MOLECULAR AND MORPHOLOGICALGENETIC DIVERSITY OF POTATO (*SOLANUM TUBEROSUM*) CLONES CONSERVED IN ETHIOPIA USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS.

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MB 300-0003/2015

A Thesis submitted to Pan African University, Institute for Basic Sciences, Technology and Innovation in partial fulfillment of the requirements for the Degree of Master of Science in Molecular Biology and Biotechnology

2017

DECLARATION

This thesis is my original work and has not been submitted to any other University for examination.

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ACKNOWLEDGEMENTS

First I want to thank God for giving strength and wisdom from t during my research work. I would like to say thank you to my supervisors for their support. I would like to acknowledge African Union (AU) and Pan African University, Institute for Basic Sciences, Technology and Innovation (PAUSTI) for giving me this scholarship. I also want to acknowledge Africa-*ai*-JAPAN JKUAT office for their financial support. I would like to say thank you to all Amhara Agricultural Research Institute plant tissue culture laboratory Bahirdar Ethiopia staffs for their support during my sample preparation and collection and the Institute for giving me a study leave and allowing me to access the potato clones and the laboratory and greenhouse facility.

I want to say thank you to all my friends and colleges who was always available and supportive, Biniam Mesfine, Amanuel, Yosef and Temitope. I am very grateful to have you as my Friends.

My deepest heartfelt gratitude to my Fiancé Mr. Gashaw Belay for his support and inspiration starting from applying for the scholarship and guiding me during my course work, research and also not letting me to give up during my hard times and also for his unconditional love.

I am deeply indebted to my family my mother, my brothers and my sister for their unconditional love and support throughout my life.

DEDICATION

This thesis is dedicated to my mother and memories of my beloved Father.

ABSTRACT

Potato (*Solanum tuberosum L.*) is the fourth most important food crop in the world after rice, wheat, and maize. Potato plays a significant role in human nutrition worldwide, where more than 320 million tons of potato is produced annually on 20 million hectares of land. In this study, a total of 53 potato genotypes which are conserved *in vitro* in Amhara Regional Agricultural Research Institute, Ethiopia, for further research and development purpose were used. These *in vitro* conserved Potatoes had not been investigated for their genetic diversity or relatedness. So, the aim of this study was to assess the molecular and morphological genetic diversity of *in vitro* conserved potato genotypes. Molecular genetic diversity was done using twelve fluorescently labeled simple sequence repeat (SSR) markers while Morphological diversity was done using morphological descriptors from CIP Potato catalog. The dendrogram analysis using the morphological data clustered the potato genotypes in to four main cluster and five sub clusters. The first cluster (CI) was the smallest cluster with 3 genotypes all from Exotic. The second cluster (CII) had 5 genotypes four Ethiopian local varieties and one from CIP. The third cluster (CIII) was the largest cluster with five sub-clusters (SC1 to SC5) It had 27 genotypes. Cluster four (CIV) contained 7 genotypes all from Ethiopian nationally released varieties. Using SSR markers a total of 79 alleles were observed with an average of 6.58 per marker. The polymorphism level of the markers varied from 3 to 10 for markers STM1053 and STM1052, respectively. The polymorphic information content of the markers ranged from 0.93 for markers STI0030 and STI0004 to 0.57 for marker STM1053 with an average value of 0.85. The highest percentage of polymorphic loci was 83.33% observed in CIP populations and the lowest was 58.33% recorded in Ethiopian local Varieties. The number of different (Private) Alleles varied from 1.792 in Ethiopian local genotypes to 3.292 in CIP genotypes. The highest genetic distance was observed between Ethiopian local varieties and the Exotic varieties which was 0.8236; the lowest genetic distance was observed between Exotic varieties and CIP Populations which was 0.6256. Principal coordinate analysis (PCoA) explained 27.63% of the total variation. Samples from CIP and the Exotics were scattered in two coordinates. The Neighbor-joining tree generated from SSR markers divided the 53 studied potato genotypes into three main clusters and 5 sub-clusters. The first cluster (C1) contained 19 genotypes with 2

sub-clusters and was dominated by samples from CIP. The second cluster (C2) contained 29 genotypes with 2 sub-clusters; all Ethiopian local varieties are clustered in this group. The third cluster (C3) constituted 5 genotypes from CIP only. The analysis of molecular variation showed that 4 % total variation was among populations and 96% of the variation was within individuals. This study revealed that there is genetic diversity among conserved potato genotypes. The information generated from this study can serve as a basis for the Ethiopian potato breeding program and *in vitro* potato conservation management strategies.

Keywords: Potato, simple sequence repeat (SSR), genetic diversity, *in vitro* conservation

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ACRONYMS

AMOVA Analysis of Molecular Variance

ARARI Amhara Agricultural Research Institute

CIP International Potato Center

CSA Central Statistic Agency

DNA Deoxyribonucleic acid

FAOSTAT Food and Agriculture Organization Statistical Database

IOB Institute of Biotechnology

IBR Institute of Biotechnology Research

ISSR Inter-Simple Sequence Repeats

LB Late Blight

PCR Polymerase Chain Reaction

PIC Polymorphic Information Content

PCoA Principal Coordinate Analysis

RFLP Restriction Fragment Length Polymorphism

RAPD Random Amplified Polymorphic DNA

SSR Simple Sequence Repeats

SNP Single Nucleotide Polymorphism

STR Short Tandem Repeats

UL Micro Liter

WHO World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Potato (*Solanum tuberosum L.*) is the fourth major important food crop in the world after rice, wheat, and maize. More than 320 million tons of potato is produced annually on 20 million hectares of land in the world. Asia and Europe are the world's major potato producing regions, accounting for more than 80 percent of world production. There has been a dramatic increase in potato production and demand in Africa (CIP 2015). Potato is an important crop for smallholder farmers in Ethiopia, serving both as a cash crop and food security crop. It is one of the root crops widely grown in the country because of increasing demand and emerging markets that have provided a great opportunity for resource-poor farmers to generate additional income (Bekele *et. al.*, 2011). The northwestern part of the country which includes the highlands of the west Amhara sub-region is the major production area. This region makes up over one-third of the total area allotted to potato in Ethiopia. About 600,000 rural households are involved in potato production in the region, according to Central Statistics Agency (CSA) (2008/2009). Thus, the country is among the top potato producers in Africa, with 70% of its arable land in the high altitude areas (> 1500 m.a.sl) suitable for potato production. The National average productivity of potato in Ethiopia is 8 tons/ha, which is below the African continent average (10.8 tons/ha) (FAOSTAT, 2008). Diseases, the lack of improved varieties, poor crop management practices, use of inferior quality seed tubers of unknown origin, inappropriate storage structure, Poor seed system, and poor research-extension linkage are among the key factors contributing to this low yield (Gebremedhin *et al.*, 2008).

1.1.1 Potato Production Constraints in Ethiopia

Just like any other crop, potato‘s production is constrained by a wide range of factors that result in low yields (Gebremedhin, 2003). The use of unimproved potato varieties and diseases are among the major production constraints. The use of local potato varieties for seed is one of the constraints of potato production in Ethiopia because the local varieties which were introduced earlier, may be of the same parentage (Haile-Michael, 1979), suggesting that the genetic base of local varieties in

the country is most probably narrow (Baye and Gebremedhin, 2013). Potato is prone to different diseases such as Bacterial wilt, Late blight which are the most devastating diseases in Ethiopia. So the lack of disease resistant varieties is the other factor for poor potato production in the country.

1.1.2 Agronomic practices of potato in Ethiopia

Planting time varies from place to place and from variety to variety. For maximum yield, potato should be planted when favorable conditions prevail for better growth and development. Farmers in northwest Ethiopia plant potato earlier in the season to escape Late Blight (LB) infection. However, this practice exposes the crop to moisture stress at an early stage of growth for which potato is very sensitive and is therefore subject to considerable loss. Tesfaye *et al.,* (2008) reported that regardless of the type of varieties, yield declined as planting date was delayed. Therefore, May 1–June 1 were recommended planting dates around Adet and similar agro-ecologies for potato cultivars that are susceptible to LB and moderately tolerant/resistant. Similarly, early June was recommended for Emdiber (Gurage zone), Holetta (central Shewa), and other similar agro-ecological areas (Berga *et al*., 1994). Semagn and Abdulwaha (2008) recommended the last week of May to mid-June as an appropriate planting time for potato in the highlands of Ankober (North Shewa) and other similar agro-ecologies.

Seed tuber size and plant population density are among the major factors affecting the production and productivity of potato. According to Berga *et al*., (1994), spacing should depend on the intended use of the crop such as for seed or ware. Closer intra-row spacing of 10 or 20 cm in rows 75 cm apart would be beneficial for seed; larger seed tubers (45–55 mm) do better than the smaller ones. Wider intra-row spacing (30 or 40 cm) were better, again in rows of 75 cm apart, is good for ware potato. Considering the amount of seed tuber required and type of output and synergy with other cultural practices, seed tuber size of 35–45 mm diameter, 60-cm inter-row spacing, and ridging once at 3–4 weeks of crop emergence were recommended for seed potato production. However, 35–45 mm diameter seed tuber, 75 cm inter-row spacing, and ridging once at 3–4 weeks from crop emergence was found to be optimum and recommended practices for ware potato production at Adet and its environs (Tesfaye *et al*.,2008).

