 X TAE buffer. Amplification product of 10µl was loaded with 2µl of 6 x loading dye. Low DNA mass ladder (2000, 1200, 800,400,200,100 base pairs) was used to estimate molecular weight and size of the fragments. Electrophoreses was carried out for 3 hours at constant voltage of 100V. Gels were stained with ethidium bromide and band detection was performed using a BIO-RAD Gel Doc.

4.3.4. Data analysis

ISSR fingerprint profiles were scored manually for each individual sample from the gel photograph. The bands were recorded as characters, present '1', absent '0' and '?' for missing data. Based on recorded bands different software's were used for analysis. POPGENE version 1.32 software (Yeh *et al.*,1999) was used to calculate genetic diversity for each accession as number of polymorphic loci, percent polymorphism, gene diversity and Shannon weaver diversity index. Analysis of Molecular Variance (AMOVA) was used to calculate variation among and within accession using Areliquin version 3.01 (Excoffier *et al.,* 2006). NTSYS (Numerical Taxonomy and Multiware Analysis System)- 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 by the following 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 mean (UPGMA) (Sneath and Sokal, 1973) was used to evaluate and compare individual sample and accession, and generates dendrogram using Numerical taxonomy and multivariate analysis system (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 genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 Software (Pavlicek *et al.*, 1999).

In addition, to examine the pattern of variation among individual samples on 2D (two dimentional) and 3D (three dimension); a Principal Coordinated Analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The calculation of Jaccard's coefficient was made using PAST software version 1.18 (Hammer *et al.,* 2001). The first three axes were used to plot the three dimensional PCO with STATISTICA version 6.0 software (Hammer *et al.,* 2001; Sta. soft, Inc.2001).

5. RESULTS

5.1. Soil analysis

Physiochemical properties of the soil sample that was collected from common bean growing field was investigated before the start of the actual experiment in the greenhouse. Table 4 shows the results of the chemical and physical analyses of the soil sample.

Table 4. Chemical and physical properties of the soil

No.	Parameters	Result
1.	CEC, cmol(+)/Kg Soil	40.83
2.	Ph	7.73
3.	EC	1.40
4.	Available phosphorus, mg/Kg soil	92.80
5.	Soluble calcium , meq/100 g soil	0.49
6.	Soluble magnesium , meq/100 g soil	0.22
7.	Soluble sodium , meq/100 g soil	0.10
8.	Soluble potassium , meq/100 g soil	0.12
9.	Percent water saturation, %	90.13
10.	Texture	Clay (30%), silt (12%) and sand (58%)

5.2. Effect of Salt (NaCl) on Common Beans

5.2.1. Effect on shoot development

Our results showed that common beans grown at lower salt concentration had higher shoot growth or Shoot Length (SL) than those treated with relatively high level of salt concentration, as expected (ANOVA, P= <0.05, Table 5, Appendix 3a).

When compared to the control, SL was high at 50 mM in all varieties except the accession collected from “Metekel (Dangure)” (Table 5). The influence of NaCl was more pronounced at 200mM salinity level. At this treatment, SL growth was lower by 79.94%, 77.32% and 53.09% for Metekel (Dangure), Harerge (Figure 5) and West Wellega, respectively, as compared to the control (Table 5). Therefore, they were found to be salt sensitive. Whereas, accessions from Shinile and Metekel (Dibate) were found to be better salt tolerant at 200mM salinity level having 38.4% and 29.85% lower SL, respectively, relative to the control. The remaining, North Omo and Hadiya, accessions had intermediate SL which were lower by 43.44% and 49.60%, respectively, as compared to the control.

Table 5. Effect of salinity on mean Shoot Length (cm) in different *P.vulgaris* accessions

Accessions	**NaCl concentration (mM)**				
	Control	**50**	**100**	**150**	**200**
North Omo	38.90± 0.37abc	59.00± 1.45b	34.16± 3.3ac	23.00± 0.57ac	22.00± 3.6ac
Hadiya	41.66± 2.18a	59.00± 5.50b	21.00± 0.57c	21.00± 1.00c	21.00± 2.77c
Metekel (Dangure)	93.00± 15.63a	24.66± 3.71b	22.00± 1.52b	18.33± 2.60b	18.66± 2.02b
Shinile	52.66± 5.23a	70.66± 2.40b	43.66± 2.72ac	43.00± 3.05ac	32.00± 7.57c
Harerge	64.66± 21.61ad	71.00± 1.52ab	37.00± 3.78dc	24.00± 1.52c	14.66± 1.20c
West wellega	54.00± 1.52a	75.00± 9.53b	46.66± 6.38a	40.00± 5.85ac	25.33± 4.17c
Metekel (Dibate)	25.66± 2.96a	29.66± 1.45a	24.66± 1.76a	19.00± 0.57b	18.16± 0.16b

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

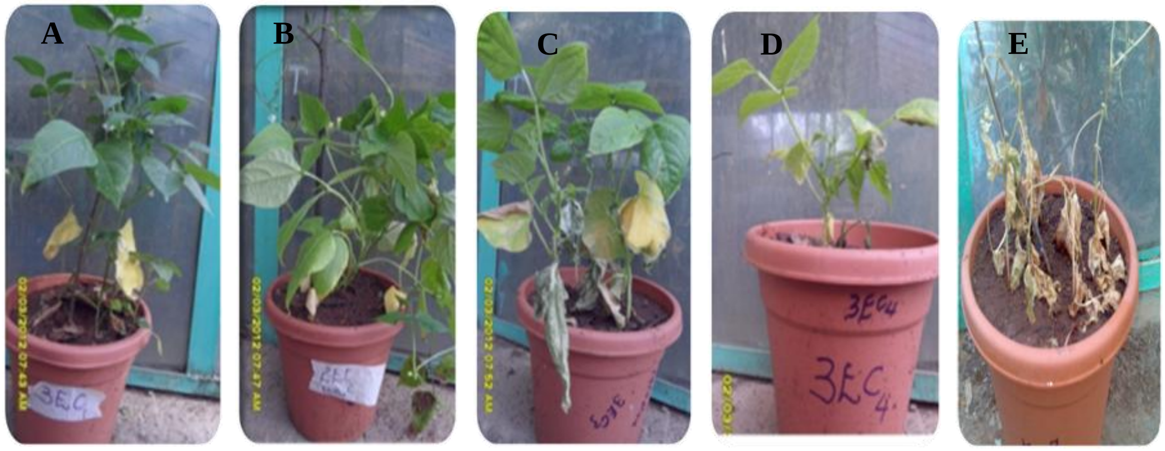

Figure 5.picture showing variation in shoot length in accession of Harerge with different salt concentrations ,A (control), B (50mmol), C (100mmol), D (150mmol) and E (200mmol),

5.2.2. Effect on Root Length (RL)

Common beans grown at lower salt concentration had higher root growth than those treated with relatively high level of salt concentration (ANOVA, p =<0.05, Appendix 3b, Table 6). When compared to the control, RL was high at 50 mM in all varieties except the accessions collected from West Wellega, Hadiya and Harerge (Table 6). On the other hand, the effect of NaCl was more noticeable in some accessions at 200mM salinity level. At this treatment level, The RL of Harerge and West Wellega was lower by 59.22% and 22.48%, respectively, as compared to the control (Table 6). Hence, at 200 mM, the highest and the lowest root growth was observed in accessions collectd from Harerge and West Wellega, respectively, as compared to control.

Table 6. Effect of salinity on root length (cm) in common bean (*P. vulgaris*) accessions

Accessions	**NaCl concentration (mM)**				
	Control	**50**	**100**	**150**	**200**
North Omo	22.66±1.76a	37.83±5.65b	23.33±5.48a	13.66±2.40a	12.00±0.88a
Hadiya	15.50±2.29a	13.00±2.33ac	8.66±1.20bc	8.66±2.66bc	7.00±0.57b
Metekel (Dangure)	19.33±4.97ac	23.66±4.17c	9.66±2.33ab	8.66±1.85b	8.33±1.85b
Shinile	22.66±1.45ac	25.00±0.57a	19.33±0.57bc	16.33±3.28b	15.66±1.20b
Harerge	25.33±2.02a	19.66±0.33a	12.00±0.57b	11.50±4.48b	10.33±1.45b
West wellega	16.33±0.33a	16.00±5.13a	16.00±1.52a	15.66±0.33a	12.66±1.45a
Metekel (Dibate)	17.83±1.30ab	19.00±3.21a	13.66±0.88ab	13.33±0.33b	13.00±1.20b

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

5. 2. 3. Effect on Root Fresh Weight (RFW)

Different concentrations of NaCl had also an effect on Root Fresh Weight (RFW) of *P.vulgaris*. RFW varied among salt treatments levels within each accession of *P. vulgaris* (ANOVA, p= <0.05, Appendix 3c). The data presented in Table 7 also revealed that RFW of most accessions was lower when salt levels increased. The influence was more pronounced at 150 mM and 200 mM salinity levels. At 200mM treatment, RFW was lower by 98.4%, 90.38% and 88.79% for Metekel (Dangure), Hadiya and Harerge, respectively, when comprared to control showing that they were relatively salt sensitive. However, accessions from West Wellega and North Omo were found to be less salt-affected at 200mM salinity level having 23.68% and 60.27% lower RFW, respectively, relative to the control The remaining, Shinile and Metekel (Dibate), accessions had intermediate RFW as compared to the control (Table 7).