Potato is naturally a heavy feeder crop. Economically feasible fertilizer rate varies with soil type, fertility status, moisture amount, other climatic variables, variety, crop rotation, and crop management practices (Smith, 1977). Research results indicated that 108/69 and 81/69 kg/ha. N/P2O5 were economically feasible and optimum rate for potato production in south Gondar and Gojam areas, respectively (Tesfaye *et al*., 2008). For optimum potato tuber yield in nitosols and light vertisols of the highland areas of north Shewa, 110 kg/ha nitrogen and 70.5kg/ha P2O5 kg/ha were recommended (Abdulwahab and Semagn, 2008). Berga *et al.*, (1994) recommended 165/90 N/P2O5 as the feasible rate for the central Shewa, and this recommendation is still in use as blanket recommendation throughout the country. In the same way, 146/138 N/P2O5 was recommended as the economic and agronomic rate of fertilizer for the highlands of Hararghe (Teriessa, 1995). These recommendations may not work for the current market, soil fertility status, and other climatic variables. Therefore, detail soil test-based fertility studies should be carried out to provide appropriate local recommendations.

In the absence of storage technologies for ware and seed potato, farmers keep potato harvest in the ground for a long period in Ethiopia. This reduces tuber yield significantly. A study on extended harvesting period in Alemaya revealed that yield of marketable tubers was reduced by 60% when tubers were harvested at 210 days after planting as compared to a harvest at 120 days (Berga, 1994). Similarly, Gebremedhin *et al*., (2001) reported significant yield reductions (70–100%) when harvesting was delayed from about 125 days to 230 days after planting.

1.1.3 Potato breeding strategies in Ethiopia

Improving the productivity of potato, which is a demanding process, has occurred by trying to widen the genetic base of potato. A selection program was started in 1973 at Alemaya College of Agriculture in collaboration with the Institute of Agricultural Research (IAR) and the International Potato Center (CIP) in Peru which is a center of diversity and maintains the world's largest bank of potato germplasm. A more coordinated improvement effort was started later in 1975. Introduction and evaluation of commercial varieties, germplasm, generation of local populations,

and, more recently, the introduction of advanced materials were some of the strategies that have been followed to develop varieties by the National Potato Research Project (formerly known as the National Potato Improvement Program). A number of variety trials were conducted in different areas of the country to address problems of different agro-ecologies (Baye and Gebremedhin, 2013).

The overall potato breeding strategy focuses on developing varieties based on the morphological information. The breeding procedure generally involves introducing clones, clonal selection, stability studies, and verification tests for official release and dissemination of new varieties for production (Gebremedhin *et al*., 2008). From 1987 to 2010 29 potato varieties were released by the research system (Berga *et al*., 1994).The clones with better performance in the nursery stages are replicated across three different environments in pre-national variety trial (PNVT) and evaluated for one season before they were promoted to the national variety trial (NVT). The clones selected from the PNVT stage are promoted to a replicated study across 16 different environments (8 locations for two years) under NVT. The challenge in this approach has been the appropriateness of the weather in 2–3 consecutive years to represent the long-term climate at each site, even though the management in each year for all varieties across sites can be assumed uniform. Moreover, the stability of clones across locations and years was evaluated to better understand the genotype and environment interactions (Gebremedhin, 2003).

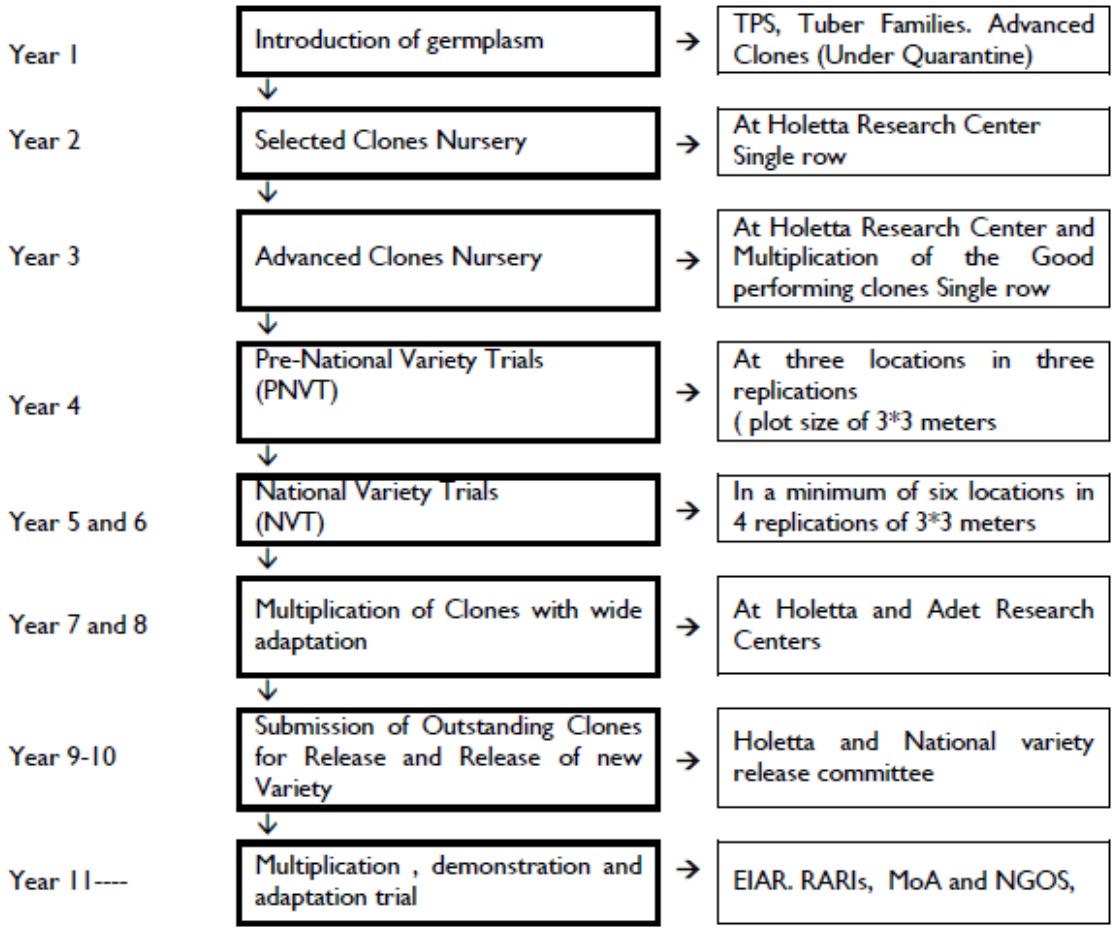

Figure 1: potato variety development /selection scheme in Ethiopia Gebremedhin, (2003)

The Ethiopia potato breeding and variety development is based on observation of morphological characters or descriptors which takes up to 11 years of trial of the germplasms, this is time consuming, laborious and expensive process. Furthermore, morphological characters are often multigenic, not available at all growth stages and influenced by environment, making it difficult to assess them quickly and objectively, and requiring repeated observations. So, to facilitate and hasten the potato breeding process, knowledge of genetic diversity of the introduced germplasms which is very crucial.

For a successful potato breeding program, genetic diversity and variability play a vital role. Genetic diversity in a population is a precondition for an effective plant-breeding program. Genetic divergence is a useful tool for an efficient choice of parents for hybridization to develop high yielding cultivars (Haydar, 2007). The importance of genetic divergence in the improvement of the crop has been stressed in both self and cross-pollinated crops (Gaur *et al.,* 1978). Evaluation of genetic difference is important to know the source of genes for a particular trait within the available germplasm (Tomooka, 1991).

In Amhara Regional Agricultural Research Institute (ARARI), Ethiopia, plant tissue culture laboratory, more than 50 potato accessions are conserved for further breeding and seed multiplication purposes. These potato genotypes were collected from local farmer's fields in the country and gained from other countries through germplasm exchange among international research institutes. However, these conserved potato accessions have not been assessed for their genetic diversity or relatedness.

Several methodologies are used to characterize and evaluate the similarity or genetic distance between genotypes Recent developments in molecular biology have opened the possibility of employing various types of molecular tools to identify and use genomic variation for improvement of several organisms (Francesco *et al.,* 2013).

Recently, molecular marker development has resulted in various molecular markers being used for different purposes by researchers. . A molecular marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. The most important markers include; Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSRs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphism (SNP). SSRs are currently considered the markers of choice in many areas of genetic diversity, due to their co-dominant and polymorphic nature (Pineda *et al.,* 2013). SSR markers have proven to be useful tools for genetic diversity studies, because they are reproducible, neutrally evolving, and multi-allelic. Accordingly, SSR markers were used in this study.

1.2. Statement of the problem

Potato is a cheap source of energy and supplies good quality food within a relatively short period of time for subsistence farmers in developing countries like Ethiopia. The yield level of potato in Ethiopia is lower than other potato growing countries of the world. Development of high yielding varieties having good keeping quality is one of the challenges of potato breeders. Genetic variable is considered as a prerequisite for crop improvement program. The quantification of genetic diversity will allow precise parental line selection for successful potato breeding program. The current potato varieties which are used by the farmers have limitations that include shortage of good quality seed tubers; lack of adaptable and disease resistant varieties, which is associated with the narrow genetic base of the crop. Therefore, to broaden the gene pool of the crop, introduction of new potato genotypes and collection of potato landraces was done and the collected germplasm was conserved at ARARI, plant tissue culture laboratory. As a short term solution, dissemination of disease-free potato varieties through tissue culture technique has been used for many years. However, tissue culture alone has not overcome the vast challenge of potato Production in Ethiopia. No attempts had been made before to investigate the genetic diversity of the conserved potato genotypes in Ethiopia. In addition, it's expensive to conserve these genotypes in a tissue culture laboratory because of the need for consumables, manpower, and space. Therefore there is need to investigate the molecular and morphological genetic diversity of the conserved germplasm to help eliminate duplication and allow for a systematic conservation strategy.

1.3 Justification

It is known that development and utilization of genetic resources, as well as germplasm conservation, depend on the understanding of the genetic diversity and relationships between varieties from target regions. Thus, understanding the molecular and morphological genetic variation between and within potato genotypes is an important step for every management strategy directed towards improvement and conservation of diverse potato genotypes in Ethiopia. Moreover, having knowledge on genetic diversity is also the basis for molecular breeding of potato to improve important traits such as stress tolerance, high yield, and quality among others. Cultivar identification can simply be made using molecular markers like simple sequences repeats (SSRs),

which give accurate identification and does not require a lot of DNA and plants can be analyzed at early age. This study has generated data on, molecular and morphological genetic diversity of the Ethiopian conserved potato accessions. The information generated from this study can serve as a basis to help improve potato productivity as well as enhance *in-situ* and *ex-situ* conservation.

1.4. General objective of the study:

The general objective of this study was to determine the molecular and morphological genetic diversity of potato genotypes conserved at ARARI, plant tissue culture laboratory, Ethiopia

1.4.1. Specific objectives

- To determine the molecular genetic diversity and population structure of conserved potato *(S. tuberosum)* genotypes in Ethiopia using SSR markers.

-To investigate the morphological diversity of the *in vitro* conserved potato (*S. tuberosum*) genotypes in Ethiopia using morphological descriptors from CIP potato catalog

1.5. Significance of the study

The molecular and morphological genetic diversity information of potato germplasm conserved in ARARI Ethiopia can be used by potato breeders for selection of the best divergent parental lines which is required for molecular breeding of potato for the development and improvement of potato varieties. Which in turn can help to fight food insecurity and poverty in the country. Improved potato production increases income for the farmers and hence improve the livelihoods of millions of Ethiopians that rely on potato. It also helps in conservation strategies because it helps to eliminate duplication in the conserved germplasm.

1.6 Hypothesis

There is no molecular and morphological genetic diversity in potato germplasms conserved at ARARI in Ethiopia.

CHAPTER 2: LITERATURE REVIEW

2.1. Origin and Distribution of Potato

Potato (*Solanum tuberosum* L.) originated in the Andes of central Peru and central Bolivia (Poehlman and Sleper, 1995). Potato was originally believed to have been domesticated independently in multiple locations, but later genetic testing of a wide variety of cultivars and wild species proved a single origin for potatoes in the area of present-day southern Peru and extreme northwestern Bolivia (from a species in the *Solanum brevicaule* complex), where they were domesticated approximately 7,000–10,000 years ago (Sponner, 2005). Following centuries of selective breeding, there are now over a thousand different types of potatoes. Over 99% of the present cultivated potatoes worldwide descended from varieties that originated in the lowlands of south-central Chile, which have displaced formerly popular varieties from the Andes (Ames & Spooner, 2008).

2.2. Taxonomy of Potato (*Solanum tuberosum L.)*

Potato (*Solanum tuberosum* L.), is a member of the *Solanaceae* family and the genus *Solanum*, it is one of the most productive and widely grown horticultural food crop in the world. The genus *Solanum* comprises of about 200 species, including over 150 tuber-bearing species that form a polyploid series from diploids (2x) to hexaploids (6x) with 75 percent of them representing diploids (Poehlman and Sleper, 1995). The common potato is an autotetraploid with a genomic constitution of 2n= 4x= 48.

2.2.1. Taxonomic hierarchy of Potato

- Kingdom: *Plantae* (*Plants*)

  Subkingdom:*Tracheobionta*(Vascularplants)

- Superdivision:*Spermatophyta* (seedplants)
- Division: *Magnoliophyta* (flowering plants)
- Class: *Magnoliopsida* (dicotyledons)
- Subclass: *Asteridae*
- Order: *Solanales*

- Family: *Solanaceae*
- Subfamily: *Solanoideae*
- Genus: *Solanum L.*
- Section: *Petot*
- Subsection: *Potatoe*
- Series: *Tuberosa*
- Species: *Solanum tuberosum L.*

Source: USDA, NRCS, 2010

2.3. Botany of potato

Potato *(S. tuberosum)* is a herbaceous plant that grows to 0.4 to 1.4 m tall and may range from erect to fully prostrate (Spooner and Knapp, 2013). Stems range from nearly hairless to densely hairy and may be green, purple, or mottled green and purple. Leaves are pinnate with a single terminal leaflet and three or four pairs of large, ovoid leaflets with smaller ones in between (Spooner and Knapp, 2013). *S. tuberosum* plants produce rhizomes (often called stolon's) that have rudimentary leaves and are typically hooked at the tip. They originate from the basal stem nodes, typically below ground, with up to three rhizomes per node (Struik, 2007). Tubers can be spherical to ovoid in shape and are swellings of the rhizome. The flesh of the tubers varies in color from white to yellow to blue and the skin varies from white through yellow to tan and from red through blue. The color of the flesh may or may not correspond to the color of the skin. The texture of the surface may vary from smooth to netted or russeted (Spooner and Salas, 2006). On the surface of the tuber are axillary buds with scars of scale leaves that are called eyes (Struik, 2007). Potatoes mostly propagate vegetatively (International year of potato, 2008). When tubers are planted, the eyes develop into stems to form the next vegetative generation.

2.3.1 Reproduction

Potato flowers are bisexual, have both male and female parts. They possess all four essential parts of a flower; calyx, corolla, male elements and female elements (Potatoes production guideline, 2013). Most commercial varieties of potato have a reduced ability to flower and breeders do not select for traits that make the flower attractive to pollinators. However, natural potato pollination remains important to sustaining the diversity of landraces. The diverse smallholder farming systems in the Andes harbor a variety of potato flowering plants that do attract pollinators, such as honeybees and bumblebees, which promote cross-pollination of potato flowers, thus increasing seed production and sustaining diversity(International year of potato, 2008).

2.4 Ecological requirements of potato

Potatoes grow well on a wide variety of soils. In some areas where potatoes are commercially grown, the soils are acid, whereas in others they are alkaline. An ideal soil for potato growing is one that is deep, well-drained and friable. Soils high in organic matter such as peat or muck, if adequately drained, can also produce potatoes. Potatoes are more tolerant to low pH than most other crops. The incidence of common scab tends to be less of a problem where soil pH is lower than 5.4 for cultivars that are susceptible to common scab, the disease is often managed by maintaining soil pH in the range of 5.0 to 5.4 (Potatoes production guideline, 2013).

Water management and/or rainfall are among the most important factors determining yield and quality of potatoes. The amount of water required for optimum growth of potatoes varies with cultivar, relative humidity, solar radiation, day length and length of growing season. However, the general rainfall requirement for cultivars in all areas is at least 460 mm. As much as 760 to 910 mm of rainfall is required in some specific production areas depending on soil type, weather conditions, and potato cultivar. Water should be applied to the soil frequently in light volumes to maintain the crop with an adequate water supply throughout all growth stages of the crop (Potatoes production guideline, 2013).

2.5 Utilization of potato

Potatoes are used for a variety of purposes, not only as a vegetable for cooking at home. Less than 50 percent of potatoes grown worldwide are consumed fresh. The rest are processed into potato food products and food ingredients; fed to cattle, pigs, and chickens; processed into starch for industry, and re-used as seed tubers for growing the next season's potato crop (Potato processing and uses, 2011).

2.5.1 Food Uses

Fresh potatoes are baked, boiled, or fried and used in a staggering range of recipes: But global consumption of potato as food is shifting from fresh potatoes to value-added, processed food products (CIP, 2011). Potato flour is used by the food industry to bind meat mixtures and thicken gravies and soups and its starch provides higher viscosity than wheat and maize starches and delivers a tastier product. It is used as a thickener for sauces and stews, and as a binding agent in cake mixes, dough, biscuits, and ice-cream. In eastern Europe and Scandinavia, crushed potatoes are heated to convert their starch to fermentable sugars that are used in the distillation of alcoholic beverages, such as vodka and akvavit (Potato processing and uses, 2011).

2.5.2 Non-Food Uses

Potato starch is widely used in the pharmaceutical, textile, wood, and paper industries as an adhesive, binder, texture agent, and filler, and by oil drilling firms to wash boreholes. Potato starch is an 100% biodegradable substitute for polystyrene and other plastics and is used to make disposable plates, dishes, and knives. Potato peel and other "zero value" wastes from potato processing are rich in starch that can be liquefied and fermented to produce fuel-grade ethanol. A study in Canada's potato-growing province of New Brunswick estimated that 44,000 tons of processing waste could produce 4-5 million liters of ethanol .In the Russian Federation and other east European countries, as much as half of the potato harvest is used as farm animal feed. Cattle can be fed up to 20 kg of raw potatoes a day, while pigs fatten quickly on a daily diet of 6 kg of boiled potatoes. (Potato processing and uses, 2011).

2.6. Nutritional content of potato

Potato tubers contain substantial amounts of essential vitamins and trace elements. It is a source of ascorbic acid (Vit-C) and some B-complex vitamins, especially thiamine, niacin and vitamin B6 (Horton and Sawyer, 1985). One hundred (100) g boiled potato in the diet provides 50 percent of the recommended daily allowance of Vitamin-C. It is also a moderate source of iron and a good source of phosphorus, magnesium, and potassium (Horton and Sawyer, 1985). Potatoes offer some protein but its content is low compared to Eggs, dairy, meat, seafood, fish, and poultry but has higher amino acids compared to Sweet potato and Cassava (Anne, 2015).

Table 2.0: Amino acid content of potato compared to sweet potato and cassava

Amino acid(mg/g crude protein)	**Potato**	**Sweet Potato**	**Cassava**
Histidine	20	13	21
Leucine	59	54	40
Lysine	60	34	41
Methionine+cystine	30	28	27
Phenylalanine+Tyrosine	78	62	41
Threonine	39	38	26
Tryptophan	14	14	12
Valine	51	45	33

World Health Organization (WHO) (1985).