Table 7. Effect of salinity (NaCl) on Root Fresh Weight (RFW) (g) in common bean (*P. vulgaris)* accessions

Accessions	**NaCl concentration (mM)**				
	Control	**50**	**100**	**150**	**200**
North Omo	1.46±0.15a	1.57±0.43a	0.72±0.16b	0.58±0.13b	0.58±0.11b
Hadiya	0.52±0.11a	1.41±0.50b	0.39±0.31a	0.08±0.01a	0.05±0.02a
Metekel (Dangure)	1.25±0.06a	1.45±0.10a	0.43±0.11b	0.04±0.01c	0.02±0.01c
Shinile	1.46±0.15a	2.66±0.64b	0.64±0.02ac	0.63±0.03ac	0.50±0.05c
Harerge	1.16±0.13a	3.71±1.41b	0.43±0.02a	0.15±0.07a	0.13±0.03a
West wellega	1.14±0.26a	2.44±0.30b	0.82±0.22a	0.86±0.14a	0.87±0.04a
Metekel (Dibate)	2.73±0.08a	6.16±0.31b	1.36±0.14c	0.65±.14d	0.68±0.07d

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

5.2.4. Effect on Root Dry Weight (RDW)

Root dry weight varied among salt treatments levels within each accession of *P. vulgaris* (ANOVA, p =<0.05, Appendix 3d, Table 8). When compared to the control, RDW was high at 50 mM in accessions collected from Harerge, Shinile and North Omo; whereas, it was low in Hadiya, Metekel (Dangure), West wellega and Metekel (Dibate) (Table 8). The influence of NaCl was most noticeable at 200mM salinity level in all accessions except Harerge and West wellega having 60.47% and 65.12% lower in RDW, respectively, as compared to control (Table 8).

Table 8. Effect of salinity (NaCl) on Root Dry Weight (RDW) (g) in common beans (*P. vulgaris)* accessions

Accessions	**NaCl concentration (mM)**				
	Control	**50**	**100**	**150**	**200**
North Omo	0.43±0.12a	0.72±0.00b	0.14±0.02c	0.02±0.00d	0.02±0.01d
Hadiya	0.43±0.06a	0.24±0.07b	0.05±0.00c	0.02±0.00c	0.03±0.01c
Metekel (Dangure)	0.50±0.03a	0.25±0.03b	0.16±0.05b	0.02±0.00c	0.03±0.01c
Shinile	0.43±0.06a	1.00±0.26b	0.17±0.05a	0.15±0.02a	0.08±0.02a
Harerge	0.43±0.06a	1.00±0.26b	0.21±0.00a	0.16±0.02a	0.16±0.00a
West wellega	0.43±0.06a	0.27±0.01c	0.19±0.00bc	0.11±0.01b	0.10±0.05b
Metekel (Dibate)	0.78±0.08a	0.45±0.03b	0.15±0.01c	0.10±0.01c	0.08±0.01c

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

5. 3. Molecular genetic diversity analysis

5. 3.1. ISSR primers and their banding patterns

Out of the Nineteen primers tested initially, seven (six di- and one tri- nucleotide) which gave relatively polymorphic, reproducible and clear banding pattern were selected for this study (Table 3). The seven primers yielded a total of 69 reliable fragments from the 96 individuals representing the 12 accessions of *P. vulgaris*, with a 100% polymorphism in all of the primers. Among these, six di-nucleotides (825, 827, 834, 836, 841 and CR-2) contributed 60 polymorphic bands whereas, the remaining tri nucleotide (866) gave nine polymorphic bands. The least polymorphic bands (6) were scored in primers 825; whereas primer 836, CR2-2 showed the highest polymorphic bands, thirteen and twelve, respectively. The remaining primers, 827, 834,841 and 866, produced eight, eleven, ten and nine polymorphic bands, respectively. The molecular size of the bands amplified using the primers were in the range of 100 bp to 800 bp; and there was a mean of 9.85 bands per primer (Table 10). Figure 6 shows the amplification pattern of primer 836. The other gel pictures of each primer used in this study are presented in appendix 2.

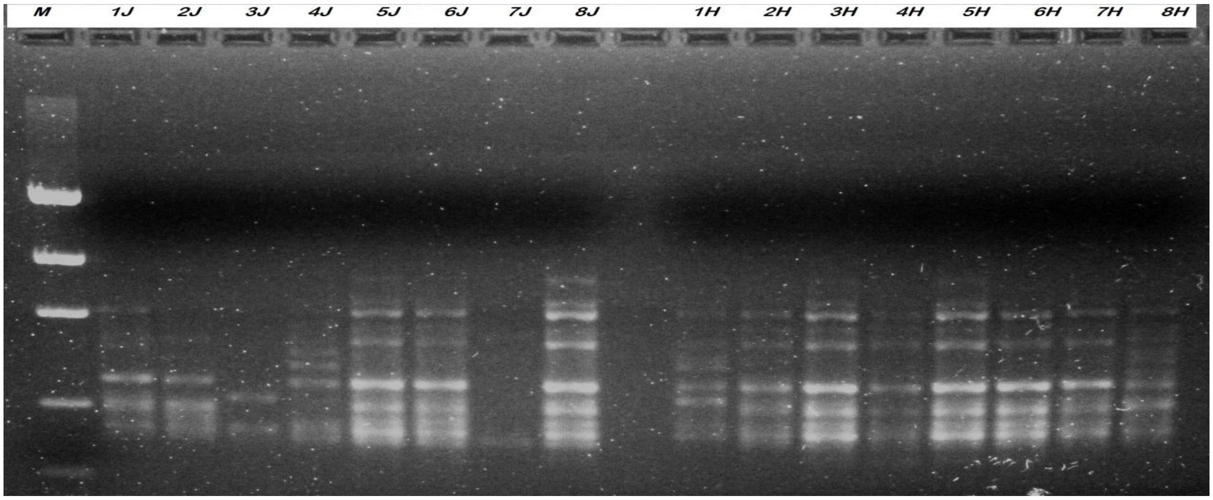

Figure 6. 12 ISSR fingerprints generated using primer 836.

Key: J1- J8, H1-H8 (both accessions from W.Wellega).

Table 9. Banding patterns generated using the seven primers, their repeat motifs and number of scored bands.

ISSR primer code	**Repeat motifs**	**Number of scorable bands**
825	ACACACACACACACACT	6
827	ACACACACACACACACG	8
834	AGAGAGAGAGAGAGAGYT	11
836	AGAGAGAGAGAGAGAGYA	13
841	GAGAGAGAGAGAGAGAYC	10
866	CTCCTCCTCCTCCTCCTC	9
CR- 2	CACACACACACACACAAG	12
Total		69

Single-letter abbreviations for mixed base positions: Y = (C, T)

5. 3. 2. Gene Diversity

Among the *P. vulgaris* accessions evaluated using ISSR markers, the highest gene diversity was obtained for Shinile (H = 0.29); while Metekel (L) was the least diverse (H=0.10). In addition, more related values were found for North Omo (H = 0.28) and Metekel (C) (H = 0.26). The overall gene diversity for the total population was 0.35 (Table 11). Relatively similar diversity patterns were also observed in Shannon Diversity Index (I). Shinile and Metekel (L) populations showed the highest and the least gene diversity values with 0.43 and 0.15, respectively (Table 11).

Table 10. Number of Scorable Bands (NSB), Number of Polymorphic Loci (NPL), Percent Polymorphism (PP), genetic diversity (H), and Standard Deviation (SD) and Shannon Diversity Index (I) for each primer.

Primers	**NSB**	**NPL**	**PP**	**H±SD**	**I±SD**
825	6	6	100	0.23± 0.13	0.38± 0.16
827	8	8	100	0.32± 0.12	0.12± 0.16
834	11	11	100	0.34± 0.12	0.52± 0.14
836	13	13	100	0.39± 0.10	0.57± 0.12
841	10	10	100	0.31± 0.14	0.48± 0.17
866	9	9	100	0.44± 0.10	0.62± 0.12
CR-2	12	12	100	0.40± 0.08	0.58± 0.09
Average	9.85	9.85	100	0.35±0.11	0.47±0.14
Over all	69	69	100	0.35±0.12	0.53± 0.15

Table 11. Number of Polymorphic Loci (NPL), Percent Polymorphism (PP), and genetic diversity (H), Shannon Index (I) and Standard Deviation (SD) for all accessions.

Accessions	**NPL**	**PP (%)**	**H±SD**	**I±SD**
North Omo (A)	49	71.01	0.28 ± 0.20	0.41± 0.28
Hadiya (B)	39	56.52	0.21 ± 0.21	0.31± 0.30
Metekel (Dangure)(C)	49	71.01	0.26± 0.19	0.39± 0.27
Shinile (D)	51	73.91	0.29± 0.20	0.43± 0.28
Harerge (E)	27	39.13	0.16± 0.21	0.24± 0.30
W. Wellega (F)	30	43.48	0.18± 0.22	0.27± 0.31
Metekel (Dibate) (G)	29	42.03	0.18± 0.22	0.26± 0.31
W. Wellega (H)	44	63.77	0.24± 0.20	0.36± 0.29
W. Wellega (I)	48	69.57	0.24± 0.19	0.36± 0.27
W. wellega (J)	49	71.01	0.23± 0.19	0.35± 0.26
Harerge (K)	32	46.38	0.17± 0.20	0.25± 0.29
Metekel (Dangure) (L)	19	27.54	0.10± 0.19	0.15± 0.27

5. 3. 3. Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) was carried out on the overall ISSR data score of *P.vulgaris* accessions without grouping by region or geographic location (Table 12). The higher percentage of variation (62.55%) revealed through AMOVA is attributed to within population; while the remaining (37.45%) was a result of among populations (P=< 0.005).

Table 12. Analysis of Molecular Variance (AMOVA) among accessions of *P. vulgaris* without grouping the populations

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**	**Significance**
Among populations	11	287.719	2.70480	37.45%	0.00
Within populations	84	379.500	4.51786	62.55%	
Total	95	667.219	7.22266	100%	

5. 3. 4. Cluster Analysis

UPGMA and Neighbor Joining (NJ) analysis were used to construct a dendrogram for the twelve accessions and 96 individuals based on 69 PCR bands amplified by six di-nucleotide and one tri nucleotide ISSR primers. The dendrogram obtained from NJ analysis illustrated that there were three major distinct clusters and two subclusters within the third major cluster (Figure 9). Most individuals from each accession tended to form their own cluster; while few individuals from each accession were distributed all over the tree. Among the twelve accessions, Metekel (L) (major cluster I), North Omo (major cluster II) and Harerge (E*)* (major cluster III, sub-cluster I) showed a relatively clear grouping.

The dendrogram generated based on UPGMA resulted in the separation of the twelve common bean populations into two main clusters. Population from Harerge (K) was an outlier which made in to group ‘I’ whereas, the rest 11 populations together grouped into cluster II. In cluster II, North Omo, Hadiya, West Wellega (F), Shinile, Harerge (E), Metekel (G), Metekel (L), Metekel (C) and West Wellega (I) formed sub cluster I and the two populations from West Wellega (H, J) formed sub cluster II. In sub cluster I, West Wellega (I) singly formed sub sub cluster I and the rest formed sub sub cluster II. Within sub sub cluster II, Hadiya-West Wellega (F); Shinile-Harerge (E) and Metekel (G) - Metekel (L) formed a strong clustering which shows that the populations were highly related (Figure 7). In addition, Jaccard similarity coefficient based pair wise comparisons of the 12 populations showed that Harerge (E) and West Wellega (F) have a similarity coefficient value of 0.53; whereas Harerge (K) with populations of Hadiya, Shinile and West Wellega (F) showed a distant relation with similarity coefficient of 0.28, 0.29 and 0.29, respectively (Table 13).

In contrast, individual based UPGMA clustering of an overall analysis showed a strong clustering of individuals with respect to their populations except certain intermixed individuals from other population (Figure 8). This result is similar to that of individual based in NJ.

Table 13. Pairwise Jaccard similarity coefficient based comparisons among 12 accessions of *P. vulgaris*

	North Omo(A)	Hadiya(B)	Metekel (Dangure)(C)	Shinile (D)	Harerge(E)	W. wellega (F)	Metekel(G)	W. wellega(H)	W. wellega(I)	W.wellega(J)	Harerge(K)	Metekel(L)
A												
B	0.485											
C	0.409	0.418										
D	0.417	0.434	0.436									
E	0.503	0.5	0.443	0.526								
F	0.454	0.527	0.402	0.480	0.531							
G	0.375	0.410	0.414	0.336	0.414	0.413						
H	0.400	0.436	0.363	0.370	0.463	0.435	0.372					
I	0.390	0.382	0.336	0.385	0.424	0.404	0.408	0.394				
J	0.408	0.365	0.370	0.392	0.424	0.377	0.297	0.437	0.36			
K	0.421	0.284	0.392	0.299	0.336	0.296	0.418	0.343	0.385	0.357		
L	0.488	0.437	0.388	0.402	0.527	0.515	0.512	0.406	0.42	0.356	0.367	

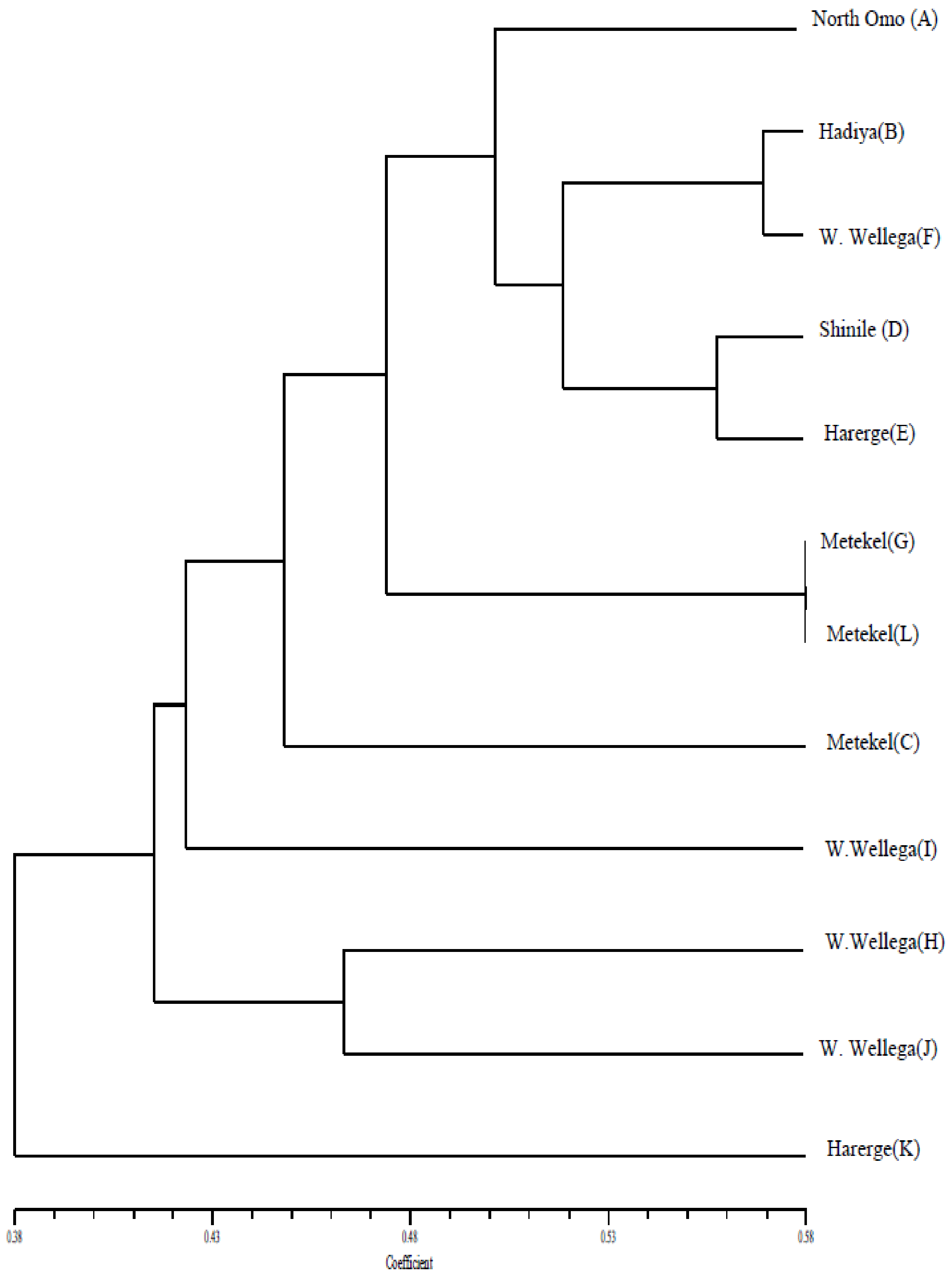

Figure 7. UPGMA based dendrogram for 12 *P.vulgaris* populations collected from different areas of Ethiopia. *Key: W.Wellega=West wellega*

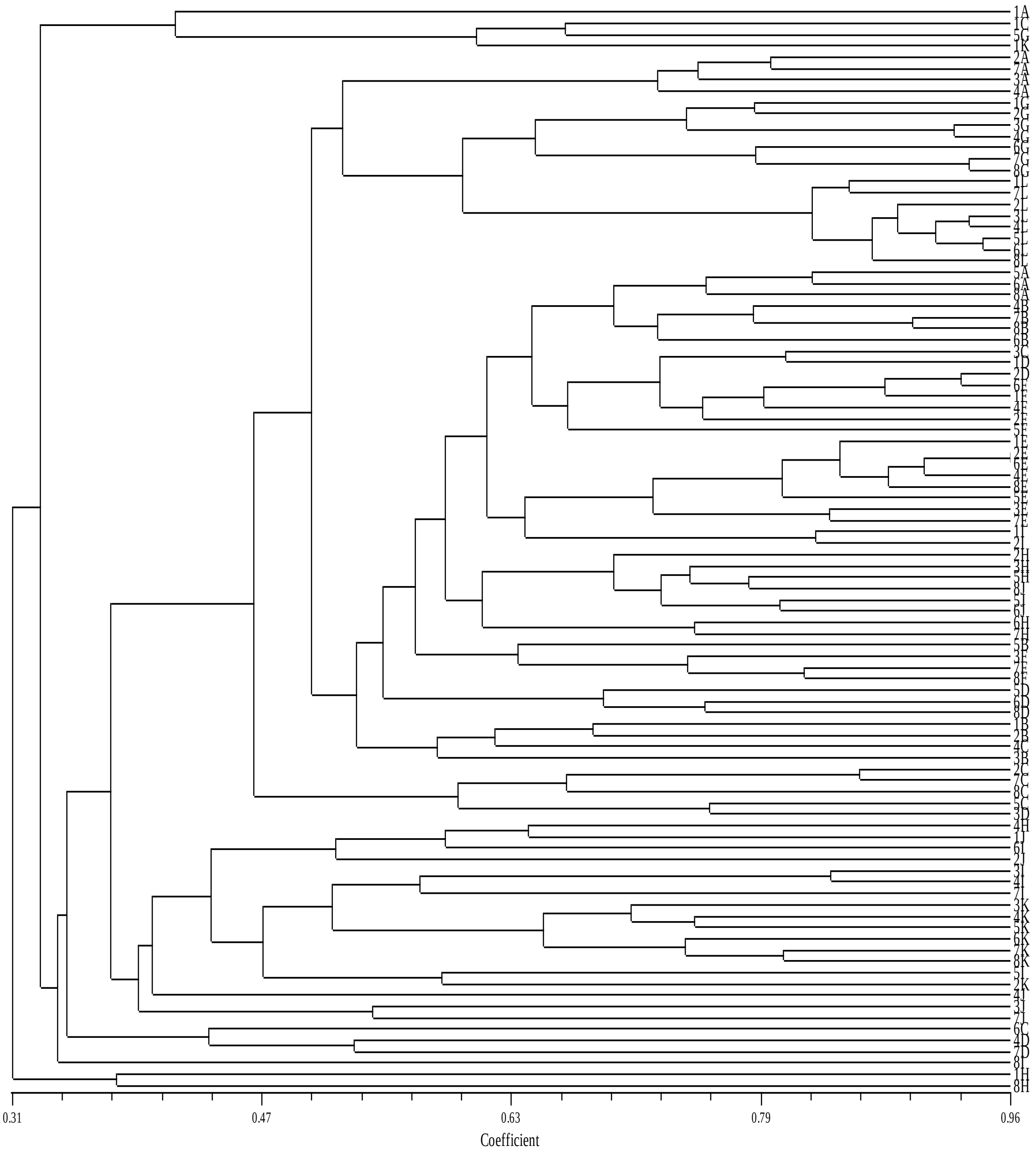

Figure 8. UPGMA based dendrogram for 96 *P.vulgaris* individuals using 7 ISSR primers.