2.7. Importance of genetic diversity of potato

The history of potato provides warning of the need to maintain genetic diversity in our staple food crops. In the 19th century, Ireland was heavily reliant on only a few varieties of potato, and those types contained no resistance to the devastating disease known as late blight. When late blight destroyed the 1845-1846 potato crop, widespread famine followed (International year of Potato, 2008). An estimated one million people starved to death and more than a million were forced to migrate abroad.

To combat pests and diseases, increase yields, and sustain production on marginal lands, today's potato-based agricultural systems need a continuous supply of new varieties. That requires access to the entire potato gene pool. But potato biodiversity is under threat as ancient varieties cultivated by Andean peoples for millennia have been lost to diseases and climate change. Therefore more the need for genetic diversity especially with uncertainties of the effects of climate change on crop productivity.

2.8 Genetic diversity and its implication in crop (Potato) improvement

Genetic diversity refers to the amount of genetic variability among individuals of a variety or population of a species (Brown, 1983). During the assessments of genetic diversity, the number of alleles and their distribution as well as effect on performance, and the overall distinctness between different populations can be determined (Rao and Hodgkin, 2002). It is clearly understood that variation arises from selection, genetic drift, and gene flow which cause allelic variation in different populations.

Knowledge of genetic variation and relationships between accessions/clones is critical to identify cultivars, understand their genetic variability for further improvement programs, and provide evidence of evolutionary forces shaping the cultivar diversities and give appropriate conservation strategies (Thormann *et al.,* 1994). This is also reported as the basis for survival and adaptation in different seasons, places and fitness (Thormann *et al.,* 1994). On the other hand, the destruction of genetic resources can be due to biotic/abiotic stresses, or isolation and habitat alteration because of slow geological and climatic change, natural catastrophes or human activities (Rao, 2004).

In order to develop a conservation strategy so as to determine what to conserve, where and how to conserve, sufficient information on the extent and distribution of genetic variability in a species is required. As a result, variability/similarity data provides relevant information on gene pools coverage and redundancy gaps which waste resources through increased cost of management (Rao & Hodgkin, 2002). Thus, knowledge of genetic diversity of plant genetic resources is an essential for efficient utilization and conservation of germplasms.

2.9. Molecular markers for diversity studies in crops

The differences that distinguish one plant from another are encoded in the plant's genetic material, the deoxyribonucleic acid (DNA). A genetic marker can be defined in one of the following ways:(a) a chromosomal landmark or allele that allows for the tracing of a specific region of DNA; (b) a specific piece of DNA with a known position on the genome; or (c) a gene whose phenotypic expression is usually easily distinguished, used to identify an individual or a cell that carries it, or as a probe to mark a nucleus, chromosome, or locus. They can also be defined as identifiable DNA sequences, found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next (Rajeev *et al.*, 2007). Approaches are beginning to have a significant impact on plant genetic resource conservation and use (Carlos *et al.*, 2014). Initially, molecular techniques were used largely for the analysis of specific genes for understanding gene action, gene mapping and the development of gene transfer technologies. More recently, the techniques have been applied to problems of direct relevance for understanding the distribution and extent of genetic variation within and among between species (Maheswaran, 2004). Recent developments in molecular biology have opened the possibility of employing various types of molecular tools to identify and use genomic variation for the improvement of several organisms (Francesco *et al.*, 2013).

Molecular markers can be classified into different groups based on: a) Mode of transmission (biparental nuclear inheritance, maternal nuclear inheritance, maternal organelle inheritance, or paternal organelle inheritance); b) Mode of gene action (dominant or co-dominant markers); c) Method of analysis (hybridization-based or PCR-based markers). These molecular markers

include: (i) hybridization-based markers such as restriction fragment length polymorphism (RFLP), (ii) PCR-based markers like randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP): inter-simple sequence repeats (ISSRs), and microsatellite or simple sequence repeats (SSRs), and (iii) sequence-based markers like single nucleotide polymorphism (SNP). For plant breeding applications, SSR markers have been proven and recommended as markers of choice because of their high polymorphic and codominance nature (Gupta & Varshney, 2000).

2.9.1. Restriction Fragment Length Polymorphism (RFLP)

The development of restriction fragment length polymorphism (RFLP) molecular markers has facilitated the mapping of plant and animal genomes. The technique is based on restriction enzymes that reveal a pattern difference between DNA fragment sizes in individual organisms. Although two individuals of the same species have almost identical genomes, they will always differ at a few nucleotides due to one or more of the following causes: point mutation, insertion/deletion, translocation, inversion, and duplication. Some of the differences in DNA sequences at the restriction sites can result in the gain, loss, or relocation of a restriction site. Hence, digestion of DNA with restriction enzymes results in fragments whose number and size can vary among individuals, populations, and species (Semagn *et al*., 2006).

The major strength of RFLP markers are high reproducibility, co-dominant inheritance, good transferability between laboratories, no sequence information required, and relatively easy to score due to the large size difference between fragments (Luiz *et al*., 2004). However, there are several limitations for RFLP analysis such as it requires high quantity and quality DNA. It depends on the development of specific probe libraries for the species; the technique is not amenable to automation; the level of polymorphism is low and few loci are detected per assay; it is time consuming, laborious, often requires the use of radioactive substances and it is expensive (Ahmed *et al.,* 2012). Therefore currently RFLP markers have been replaced by other suitable markers.

2.9.2. Inter-Simple Sequence Repeats (ISSRs)

ISSRs involve amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite directions. ISSRs are semi-arbitrary markers amplified by polymerase chain reaction (PCR) in the presence of one primer complementary to a target microsatellite. Each band corresponds to a DNA sequence bordered by two inverted microsatellites (Tsumara *et al.,* 1996).

ISSRs use longer primers (15–30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of high annealing temperatures leading to higher stringency. The amplified products are usually 200–2000 bp long and amenable to detection by both agarose and polyacrylamide gel electrophoresis. It does not require genome sequence information; it leads to Multilocus, highly polymorphic patterns and produces dominant markers (Mishra *et al.,* 2003). The ISSR technique is simple, quick, and the use of radioactivity is not required. ISSR markers usually show high polymorphism. However, like RAPDs, dominant inheritance and homology of co-migrating amplification products are the main limitations of ISSRs.

2.9.3 Random amplified polymorphic DNA (RAPD)

RAPD (random amplified polymorphic DNA) was the first to amplify DNA fragments from any species without prior sequences information. The RAPD protocol usually uses a 10 bp arbitrary primer at constant low annealing temperature (generally34 – 37 o C). Although the sequences of RAPD primers are arbitrarily chosen, two basic criteria indicated by (Williams *et al*., 1990) must be met: a minimum of 40% GC content (50 - 80% GC content is generally used) and the absence of palindromic sequence (a base sequence that reads exactly the same from right to left as from left to right). Because G-C bond consists of three hydrogen bridges and the A-T bond of only two, a primer-DNA hybrid with less than 50% GC will probably not withstand the 72 o C temperature at which DNA elongation takes place by DNA polymerase. The resulting PCR products are generally resolved on 1.5- 2.0% agarose gels and stained with ethidium bromide (EtBr); polyacrylamide gels in combination with either AgNO3 staining (Huff *et al*., 1993). Radioactivity or fluorescently labeled primers or nucleotides are sometimes used (CorleySmith *et al*., 1997).

Despite its low resolving power, the simplicity and low cost of agarose gel electrophoresis has made RAPD more applicable (Vejl, 1997). Most RAPD fragments result from the amplification of one locus, and two kinds of polymorphism occur: the band may be present or absent, and the brightness (intensity) of the band may be different. Band intensity differences may result from copy number or relative sequence abundance (Devos & Gale, 1992) and may serve to distinguish homozygote dominant individuals from heterozygotes, as more bright bands are expected for the former (Hollingsworth *et al*., 1998). RAPD has three limitations Reproducibility, Dominant inheritance, and Homology. The other limitation of RAPD markers is that the majority of the alleles segregate as dominant markers, and hence the technique does not allow identifying dominant homozygotes from heterozygotes (Weller and Reddy, 1997). The RAPD assays produce fragments from homozygous dominant or heterozygous alleles. No fragment is produced from homozygous recessive alleles because amplification is disrupted in both alleles (Pammi *et al*., 1994).

2.9.4 Amplified fragment length polymorphism (AFLP)

AFLP technique combines the power of RFLP with the flexibility of PCR-based technology by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh, 1998). The key feature of AFLP is its capacity for "genome representation": the simultaneous screening of representative DNA regions distributed randomly throughout the genome. AFLP markers can be generated for DNA of any organism without initial investment in primer/probe development and sequence analysis. Both good quality and partially degraded DNA can be used for digestion but the DNA should be free of restriction enzyme and PCR inhibitors (Blears *et al*., 1998).The first step in AFLP analysis involves restriction digestion of genomic DNA (about 500 ng) with a combination of rare cutter (EcoRI or PstI) and frequent cutter (MseI or TaqI) restriction enzyme Double-stranded oligonucleotide adaptors are then designed in such a way that the initial restriction site is not restored after ligation (Mueller *et al*.,1996). Such adaptors are ligated to both ends of the fragments to provide known sequences for PCR amplification. PCR amplification will only occur where the primers are able to anneal to fragments which have the adaptor sequence plus the complementary base pairs to the additional nucleotides called selective nucleotides AFLP

fragments are visualized either on agarose gel or on denaturing polyacrylamide gels with autoradiography, The advantages of AFLP include: It is highly reliable and reproducible (Lin *et al*., 1996)..It does not require any DNA sequence information from the organism under study (Jones *et al*., 1997). It is information-rich due to its ability to analyze a large number of polymorphic loci simultaneously (effective multiplex ratio) with a single primer combination on a single gel as compared to RAPDs and RFLPs (Powell *et al*., 1996).The limitations of AFLP includes: It requires more number of steps to produce the result, It requires template DNA free of inhibitor compounds that interferes with the restriction enzyme (Milbourne *et al*., 1997). The technique requires the use of polyacrylamide gel in combination with AgNO3 staining, radioactivity, or fluorescent methods of detection, which will be more expensive and laborious than agarose gels. It involves additional cost to purchase both restriction and ligation enzymes as well as adapters. Like RAPD, most AFLP loci are dominant, which does not differentiate dominant homozygotes from heterozygotes. This reduces the accuracy of AFLP markers in population genetic analysis, genetic mapping, and marker assisted selection (Russell *et al*., 1997).

2.9.5. Simple Sequence Repeats (SSRs)

Microsatellites, Simple Sequence Repeats (SSRs), or Short Tandem Repeats (STRs), are repeating sequences of 1-6 base pairs of DNA. SSR allelic differences are, therefore, the result of variable numbers of repeat units within the microsatellite structure. SSR markers are highly informative due to co-dominance, multiallelism, heritability, abundance and wide coverage of the genome. Another advantage is the conservation of flanking regions across generations, which allows repeated use of the technique (Francesco *et al.,* 2013).

Although SSR markers are developed for use in a single species, it is possible to extend known markers for use in related species. This is possible because the flanking regions are conserved and the number of duplications is variable. Therefore, once an SSR marker is available in a related species, attempting to transfer known markers can be advantageous for the individual who does not have original developed SSR markers. The availability of new microsatellite markers is important to effectively contribute to the genetic analysis of potatoes. The development of microsatellite markers involves the use of SSR-enriched libraries. This process is still time-

consuming and expensive and also requires much discovery and optimization for each species before use (Cubry *et al.,* 2014). SSR markers have been used in molecular characterization of potato cultivars because they are co-dominant and easily reproducible, and have a frequent and random distribution, allowing a wide coverage of the genome. The high level of variation detected with microsatellites increases the resolution for genealogy and germplasm genetic diversity studies and reduces the number of markers required to distinguish between genotypes (Borém & Caixeta, 2006).

Rocha *et al*., (2010), using six RAPD and three SSR markers, identified 16 cultivars of potato. The author observed that SSR markers were more efficient than RAPD markers since the three SSR markers allowed the distinction of all cultivars studied, compared with the six primers used for RAPD. Several studies have used SSR markers for the characterization of potato cultivars and accessions, such as (Braun & Wenzel, 2004); Braun *et al.,* 2004; Chimote *et al.,* 2004; Ghislain *et al.,* 2006; Barandalla *et al.,* 2006; Mathias *et al.,* 2007; Ispizúa *et al.,* 2007; Fu *et al.,* 2009; Coombs *et al*., 2004; Muthoni *et al*., 2014 and Biniam *et al*., 2016) as well as of other species, e.g. soybean (Garcia *et al*., 2007).

CHAPTER THREE: Materials and Methods

3.1. Plant material

A total of 53 Potato (*Solanum tuberosum L.*) clones were collected from Amhara Regional Agricultural Research Institute (ARARI), which are maintained at the plant tissue culture laboratory via slow growth conservation media. These genotypes were introduced from different parts of the world for research and development purposes (Table 3.0). A total of four populations were created based on the origin of germplasm (Ethiopia Local Varieties with 4 genotypes, Ethiopia Nationally released Varieties with 9 genotypes, CIP with 25 genotypes and Exotic with 15 genotypes).

Table 3.0 List of potato genotypes used in this study with their origins

SN	Clone/variety name	Origin	SN	Clone/variety name	Origin
1	AB	ETL	28	399048.39	CIP
2	EL	ETL	29	399051.1	CIP
3	AKO7	ETL	30	399053.11	CIP
4	DUll	ETL	31	399064.12	CIP
5	Belete	ETRV	32	399064.3	CIP
6	Gudene	ETRV	33	399062.119	CIP
7	Jalena	ETRV	34	399075.22	CIP
8	Gera	ETRV	35	399075.22	CIP
9	Guassa	ETRV	36	395169.4	CIP
10	Shankola	ETRV	37	395169.17	CIP
11	Zenegena	ETRV	38	395112.36	CIP
12	Gorbela	ETRV	39	Algeria	Exotic
13	Dagme	ETRV	40	Kenebc	Exotic
14	395096.2	CIP	41	Atlantic	Exotic
15	395112.19	CIP	42	M171	Exotic
16	395112.32	CIP	43	Granola	Exotic
17	395114.5	CIP	44	DRN	Exotic
18	395015.6	CIP	45	NYIZY/1015	Exotic
19	396009.239	CIP	46	PMPR#2	Exotic
20	396009.258	CIP	47	R157IY	Exotic
21	396012.266	CIP	48	M182/21	Exotic
22	395017.14	CIP	49	W5015-12	Exotic
23	396026.103	CIP	50	RD14-20	Exotic
24	396027.111	CIP	51	H	Exotic
25	396031.108	CIP	52	HG	Exotic
26	396037.215	CIP	53	S176/1	Exotic
27	396240.23	CIP			

Where: ETL= Ethiopian local varieties, ETRV= Ethiopian Nationally released varieties, CIP= International Potato Center and Exotic=Potatoes their passport date were not available.

3.2. Plant material collection and procedures for molecular genetic diversity study

Potato plantlets were transferred from conservation media to a standard (Murashige &Skoog, 1962) proliferation media in order to have enough plant samples for DNA extraction. After one month in proliferation media, the potato plantlets were transferred to ARARI greenhouse for primary and secondary acclimatization. The primary acclimatization media contained sterilized sand sprayed with Ridomil gold. The plantlets were covered by cheesecloth to reduce water loss. After two weeks of acclimatization, primary acclimatized plantlets were transplanted to secondary acclimatization stage. In secondary acclimatization, the plantlets were grown in plastic pots containing sterilized soil, red ash, sand and manure in the ratio of (2:1:1:1) respectively. Three weeks after transplanting, fresh young leaves were collected and put in zip lock plastic bags containing silica gel to hasten dehydration of leaves and ready for the downstream process of DNA isolation.

3.2.1 DNA extraction

In order to assess the molecular genetic diversity within and among conserved potato genotypes; total genomic DNA was extracted from the silica gel dried leaves of the 53 samples using QIAGEN DNeasy plant mini kit following the manufacturers' instructions (Appendix 1). DNA isolation was performed at Institute of biotechnology research laboratory (IBR) of Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya.

3.2.2 Genomic DNA quality and quantity detection

The quality of DNA was determined by 0.8% agarose gel electrophoresis and its concentration was determined by a nanodrop. To visualize genomic DNA under UV light, the gel was stained with 2ul ethidium bromide. Gel visualization and image capturing were done by using a digital gel doc system. Furthermore, the concentration and purity of the isolated genomic DNA were quantified by using NanoDrop (Nanodrop®2000c) spectrophotometer at an absorbance of 260 nm, 230 nm, and 280 nm. OD ratios A260/A280, A260/A230 were calculated automatically to evaluate the quality and quantity of genomic DNA. Overall the concentration of DNA was within a range of 12.45-123.44(ng/µl). (Appendix 2). Finally, the concentrations of the extracted genomic DNA

from each of the 53 samples was normalized to 10 ng μL^{-1} by diluting with distilled sterilized water and later stored at –20°C until it used in the PCR assay.

3.2.3 Primer selection and polymerase chain reaction (PCR)

Based on the polymorphic information content (PIC) value and functionality of the primers (Biniam *et al.*, 2016), twelve sets of M13 tailed fluorescent labeled primer were selected (Table 3.1). The SSR-PCR assay was carried out in Institute of biotechnology research laboratory (IBR) of Jomo Kenyatta University of Agriculture and Technology, Juja Kenya. The PCR amplifications were carried out in 10µl reaction volume containing 1.5 µl template DNA, 5 µl 2x PCR master mixes, 0.2 µl of each primer, 1 µl M13 tail and ddH2O.The SSR locus amplification was carried out using the following PCR conditions: initial denaturation at 95 ^{0}C for 3 minutes; 35 cycles, each consists of 94^{0}C for 30 sec, 55^{0}C and 60^{o}C (depending on the primers annealing temperature) for 1 minute, 72^{0}C for 2 minutes; final extension at 72^{0}C for 15 minutes; holding at 4^{0}C.

3.2.4 Gel electrophoresis of PCR products

The PCR amplicon quality was determined using 1.5% agarose gel electrophoresis which ran for 35 minutes at 130V using 0.5x TBE buffer. The gel was observed using a digital gel doc system. Microsatellite alleles were detected for their amplification and correctness, hence true amplicons were subjected to capillary electrophoresis system (Appendix 3).