Key: A =North Omo, B= Hadiya, C= Metekel, D=Shinile, E=Harerge, F=West wellega, G= Metekel, H=West wellega, I= West wellega, J =West wellega, K= Harerge, L= Metekel

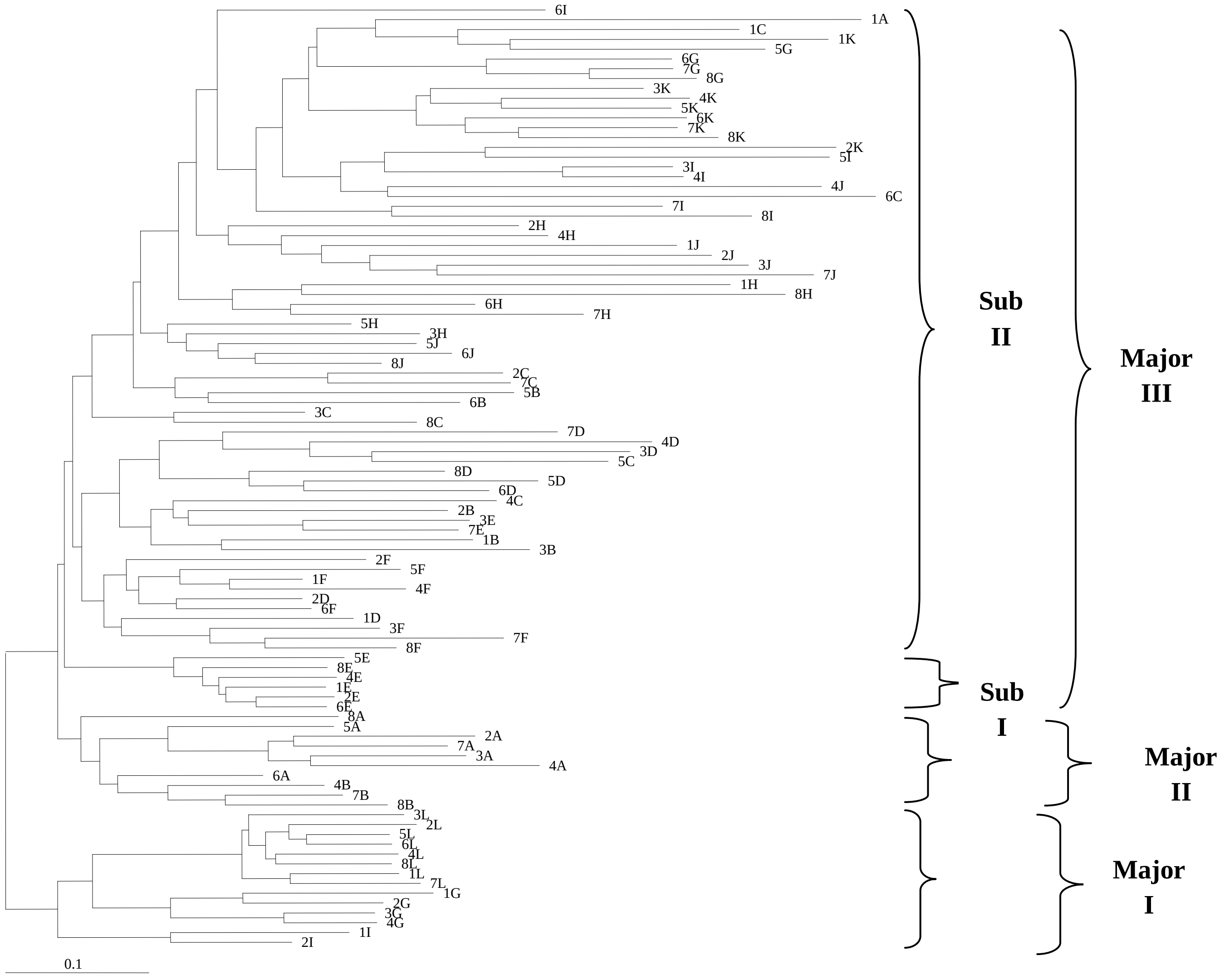

Figure 9. Neighbor-joining analysis of 96 individuals.

Key: A =North Omo, B= Hadiya, C= Metekel, D=Shinile, E=Hararge, F=West Wellega, G= Metekel, H=West Wellega, I= West Wellega, J =West Wellega, K= Hararge, L= Meteke

5. 3.5. Principal Coordinate analysis (PCO)

The first three coordinates of the PCO having eigen values of 4.9546, 3.4101 and 2.2552 with a variance of 12.224%, 8.4133% and 5.56%, respectively, were used to reveal the grouping of individuals using two and three coordinates (Figure 10 and 11). In the three dimensional (3D) Figure 11, most individuals from Metekel (L) tended to form their own grouping. The remaining individuals from each accession did not show a clear assembly rather they were inter-mixed with each other. Moreover, mixed individuals did not show separate group within each other. Almost similar result was observed from the use of two coordinates (Figure 10) as well as three coordinates.

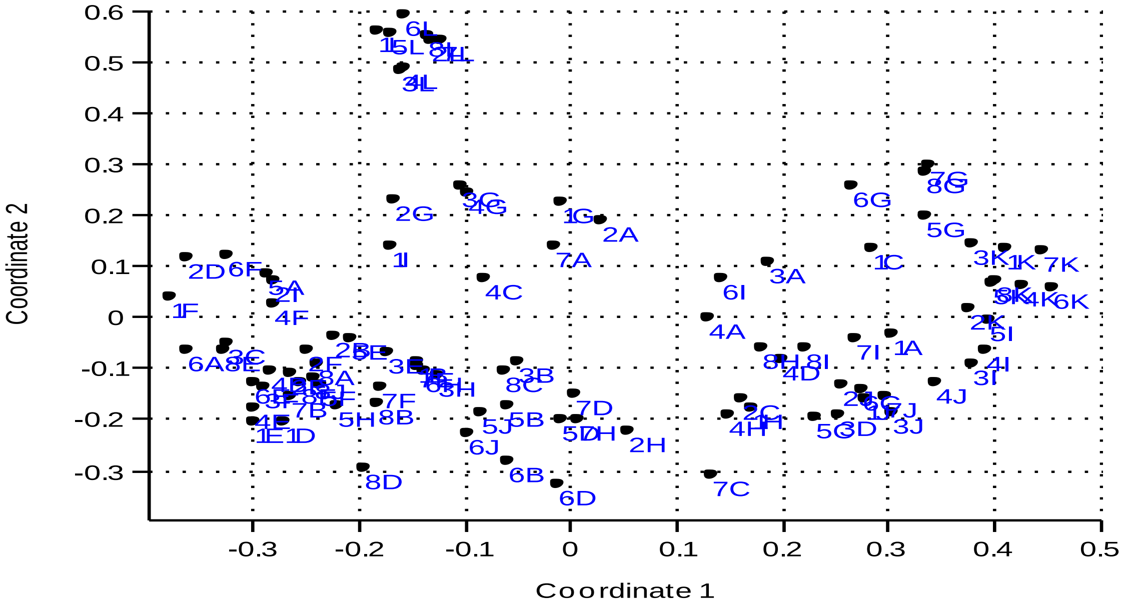

Figure 10. Two dimensional representation of principal coordinate analysis of the genetic relationships among 96 individuals of twelve *P.vulgaris* accessions.

Key: *A =North Omo, B= Hadiya, C= Metekel, D=Shinile, E=Harerge, F=West wellega, G= Metekel, H=West wellega, I= West wellega, J =West wellega, K= Harerge, L= Metekel*

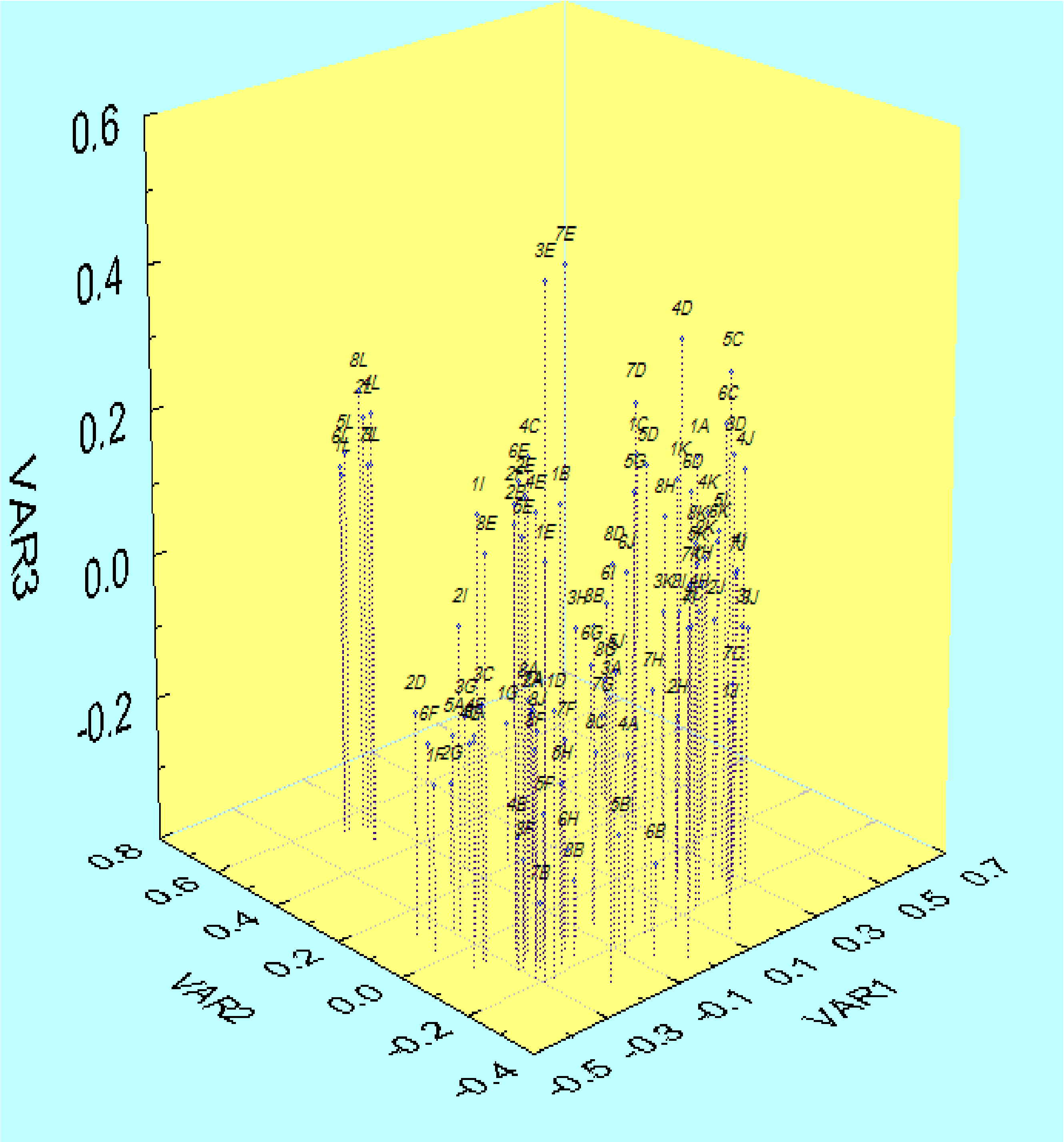

Figure 11. Three dimensional representation of principal coordinate analysis of genetic relationships among 96 individuals of twelve *P.vulgaris* accessions.