Table 3.1. Characteristics of the 12 SSR markers used in the study

SN	Marker	Motif	Forward	Reverse	Size(bp) range	Annling Temperature in ^{0}c	Dye
1	STG0016	(AGA)n	AGCTGCTCAGCATCAAGAGA	ACCACCTCAGGCACTTCATC	137-174	55	Ned
2	STM5114	(ACC)n	AATGGCTCTCTCTGTATGCT	GCTGTCCCAACTATCTTTGA	297-325	55	Ned
3	STM1053	(TA)n(ATC)n	TCTCCCCATCTTAATGTTTC	CAACACAGCATACAGATCATC	170-196	55	Ned
4	STI0012	(ATT)n	GAAGCGACTTCCAAAATCAGA	AAAGGGAGGAATAGAAACCAAAA	183-234	55	Pet
5	STI0032	(GGA)n	TGGGAAGAATCCTGAAATGG	TGCTCTACCAATTAACGGCA	127-148	55	Vic
6	STI0004	(AAG)n	GCTGCTAAACACTCAAGCAGAA	CAACTACAAGATTCCATCCACAG	83- 126	55	Vic
7	STM0031	(AC)n ... (AC)n(GCAC) (AC)n(GCAC) n	CATACGCACGCACGTACAC	TTCAACCTATCATTTTGTGAGTCG	168-211	60	Vic
8	STM1104	(TCT)n	TGATTCTCTTGCCTACTGTAATCG	CAAAGTGGTGTGAAGCTGTGA	178-199	55	Pet
9	STM1052	(AT)n GT(AT)n(GT)n	CAATTTCGTTTTTTCATGTGACAC	ATGGCGTAATTTGATTTAATACGTAA	214-263	55	Pet
10	STM1106	(ATT)n	TCCAGCTGATTGGTTAGGTTG	ATGCGAATCTACTCGTCATGG	151-214	55	Vic
11	STM0037	(TC)n(AC)n AA(AC)n(AT)n	AATTTAACTTAGAAGATTAGTCTC	ATTTGGTTGGGTATGATA	87-133	55	Vic
12	STI0030	(ATT)n	TTGACCCTCCAACTATAGATTCTTC	TGACAACTTTAAAGCATATGTCAGC	94- 137	55	6-Fam

3.2.5 Allele scoring and genetic data analysis

Raw data from capillary electrophoresis output was exported to Microsoft excel computer program to conduct locus analysis and allele calling (score allele peaks) using Gene Mapper v4.1. Finally, molecular data matrix was constructed for 53 potato genotypes and 12 markers based on allele size (bp) of the loci. Different diversity and phylogenetic software were used for molecular data analysis. Allelic data were used for computation of different genetic analysis. Polymorphic information content (PIC), number of alleles, allele frequency, and gene diversity analyses were conducted using Power Marker V: 3.0 software **(**Liu and Muse, 2005). To examine Population's genetic pattern; percentage polymorphism, allele number, observed and expected heterozygosity, allelic range, gene number and diversity, and Shannon's Information Index, GenAlEx v6.5(Peakall and Smouse 2012) software was used. To examine and visualize the patterns of variation among

53 individuals of potato on three-dimensional plots, a principal coordinate (PCO) analysis was performed using GenAlEx v6.5 (Peakall and Smouse 2012). To examine genetic relationships among individual potato genotypes, Neighbor-Joining (NJ) was constructed using DARwin software v6.0 (Perrier and Jacquemoud-Collet 2006). The 53 conserved potato genotypes with a set of 12 SSR loci were used to investigate the genetic structure and number of subpopulations in the collection used in this study Structure v2.3.4 (Pritchard *et al*., 2000) software were used. To investigate the distribution of variation among and within the studied populations, analysis of molecular variance (AMOVA) was calculated using Genalex V6.5 (Peakall R and Smouse PE, 2012).

3.3. Morphological Data collection and analysis

The morphological data of the conserved potato genotypes were collected Based on the CIP potato catalog morphological descriptors. The 42 potato germplasms were analyzed for their morphological diversity the remaining 11 exotic potato germplasms were not included in this analysis because their morphological characteristics were not available from CIP potato catalog. The data matrix was constructed based on the morphological characteristics and the genogram was generated using R V: 3.1.3 (Schulte *et al*., 2012) software.

Chapter Four: RESULTS

4.1. Molecular Genetic diversity and Structure analysis of the *In vitro* conserved potato germplasms

4.1.1. DNA Quality Detection

Total genomic DNA extracted from silica gel dried potato leaves were checked using 0.8% agarose gel electrophoresis.

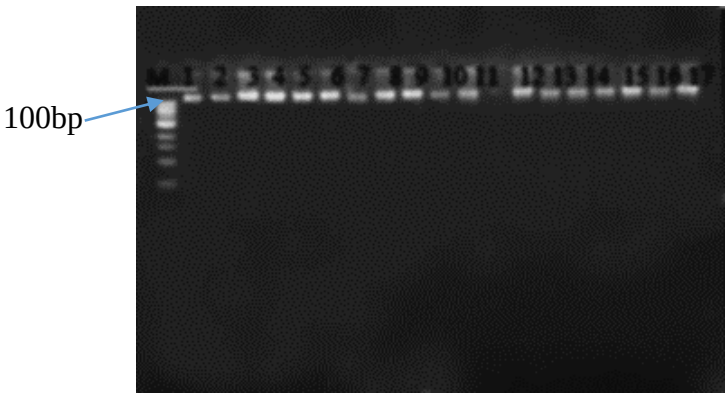

Plate 4.1. Gel photo of extracted DNA M=100bp DNA ladder from 1-17 Represents samples.

4.1.2 PCR Products Confirmation

The 12 sets of M13 tailed primer PCR products were confirmed for their amplification on 1.5%agarose gel electrophoresis.

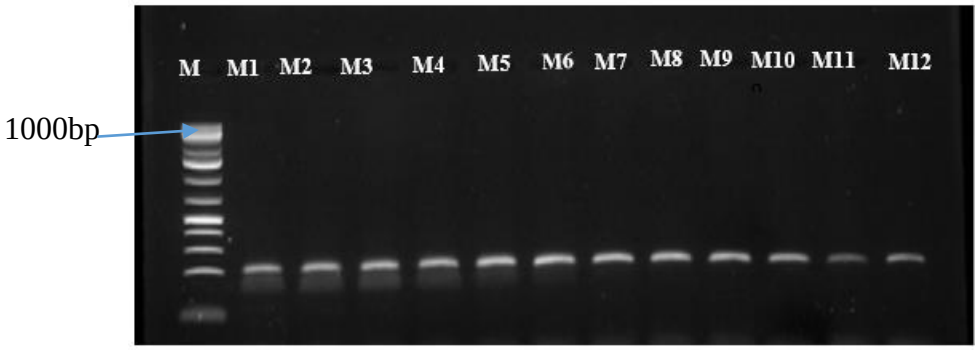

Plate 4.2. PCR Product gel test M= 1kb plus DNA ladder and M1-M12 the 12 SSR Makers

4.1.3. Number of alleles, polymorphic information content (PIC) and gene diversity

The twelve SSR markers generated a total of 79 alleles which were used to estimate the genetic diversity among the 53 conserved potato genotypes. The number of alleles revealed by each marker ranged from three for locus STM1053 to ten for locus STM1052 with an average of 6.58 per marker (Table 4.0). The PIC value for the SSR loci ranged from 0.57 for locus STM1053 to 0.93 for locus STI0030 and STI0004 with a mean value of 0.85. Marker STM0037 had the highest gene diversity of 0.9363, while STM1104 had the lowest value of gene diversity of 0.64. The mean gene diversity was 0.8678.The allele frequency ranged from 0.094 to 0.415.The highest allele frequency was obtained by marker STM1104 and the lowest by marker STM0037. This shows that when the allele frequency is low, gene diversity is high**.**

Table 4.0. Summary of 12 SSR loci allele frequency, number of alleles identified, gene Diversity and PIC values of the conserved potato genotypes in Ethiopia

Marker	Allele frequency	Number of alleles	Gene diversity	PIC
STG0016	0.1698	8	0.9156	0.91
STM5114	0.3208	4	0.8046	0.78
STM1053	0.2264	3	0.9035	0.57
STI 0012	0.2264	7	0.8950	0.89
STI0032	0.2453	4	0.9000	0.74
STI0004	0.1132	7	0.9377	0.93
STM0031	0.3774	4	0.7725	0.88
STM1104	0.4151	7	0.6429	0.90
STM1052	0.2075	10	0.9114	0.89
STM1106	0.1887	8	0.8886	0.89
STM0037	0.0943	9	0.9363	0.90
STI0030	0.2453	8	0.9057	0.93
Mean	0.2358	6.58	0.8678	0.85

4.1.4. Genetic diversity analysis of populations

In this study, the percentage of polymorphic loci indicated that; the population from CIP had the highest percentage of polymorphic loci with 83.33% while the lowest percentage of polymorphic loci was observed in Ethiopia local variety population with 58.33%. The Ethiopia Local Variety population had the lowest number of different alleles (1.792) whereas CIP population had the highest number of different alleles (3.292). Similarly, the lowest (1.542) and highest (2.412) number of effective alleles was also recorded in Ethiopian local variety and CIP populations, respectively. The Shannon's Information Index value for the populations ranged from 0.589 for population of Ethiopian local variety to 0.944 for CIP population with a mean value of 0.8. Observed Heterozygosity of the populations ranged from 0.583 for Ethiopian local variety to 0.829 for CIP population. In addition, CIP population had the highest unbiased expected heterozygosity of 0.639, while Ethiopian local variety had the lowest value again of 0.455. Mean unbiased heterozygosity was 0.56 (Table 4.1).

Generally, Ethiopian national release and Exotic populations showed the moderate result for most parameters as compared to CIP and Ethiopian local varieties. The Ethiopian national release had 70.83% of polymorphic loci while Exotic had 75 % percentage of polymorphic loci. Ethiopian nationally released varieties had 2.625 and Exotic had 3.000 different alleles. The Ethiopian nationally released varieties had 2.242 and Exotic had 2.321 effective alleles. The Shannon's Information Index value for the Exotic was 0.867 and 0.821 for Ethiopian national released varieties. The exotic population had 0.747 Observed Heterozygosity and 0.548 of unbiased expected heterozygosity, while the Ethiopian national released varieties had 0.708 of Observed Heterozygosity and 0.585 unbiased expected heterozygosity. In all populations observed heterozygosity was higher than expected heterozygosity which is the implication of high gene diversity which suggested for maximum heterosis.