Key: *A =North Omo, B= Hadiya, C= Metekel, D=Shinile, E=Hararge, F=West wellega, G= Metekel, H=West wellega, I= West wellega, J =West wellega, K= Hararge, L= Metekel*

6. DISCUSSIONS

6.1. Soil and its physiochemical properties

Physiochemical properties of the soil revealed that it had Cation Exchange Capacity (CEC) of 40.83 cent mole per kilogram (cmol/ kg), low EC (1.4 dS/m) and falls in sandy clay loam soil class. Based on the above physiochemical parametres, CEC and EC, the soil can be considered as non saline (Bashour and Sayegh, 2007; Horneck *et al.*, 2007). Quantitatively, the soil type composed of 30% clay, 12% silt and 58% sand. In addition, it had the following chemical elements: P (92.80 mg/Kg), Ca (0.49 meq/100g), Mg (0.22 meq/100g), Na (0.10 meq/100g), K (0.12 meq/100 g) and 90.13% water saturation.

On the other hand, there have bean a major salinity and alkalinity problems in the semi-arid and arid low lands of Ethiopia (Tamirie Hawando, 1994 and 2000). Fore example, a study reported by Meron Nigussie (2007) from various cites of central Rift Valley, South Estern shore of Lake Ziway, sugusted that the soil contains higher concentration of Na (> 45 meq/100g). This affirms the presence of salinity in the semi-arid and arid low lands of Ethiopia.

There fore, soil analysis was considered to be useful to show that the change in growth patterns of common beans (in all the growth parameters such as shoot length, root length, etc.) were from the effect of the treatments (application of different conc. levels of NaCl) but not from the soil used to grow common beans.

6.2. Effect of NaCl concentration on common beans

Generally, there was no significant difference among parameters of shoot and root lengths as well as fresh and dry weight of roots at different salt treatment levels of *P.vulgaris* varieties. In other words, all varieties responded in a similar way with respect to the above parameters. SL and RL are the most important features for salt stress assessment because roots are in direct contact with soil which absorb water and transport it to the other parts of the plant. According to Neumann (2005) salinity can rapidly inhibit root growth; hence plant's uptake capacity of water and essential minerals from soil may be lower. As a result, normal physiological and biochemical function of the plant will be impaired.

The effect of 50mM salinity level was not intense with respect to all parameters considered (SL, RL, RFW and RDW). However, most of them were quite salt sensitive at 200mM salinity level. For instance, rising in salinity level caused more reduction in SL in most populations and the drop was quite sharp and rapid in Metekel (Dangure) (79.94%) and Harerge (77.32%) at 200mM as compared to the control (Fig.4). Similar results to our findings were reported in *P. vulgaris* (Kinfemichael Geressu, 2011), *Zea mays* (El-Sayed, 2011b), *Sorghum bicolor* L. Moench (Kinfemichael Geressu and Melkamu Gezaghegne, 2008) and *Vigna mungo* (Mensah and Ihenyen, 2009). Whereas, accessions from Shinile and Metekel (Dibate) were found to be better salt tolerant at 200 mM salinity level having 38.4% and 29.85% reduction in their SL of the control. In addition, decrease in the shoot or height of plants grown in medium saline soils has been reported *in Vicia faba* (Cordovilla *et al.,* 1996), *P. aureus* (Mirza and Mahmood, 1986) and *Cicer arietinum* (Singla and Garg, 2005).

Reduction in growth of plants under high salinity is obvious because it increases the energy requirement of the plant necessary to combat the osmotic and ionic stress for normal cellular maintenance. As a result, relatively less energy is available for growth processes (O'Leary, 1986). NaCl did not show any adverse effects on plant growth during the first weeks of treatment in *Leucaena leucocephala* plants (Anthraper and Du Bio., 2003). However, after 14 weeks of treatment distinct differences began to occur between the various treatments levels and NaCl concentration of greater than 50 mM had a great impact on the over all activity of the plant. A study by Singleton and Bohlool (1984) on soybeans supports this trend showing that NaCl concentration greater than 0.026 mol/L had a depressive effect on growth. A depressive effect at NaCl concentration greater than 50 mM/L was also observed in *C. arietinum* (El-Sheikh and Wood, 1990; Singla and Garg, 2005) and in peas and *Vicia faba* (Delgado *et al*., 1994). The above mentioned, which are closely related to common bean, reports were in agreement with our results.

Keck *et al*. (1984) have reported that salinity due to irrigation had greater effect on shoot growth than root growth in alfalfa. Similar observations were made by Mahmood and Mahmood (1989) in *Prosopis juliflora*. There is a strong relationship between our findings and the reports mentioned above; that is to say, there was a less detrimental effect of salinity on root than shoot growth. So, our results, salinity which caused greater effect on shoot than root growth, corroborate with the results of Keck *et al*. (1984), Mahmood and Mahmood (1989), Singla and Garg (2005), Anthraper and Du Bio(2003), El-Sheikh and Wood (1990), Singleton and Bohlool (1984) and Delgado *et al*. (1994).

Our results show that North Omo, Metekel (Dangure), Shinile and Metekel (Dibate) grew longer root at low salt (50mM**)** solution as compared to the control (Table 6) and these results are similar to studies of Wang *et al*.(2005) who confirmed that the roots of pakchoi (vegetable) treated with low salinity were better developed than the control. However, the RL and SL of *P.vulgaris* showed a reduction rate of 22.48% to 59.22% and 29.85% to 79.94% at 200mM treatment level respectively as compared to control. These observations support the findings of Wignarajah (1990) that salinity affected shoot growth more than root growth. Bayuelo-Jimenez (2002) also reported that salt-tolerant species of *Phaseolus* maintained relatively high root growth even at 180 mM (nutrient solution) NaCl.

The consequent increase in root to shoot growth seems to be associated with increased salinity tolerance in these species. It is possible that under salt stress the plant spends more photosynthetic energy on root production, in search of water and /or reducing water loss and thus maintains a relatively high water relations (Kafkafi, 1991). Although root production was found to be continuous, plants apparently used this process as an avoidance mechanism to remove excess ions and delay the onset of ion accumulation. In contrast, the root growth of less salt tolerant cultivars may not be continuous. Therefore, avoidance of salinity by intensive root development may depend on species or genotypes.

In the current study, high RFW was observed in all accessions at 50mM treatment level. Besides the RFW of Metekel (Dangure), Hadiya and Harerge were strongly inhibited at 200mM treatment level; even though it was less in West Wellega and North Omo as compared to control Previous reports in legumes, such as in, *Vicia faba* (Yousef and Sprent, 1983; Zahran and Sprent, 1986), *Glycine max* (Grattan and Maas, 1988) and *P. vulgaris* (Wignarajah, 1990), indicated that salinity was found to reduce shoot and root weights. These reports are similar to our findings (Table 7). Likewise, Shannon and Grieve (2000), noticed that salinity reduced fresh weight of vegetables; and Jeannette *et al.* (2002) reported that RFW of *Phaseolus* species was significantly reduced with increasing salt stress. It has been reported that the reduction in fresh weights in plants is because of the proportional increase in Na^+ concentration, which could mean that an ionic effect was being manifested. It is also assumed that addition of NaCl up to higher concentration reduces the water potential in the medium which hampers water absorption and thus reduces plant growth. Sagi *et al.* (1997) found the adverse effects of salinity stress on shoot and root growth.

Moreover, decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of common bean, which affects the growth rate, and this is often associated with a decrease in the electron transport activities of photosynthesis (Kirst, 1989) and inhibition of Photosystems II (PSII) activity (Kao *et al.*, 2003). In general, salinity reduces leaf number, leaf area, shoot and root dry weight, leading to low yields (Essa, 2002; Hamdy *et al.*, 1993; Li *et al.*, 2006; Sharifi *et al.*, 2007).

Dry weights of roots also declined with increasing level of salinity (Table 8). There are significant differences among the different treatments in RDW (Table 8). At 200mM Metekel (Dibate), Metekel (Dangure) and Hadiya were more salt-affected. However, accessions from West wellega were less salt-affected in comparison with the control. Generally, salt tolerance mechanism in all parameters, SL, RL, RFW and RDW, is a very complex process as reported by Udvardi *et al.* (2007).

6.3. Molecular genetic diversity

In the present study, twelve accessions of *P.vulgaris* were analyzed using seven ISSR primers and they gave 69 scorable and reproducible bands. Since each fragment is assumed to represent a genetic locus, it is possible to estimate percent polymorphism and other classical population parameters. All the bands generated were polymorphic and they also showed high polymorphism. Sadeghi and Cheghamirza (2012), using ISSR markers, reported very similar results to our findings. The genetic diversity parameters: percent polymorphic loci, gene diversity (H) and Shannon's diversity index (I) indicated that accessions of *P.vulgaris* such as: Shinile, North Omo, Metekel (C) and West Wellega (H) having 0.29, 0.28, 0.26 and 0.24 gene diversity, respectively were indeed high. Similarly, the result from Shannon diversity index illustrates that individuals from Shinile accession were the most diverse (I=0.43) as compared to the other accession. The overall total Nei's gene diversity and Shanon's diversity index were high, 0.35 and 0.53, respectively. This result is in agreement with other bean species from tropical, subtropical or warm temperate regions that have been recently studied with ISSR markers, such as *Vicia faba* (Abdel-Razzak *et al.*, 2012), *Dalbergia cochinchinensis* (Hien and Phong, 2012).