Table 4.1. Population's genetic pattern estimates of *in vitro* conserved potato populations based on some genetic parameter

Population	%P	Na	Ne	I	Ho	uHe
Ethiopian Local Varieties	58.33	1.792	1.542	0.589	0.583	0.455
Ethiopian Nationally released varieties	70.83	2.625	2.242	0.821	0.708	0.585
CIP	83.33	3.292	2.412	0.944	0.829	0.639
Exotic	75.00	3.000	2.321	0.867	0.747	0.548
Mean	71.87	2.67	2.12	0.8	0.72	0.56

Where: %P= Percentage of Polymorphic Loci, Na = No. of Different Alleles, Ne = No. of Effective Alleles, I = Shannon's Information Index, Ho = Observed Heterozygosity, uHe = Unbiased Expected Heterozygosity

4.1.5. Genetic distance between conserved potato populations

The Nei's Pairwise genetic distance between populations of conserved potato (*S. tuberosum*) is shown in Table 4.2. The analysis showed moderate to high genetic distance that ranges from 0.6256 (between CIP and Exotic population) to 0.8236 (between Ethiopian local variety and Exotic populations). Overall, CIP and Exotic populations showed low genetic distance (62.56%); whereas, Ethiopian local and Exotic population were more divergent (82.36%) than others.

Table 4.2. Nei's Pairwise genetic distances between potato conserved populations in Ethiopia.

Populations	CIP	ETL	ETRV	Exotic
CIP	0.0000			
ETL	0.7550	0.0000		
ETRV	0.6752	0.7315	0.0000	
Exotic	0.6256	0.8236	0.7093	0.0000

Where: ETL= Ethiopian local variety, CIP= center of international potato, ETRV= Ethiopian national released variety

4.1.6. Cluster Analysis

The neighbor-joining cluster analysis divides the 53 studied potato genotypes into three main clusters and 5 subclusters (Figure 4. 1). The first cluster (C1) contains 19 genotypes with two sub-clusters (SC1 and SC2) from different populations (12 from CIP, 6 from Exotic and 1 from Ethiopian nationally released variety). The second cluster (C2) is the largest cluster with three sub-cluster (SC3, SC4, and SC5) which contains 29 genotypes (9 genotypes from exotic, 8 from Ethiopian nationally released varieties, 4 Ethiopian local varieties and 8 from CIP). Cluster three (C3) which is the smallest cluster, it contains 5 genotypes all from CIP, it indicates the uniqueness of these CIP genotypes from others.

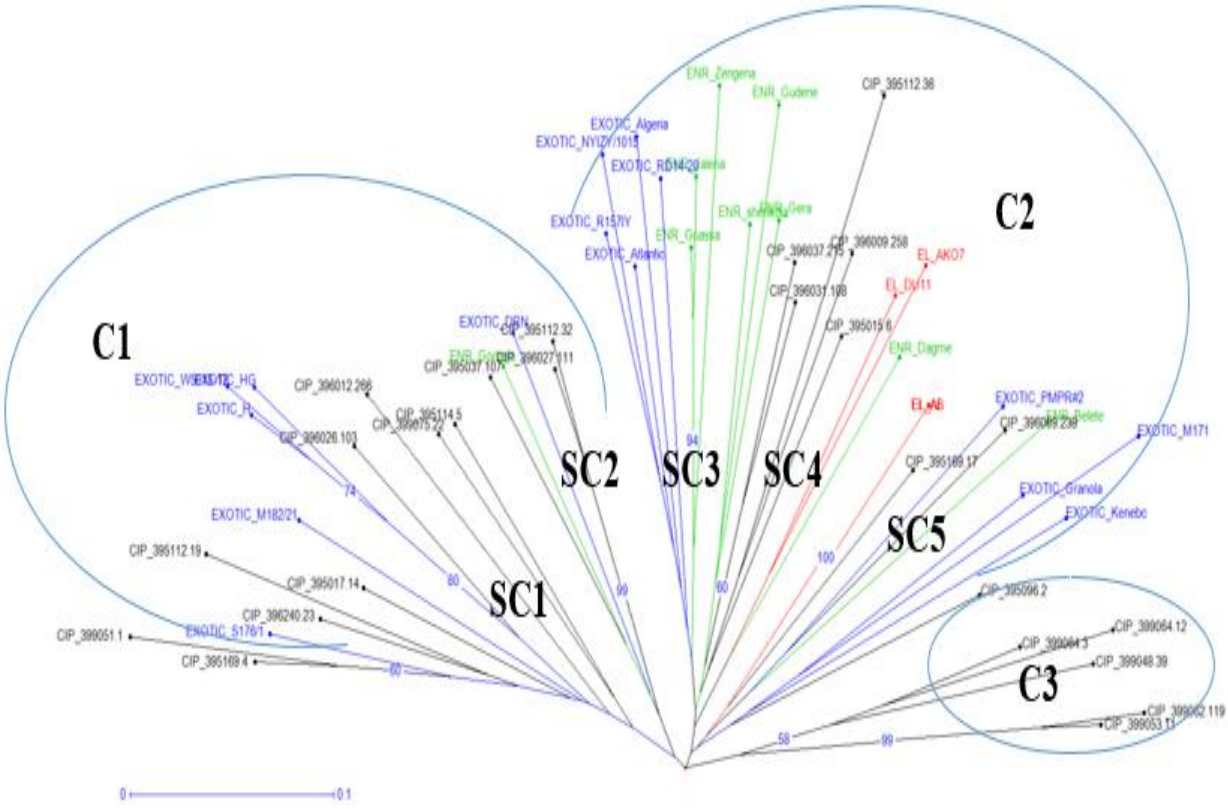

Figure 4.1. Neighbor-joining tree for 53 potatoes (solanum tuberosum) samples. Bootstrap value >50 from 1000 replication

4.1.7. Principal Coordinate Analysis (PCoA)

The genetic relatedness among the conserved potato genotypes was further investigated using three-Dimensional (3D) principal coordinate analysis (PCoA) (Figure 4.2). The three coordinates explained 27.63% of the total variation, (the first axis shows 12.05%, the second shows 8.52% and the third axis shows 7.06); however, the samples were not grouped based on their origins, Exotic and CIP samples were scattered in two coordinates. The Ethiopian national released varieties were scattered in the three coordinates. One Ethiopian nationally released variety clustered in the first coordinate with Ethiopian local varieties while eight of Ethiopian nationally released varieties clustered with Exotic and CIP populations. All of the Ethiopian local varieties were clustered in the first coordinate.

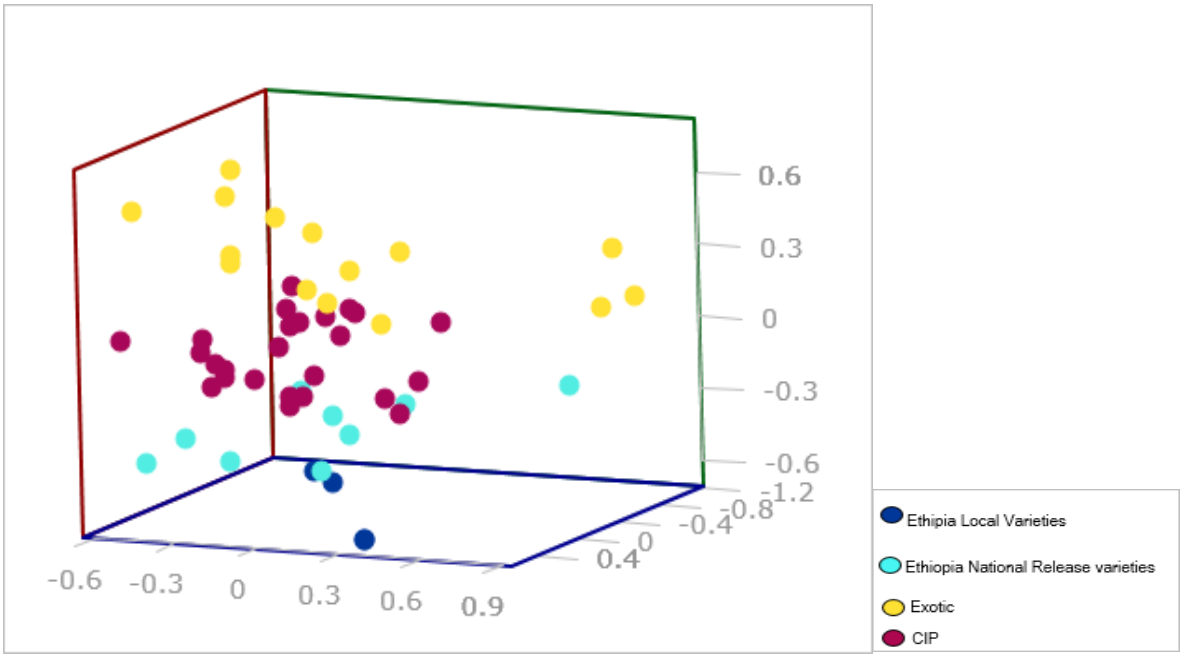

Figure 4.2 Principal coordinate analysis of conserved potato population in Ethiopia

4.1.8. Genetic structure analysis

To examine the genetic structure of the 53 conserved potato genotypes in Ethiopia, the genotypic data for 12 SSRs were analyzed using a model-based approach implemented in STRUCTURE (Pritchard *et al*., 2000). These results showed that all subpopulations contain individuals collected from all grouped populations, indicating the absence of origin based genetic structuring with Fst=0.001 which shows low differentiation.