In this study, all the diversity parameters verify that there is medium to high gene diversity in accessions of *P.vulgaris.* So, high priority should be given to areas with high genetic diversity since they contribute more accessions for future collection, breeding and conservation activities. For example, among the twelve accessions used in this study, higher genetic diversity was shown in the individuals coming from the Shinilie area.

Analysis of molecular variance (AMOVA) in *P.vulgaris* accessions showed high within accessions genetic diversity (62.55 %) as compared to inter accession difference (37.45%). This is relatively close to the result of previous study having 55-60% and 40-45% genetic diversity within and among wild populations of *P.vulgaris* L. from Mexico, respectively (Cruz *et al.*, 2004). A number of reasons may contribute for the high levels of genetic diversity within accessions. These could be high seed exchange among community, human activities during harvest and marketing and presence of large population size. Hence, these may lead to the intermixing of populations. In general, out breeding species with more or less continuously distributed populations, the proportion of internal variation is often above 70%; while below 30% is attributed to inter population difference (Hamrick *et al.*, 1992).

The current study using UPGMA of Jaccard's coefficient of similarity showed that accessions from Shinile and Harerge (E) as well as the nearby localities of Metekel (G, L) and West Wellega (H, J) constituted a cluster and this may be attributed to the ease of seed movement between the areas because of geographical and cultural factors. The formation of a cluster by the twelve accessions could be explained by high gene flow from one region to the other due to various factors. For instance, seeds of some common bean germplasms might have been transported to and from for repeated drought affected areas as a donation by geverment or non geverment organizations as well as during marketing. In the other hand, the separation and formation of a cluster by the twelve accessions may suggest that accessions may have been isolated from each other for a longer period in time and limited gene flow due long distance.

Genetic variability is useful for defining heterotic patterns in hybrid breeding and for relating the observed pattern with presence of certain economically important traits. Such information can be used to design effective germplasm conservation and for setting germplasm collection task as well as to guess or predict the risk of genetic erosion in certain areas. So, understanding the genetic diversity of *P.vulgaris* will enable for further breading and conservation.

High gene diversity was observed in accessions collected from Shinile (H= 0.29) and seed coming from North Omo (H= 0.28). They also showed comparatively high salt tolerance with respect to parameters, SL, RL, RFW and RDW; whereas low in gene diversity and salt tolerance was observed in a population of Harerge (E). Moreover, individuals with less genetic diversity

(H=0.1098) but comparatively with low salt influence was observed in Metekel (L). Others, W. Wellega (F), Hadiya (B) and Metekel (Dangure)(C), had different contrasting values with respect to gene diversity and salt response; that is, accessions with high genetic diversity but with low salt tolerance and vice versa.

7. CONCLUSION

The current study showed that Root Length (RL) was affected less by salt than Shoot Length (SL) with in the seven common bean accessions. Lower salinity levels such as 50mM have facilitated the growth in most common bean varieties; while the growth of RL and SL showed declined with increasing salinity levels.The impact of salinity on common bean varieties became intense upon salinity level increament. Among accessions, Metekel (Dangure) and Harerge were salt sensitive during shoot and root growth. Thus these varieties should not be cultivated even on slightly saline soils. However, Shinile and Metekel (Dibate) were relatively salt tolerant during shoot and root development. So, these could be cultivated effectively on moderately saline soils. Moreover, W.Wellega (F) was sensitive to shoot growth but showed effective growth in root length as well as fresh and Dry Root Weight.

In addition, the current study shows all the twelve accessions of *P. vulgaris* possess a wide range of genetic diversity, which will be important for future collection, conservation and improvement strategies. Accessions from Shinile and Metekel (L) showed a relatively high to low level of genetic diversity, respectively. Therefore, conservation effort should focus on Shinile accession and then consider less diverse populations.

Clustering and PCO analysis for the *P.vulgaris* accessions suggested that accessions originated from similar or nearby geographic areas except Hadiya with West Wellega (F) formed their own cluster. Individuals from Harerge (K) formed a distinct group which shows the genetic distance among accessions from the entire regions. In addition, there was a strong association between individuals within each accession.

The highest proportion of genetic variation was attributed to within accession than among accessions. This confirms that there was a high level of gene flow between individuals of each accession collected from different localities.

To sum up, accessions collected from Shinile and North Omo were both salt tolerant and genetically diversified; whereas low gene diversity and salt tolerance was observed in a population of Harerge (E).

8. RECOMMENDATIONS

- ✓ This research addresses the genetic diversity of *P. vulgaris* accessions along with its salt tolerance. However, it only considered few accessions from lowland areas of Ethiopia. Hence, the genetic diversity and salt tolerance of Ethiopian accessions from different agro-ecology should be studied in detail.
- ✓ In this study the genetic diversity analysis of *P.vulgaris* accessions was done using ISSR markers. Therefore, to generate more information, morphological markers and other molecular markers should be used.
- ✓ To evaluate the physiological and morphological responses of locally adapted common bean varieties for salinity stress, it is suggested to use more robust methodologies, in terms of time and resources, for screening its salinity tolerance. These include physiological markers such as survival rates, ion concentrations; also more control experiments with various parameters
- ✓ More research is also needed to study the levels and causes of common bean diversity as well as its contribution to productivity and stability of the crop in each geographical region.
- ✓ For further select of better salt tolerant common bean cultivars, quantitative trait locus analysis should be studied
- ✓ To assure the effect of NaCl on common beans which were tested in the green house, should be also evaluated on salt affected common bean growing fields.

- ✓ Germplasms collected from Shinile and North Omo can be used for cultivation for moderately salt affected areas as well as for breeding and conservation.

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10. APPENDICES

Appendix.1. DNA Extraction Protocol

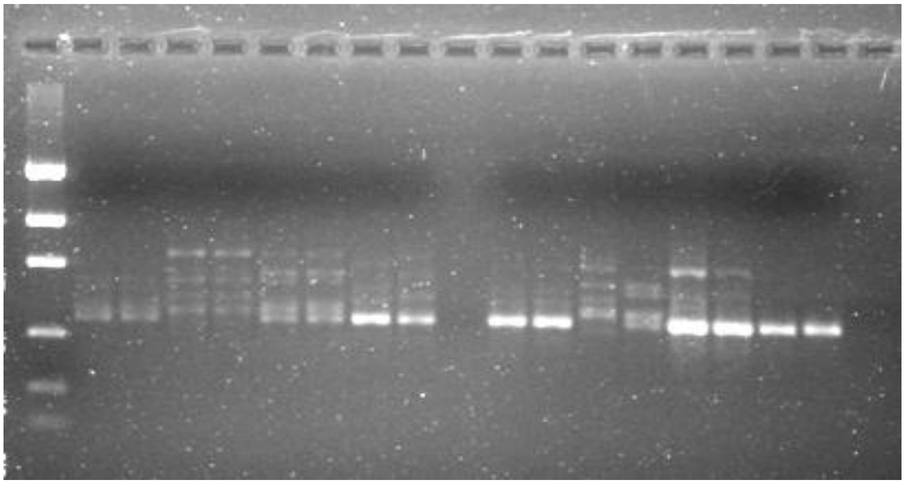
- Homogenize leaf tissue in liquid nitrogen to fine powder.
- Weigh 300mg of homogenized leaf in a 2ml eppendorf tube.
- Add 750 µl extraction buffer (EB) and 100 µl SDS (10%).
- Incubate at 65°Cfor 30mnts in water bath.
- Add 250 µl KAc and mix.
- Put the tubes in ice for at least 30 minutes (possible to take break).
- Centrifuge 13000 revolutions per minute (rpm) for 18 minutes.
- Transfer supernatant to sterile eppendorf tube.
- Add 1 volume of cold isopropanol (preserved at -20°C)
- Centrifuge at 13000rpm for 13mnts and discard the supernatant.
- Dissolve the pellet in 125µl TE and after the pellet have dissolved entirely (if not can be put in water bath at 65°C to facilitate dissolution) and add 125 µl CTAB.
- Incubate at 65°C for 15mnts in water bath.
- Extraction with one volume of chloroform.
    - Add 1 volume of chloroform
    - Centrifuge at 13000rpm for 18mnts
    - Collect the supernatant in new eppendorf tube (pool the same sample in one tube
- Repeat the chloroform extraction.
- Precipitate with 1 volume of cold isopropanol and store at 4°C over night.
- Centrifuge at 13000rpm for 18 minutes.
- Discard the supernatant.
- Dilute the pellet in 100 µl of TE and store at -20.
- Add 2.5 µl RNAse (1mg/ml) in 100 µl of TE-DNA.
- Incubate the sample at 37°C for 30 minutes.

Store the sample at -20 for next (PCR) step

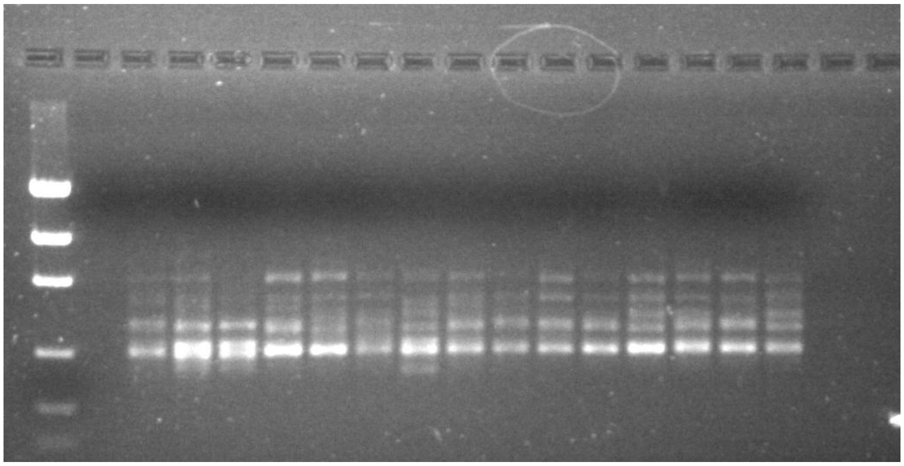
Appendix 2

ISSR fingerprints of *P. vulgaris* by different primers

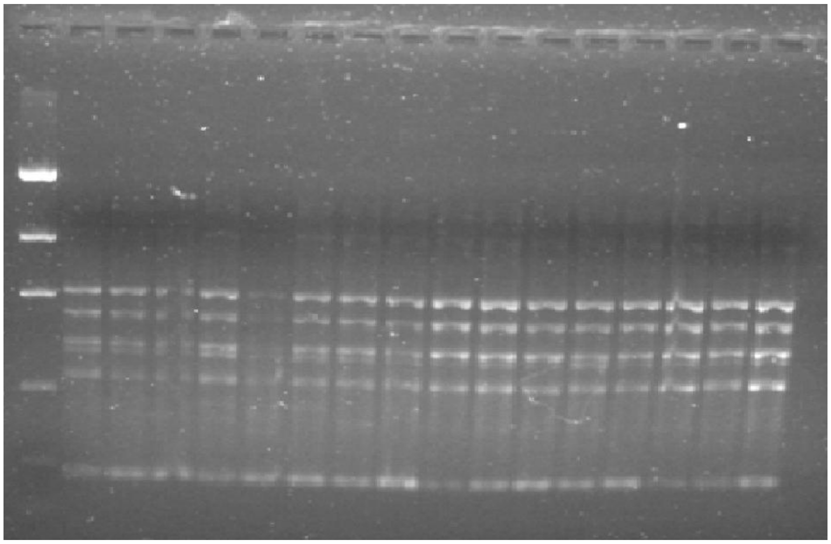
Primers 825



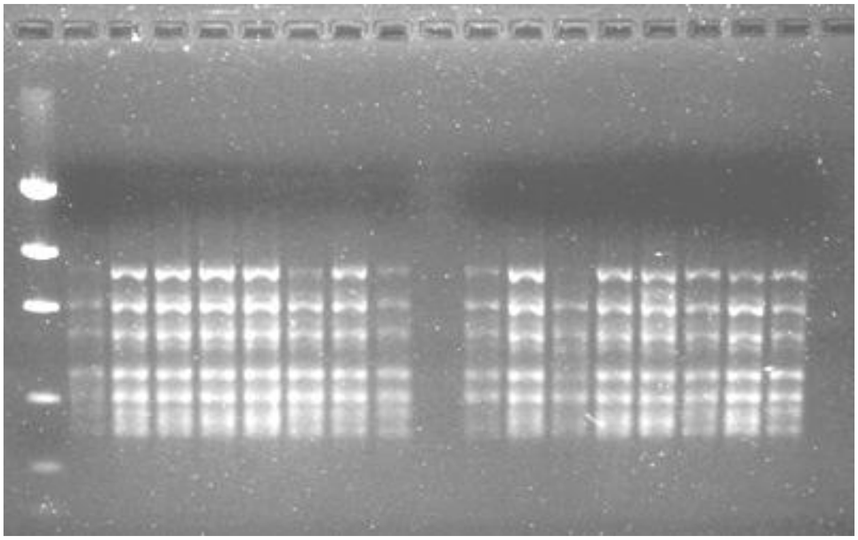
Primers 827



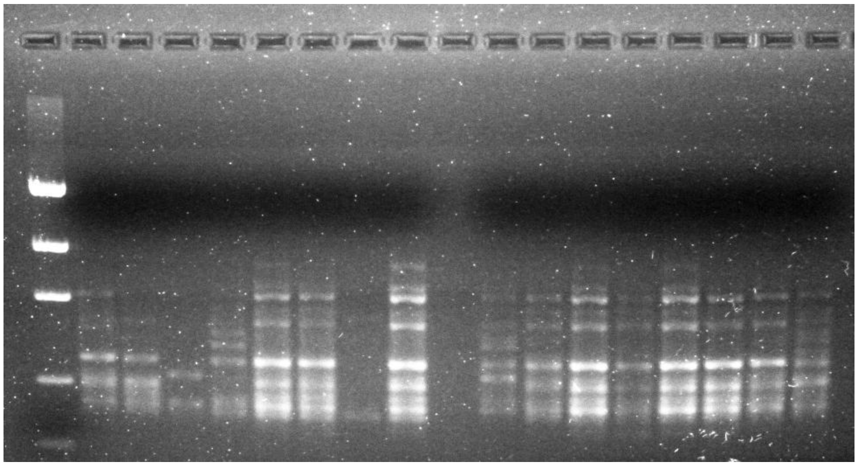
Primers 834



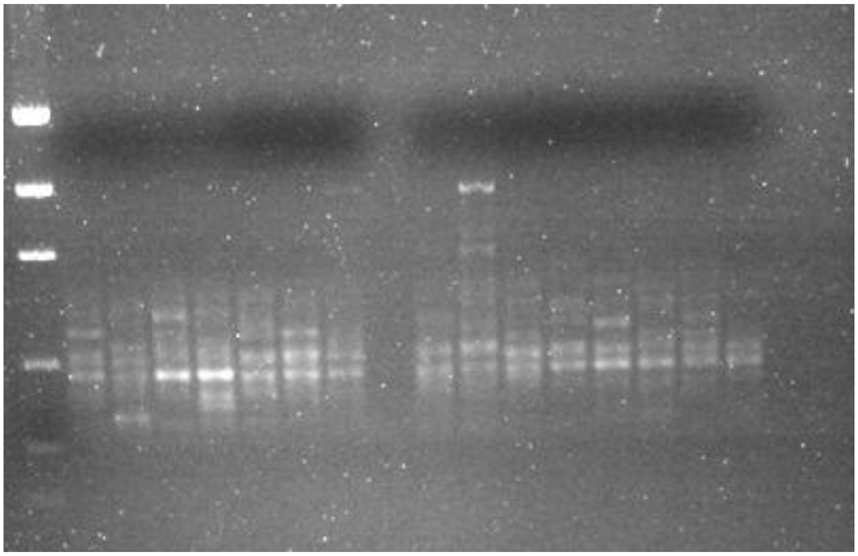
Primers 836



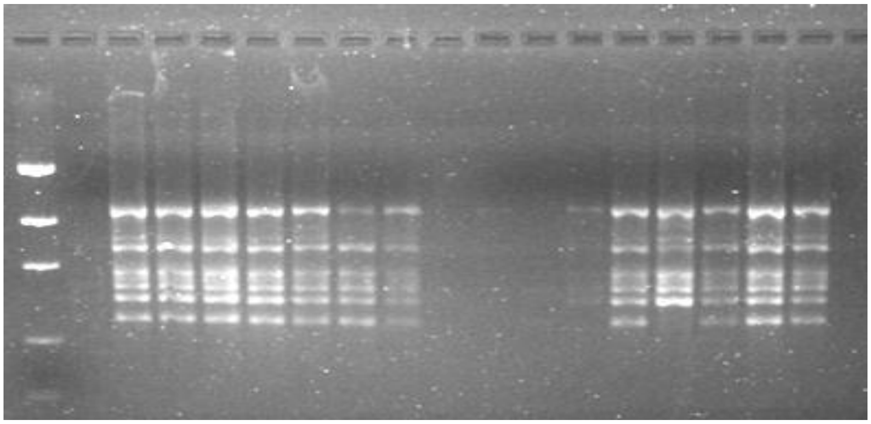
Primers 836



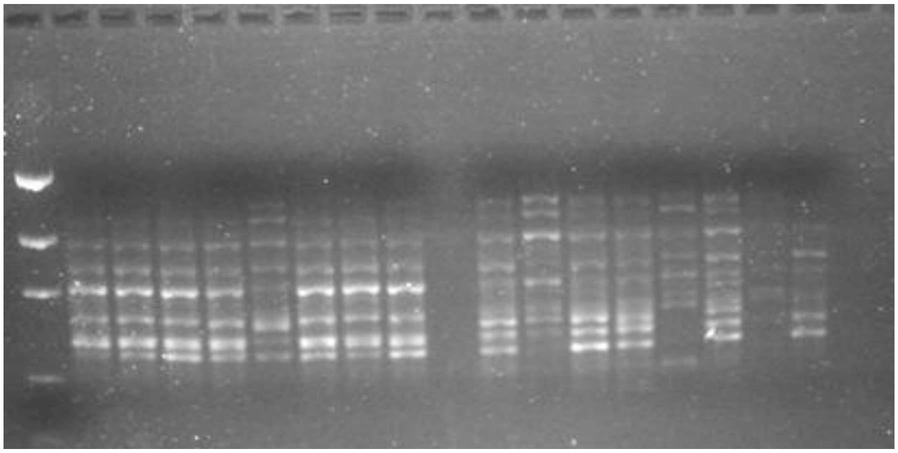
Primers 841



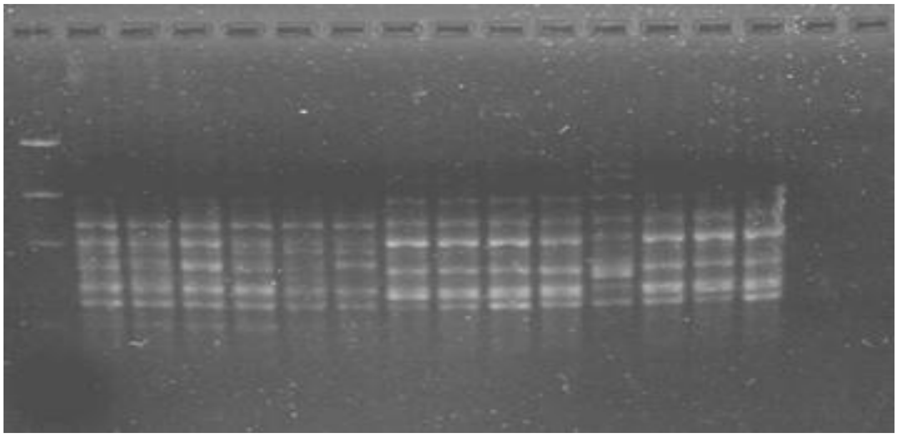
Primer 866



Primer CR-2



Primer CR-2



Appendix.3a

ANOVA table for the response of common bean (*P. vulgaris)*, at different NaCl treatments on shoot length.

Accession		Sum of Squares	df	Mean Square	F	Sig.
North Omo	Between Groups	2712.151	4	678.038	4.768	0.021
	Within Groups	1422.027	10	142.203		
	Total	4134.177	14			
Hadiya	Between Groups	3536.600	4	884.150	33.385	0.000
	Within Groups	264.833	10	26.483		
	Total	3801.433	14			
Metekel (Dangure)	Between Groups	14713.733	4	3678.433	21.05	0.000
	Within Groups	1412.000	10	141.200		
	Total	16125.733	14			
Shinile	Between Groups	2503.600	4	625.900	9.719	0.002
	Within Groups	644.000	10	64.400		
	Total	3147.600	14			
Harerge	Between Groups	7351.600	4	1837.900	6.283	0.009
	Within Groups	2925.333	10	292.533		
	Total	10276.933	14			
West wellega	Between Groups	4033.067	4	1008.267	9.040	0.002
	Within Groups	1115.333	11	111.533		
	Total	5148.400	15			
Metekel (Dibate)	Between Groups	278.267	4	69.567	8.074	0.004
	Within Groups	86.167	10	8.617		
	Total	364.433	14			

Appendix.3b

ANOVA table for the response of common bean (*P. vulgaris)* at different NaCl concentrations on root length

Accession		Sum of Squares	df	Mean Square	F	Sig.
North Omo	Between Groups	1247.400	4	311.850	7.238	0.005
	Within Groups	430.833	10	43.083		
	Total	1678.233	14			
Hadiya	Between Groups	161.400	4	40.350	3.434	.052
	Within Groups	117.500	10	11.750		
	Total	278.900	14			
Metekel (Dangure)	Between Groups	603.600	4	150.900	4.610	0.023
	Within Groups	327.333	10	32.733		
	Total	930.933	14			
Shinile	Between Groups	194.933	4	48.733	5.415	0.014
	Within Groups	90.000	10	9.000		
	Total	284.933	14			
Harerge	Between Groups	194.933	4	48.733	5.415	0.014
	Within Groups	90.000	10	9.000		
	Total	284.933	14			
West wellega	Between Groups	27.333	4	6.833	0.367	0.827
	Within Groups	186.000	11	18.600		
	Total	213.333	15			
Metekel (Dibate)	Between Groups	91.267	4	22.817	2.648	0.096
	Within Groups	86.167	10	8.617		
	Total	177.433	14			

Appendix 3c.

ANOVA table for the response of common bean (*P. vulgaris)* at different NaCl concentrations on Root Fresh Weight (RFW).

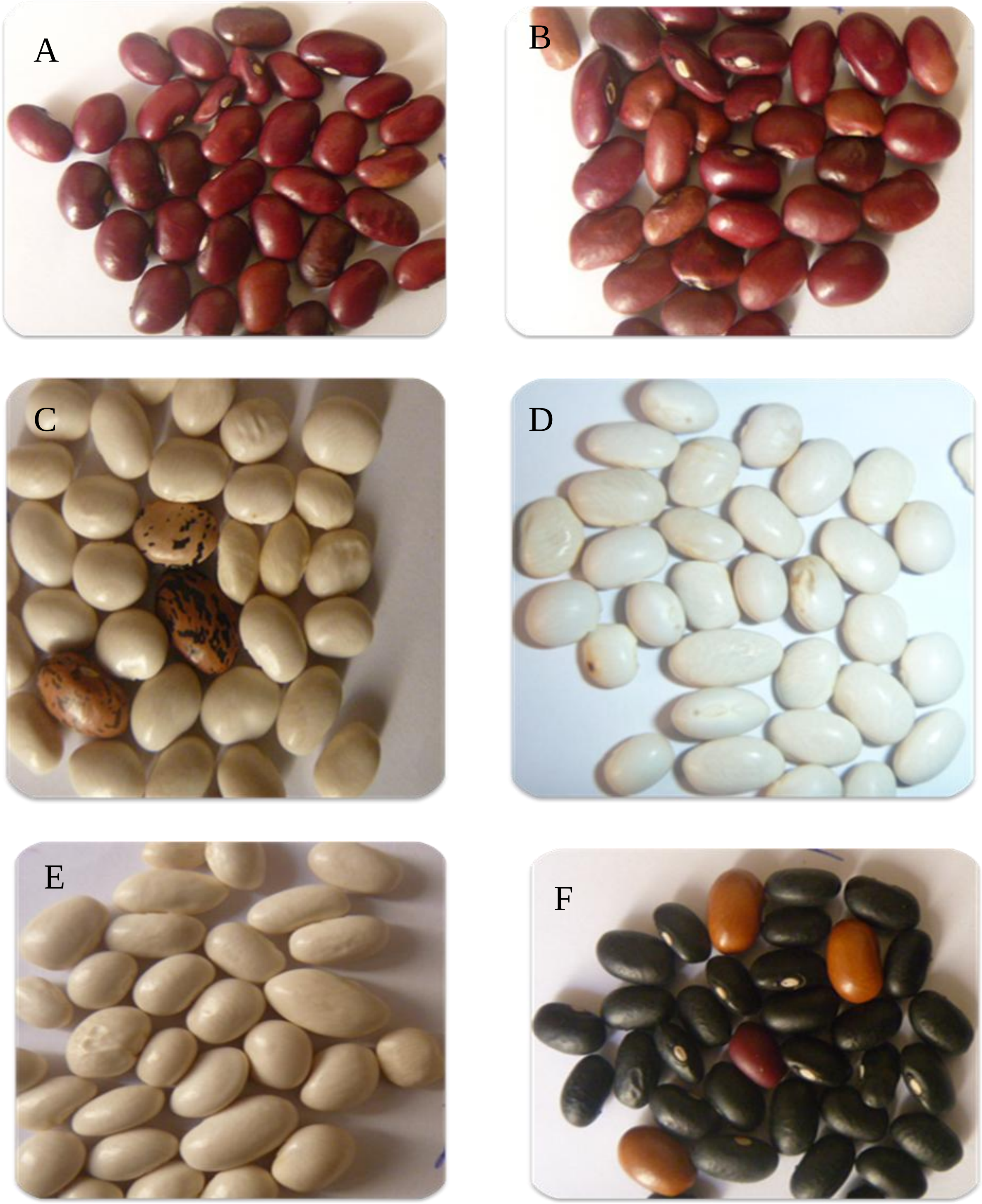
Accession		Sum of Squares	df	Mean Square	F	Sig.
North Omo	Between Groups	2.899	4	0.725	4.513	0.024
	Within Groups	1.606	10	0.161		
	Total	4.505	14			
Hadiya	Between Groups	3.633	4	0.908	4.079	0.032
	Within Groups	2.226	10	0.223		
	Total	5.859	14			
Metekel (Dangure)	Between Groups	5.451	4	1.363	78.867	0.000
	Within Groups	0.173	10	0.017		
	Total	5.624	14			
Shinile	Between Groups	9.969	4	2.492	9.472	0.002
	Within Groups	2.631	10	0.263		
	Total	12.600	14			
Harerge	Between Groups	27.372	4	6.843	5.604	0.012
	Within Groups	12.210	10	1.221		
	Total	39.581	14			
West wellega	Between Groups	5.721	4	1.430	10.175	0.001
	Within Groups	1.406	10	0.141		
	Total	7.127	15			
Metekel (Dibate)	Between Groups	64.065	4	16.016	171.041	0.000
	Within Groups	0.936	10	0.094		
	Total	65.001	14			

Appendix.3d

ANOVA table, Response of common bean (*P. vulgaris)* at different NaCl treatments on Root Dry Weight (RDW).

Accession		Sum of Squares	Df	Mean Square	F	Sig.
North Omo	Between Groups	1.086	4	0.271	76.877	0.000
	Within Groups	0.035	10	0.004		
	Total	1.121	14			
Hadiya	Between Groups	0.376	4	0.094	13.649	0.000
	Within Groups	0.069	10	0.007		
	Total	0.445	14			
Metekel (Dangure)	Between Groups	0.460	4	0.115	31.675	0.000
	Within Groups	0.036	10	0.004		
	Total	0.497	14			
Shinile	Between Groups	1.695	4	0.424	9.174`	0.002
	Within Groups	0.462	10	0.046		
	Total	2.158	14			
Harerge	Between Groups	1.518	4	0.380	8.596	0.003
	Within Groups	0.442	10	0.044		
	Total	1.960	14			
West wellega	Between Groups	0.189	4	0.047	6.083	0.001
	Within Groups	0.077	10	0.008		
	Total	0.266	15			
Metekel (Dibate)	Between Groups	1.100	4	0.275	51.223	0.000
	Within Groups	0.054	10	0.005		
	Total	1.154	14			

Appendix 4-A common bean seeds



Key: *A =North Omo, B= Hadiya, C= Metekel, D=Shinile, E=Harerge, F=West wellega, G= Metekel, H=West wellega, I= West wellega, J =West wellega, K= Harerge, L= Metekel*

Appendix 4-B

Key: 1-2, Early growth; 3-8, during NaCl treatment of *P. vulgaris*

Appendix 4-C

Common bean after NaCl treatment


1


2


3


4


5


6


7


8

Key: *1_Metekel (Dangure), control; 2_Harerge, control; 3_ Shinile, control; 4_ Metekel (Dibate), control; 5_Metekel (Dangure), 150mM; 6_Shinile, 150mM; 7_ Harerge, 150mM; 8_ Metekel (Dibate), 150mM*

Appendix 5.

Key: A_ plant material; B and C_ samples in water bath; D_ Centrifugation; E_ Pellet; F_ Sample in PCR; G_ loading PCR product; H_ electrophoresis and I_ DNA bands in gel DOC.