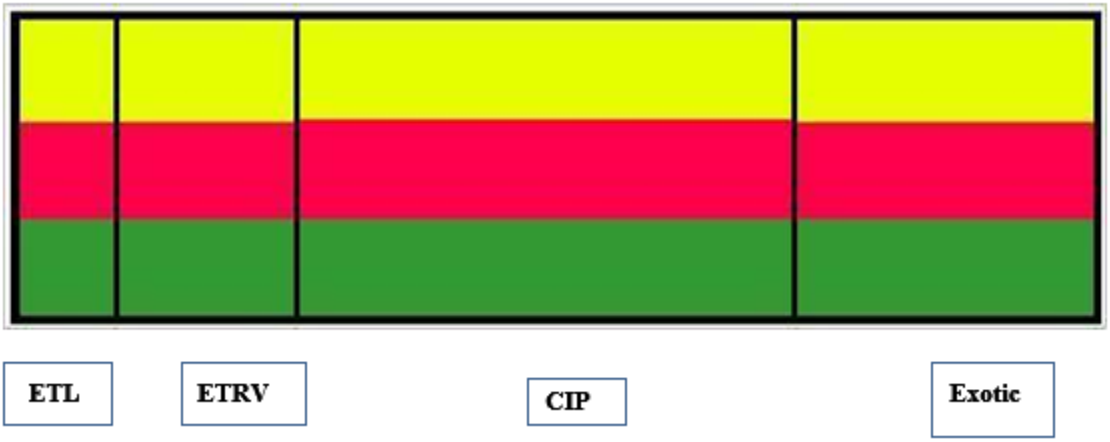

Figure 4.3 The three sub-Population inferred from structure analysis. The yellow color represents K=1, Red represents k=2 and Green K=3 Where k = subpopulations based on individual membership coefficient

4.1.9. Analysis of Molecular Variance (AMOVA)

The Analysis of Molecular Variation revealed that 4 % of the variation resulted from the difference among populations, while 96% of the variation was from within individual (Table 4.3). This implies that there is high genetic diversity within individuals in the populations.

Table 4.3: AMOVA showing the distribution of genetic diversity within and among populations of *in- vitro* conserved potato genotypes in Ethiopia

Source	df	SS	MS	Variation%
Among Pops	3	35.963	11.988	4
Within Indiv	53	389.500	7.349	96
Total	56	425.462	-	100

Where: DF =degrees of freedom; SS= sum of squares and MS=mean square

4.2. Morphological diversity of the In vitro conserved potato genotypes

The morphological characteristics of the In vitro conserved potato genotypes based on the CIP potato catalog**.**

Table 4.4 Morphological characteristics of In vitro conserved potato germplasms

Clone/Variety name	Tuber Skin Color	Tuber Shape	Tuber Flash color	Tuber Eye depth	Origin
AB	Red	Round	Nd	Deep	ETL
EL	Nd	Oval-to-oblong	Nd	Nd	ETL
AKO7	Nd	Nd	Nd	Shallow	ETL
DU11	Purple	Round	Nd	Nd	ETL
Belete	White-cream	Oblong	Cream	Shallow	ETRV
Gudene	Yellow	Nd	White	Nd	ETRV
Jalena	Nd	Nd	White	Nd	ETRV
Gera	White	Nd	White	Nd	ETRV
Guassa	Nd	Nd	White	Nd	ETRV
Shenkolla	White	Nd	White	Nd	ETRV
Zengena	Nd	Nd	White	Shallow	ETRV
Gorbela	Purplish	Nd	White	Nd	ETRV
Dagme	White-cream	Oblong	Cream	Shallow	ETRV
395112.32	Pink	Long-oblong	Cream	Shallow	CIP
395015.6	Red	Oblong	Cream	Shallow	CIP
396009.258	White-cream	Oblong	Pale yellow	Slightly deep	CIP
395017.12	White-cream	Oblong	Cream	Shallow	CIP
396027.111	White-cream	Oblong	Cream	Shallow	CIP
396037.215	White-cream	Oblong	Yellow	Shallow	CIP
399048.39	Red	Rounded	Intense Yellow	Shallow	CIP
399051.1	Red	Oblong	Yellow	Shallow	CIP
399053.11	Red	Long-oblong	Pale yellow	Shallow	CIP
399064.12	White-cream	Oblong	Pale yellow	Slightly deep	CIP
399064.3	Red	Long-oblong	Pale yellow	Slightly deep	CIP
399062.119	Red	Long-oblong	Pale yellow	Shallow	CIP
399075.22	Yellow	Long-oblong	Yellow	Shallow	CIP
395169.4	White-cream	Oblong	Pale yellow	Shallow	CIP
395169.17	White-cream	Oblong	Cream	Shallow	CIP
395112.36	Red	Oblong	Pale yellow	Shallow	CIP
395037.107	White-cream	Oblong	Pale yellow	Shallow	CIP
395096.2	White-cream	Long-oblong	Yellow	Shallow	CIP
395112.19	Pink	Rounded	Cream	Shallow	CIP
395114.5	White-cream	Long-oblong	Cream	Shallow	CIP
396009.239	White-cream	Oblong	Pale yellow	Shallow	CIP
396012.266	Purple	Oblong	Pale yellow	Shallow	CIP
396026.103	Red	Oblong	Cream	Shallow	CIP
396031.108	White-cream	Oblong	Cream	Shallow	CIP
396240.23	White-cream	Long-oblong	Pale yellow	Shallow	CIP
Kennebec	White	Nd	White	Shallow	Exotic
Atlantic	White - Yellow	Round	White cream	Shallow	Exotic
Granola	White - Yellow	Oval to round	White	Shallow	Exotic
DRN	Red	Oval to round	White	Shallow	Exotic

Where: ETL= Ethiopian local varieties, ETRV= Ethiopian Nationally released varieties, CIP= International Potato Center and Exotic=Potatoes their passport date were not available Nd=not defined.

4.2.1. Cluster analysis

The dendrogram produced based on the morphological characteristics of the potato germplasms grouped the 42 potato genotypes into four main clusters (Figure 4.4).The first cluster (CI) was the smallest cluster with 3 genotypes all from Exotic. The second cluster (CII) had 5 genotypes four Ethiopian local varieties and one from CIP. The third cluster (CIII) was the largest cluster with five sub-clusters (SC1 to SC5). Cluster three contained 27 genotypes (24 genotypes from CIP, 2 genotypes from Ethiopian nationally released and one from Exotic. Cluster four (CIV) had 7 genotypes all from Ethiopian nationally released varieties.

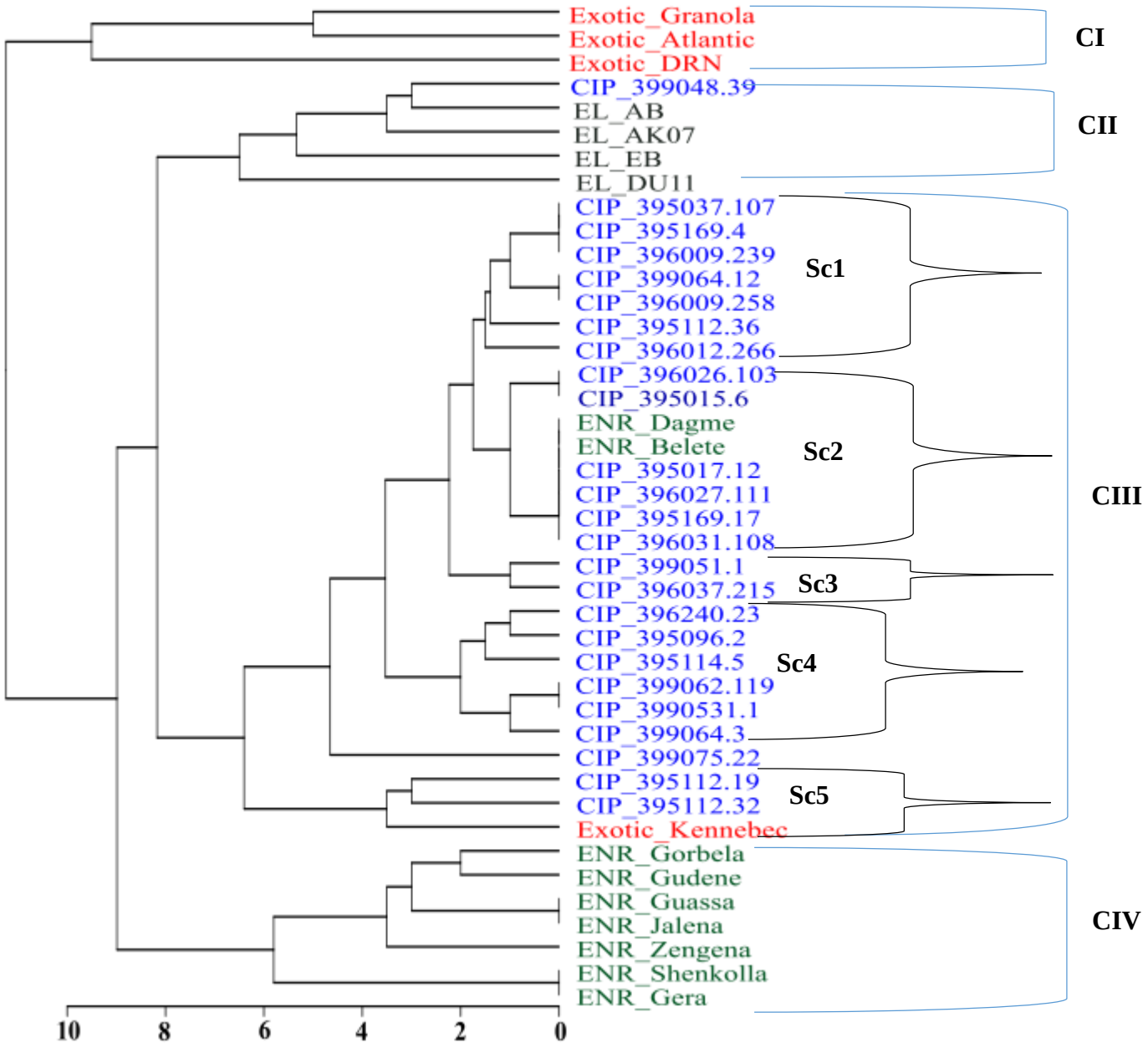

Figure 4.4.Dendrogram of the hierarchical clustering based on Bray-Curtis dissimilarity of the In vitro conserved potato genotypes.

CHAPTER FIVE: DISCUSSION

5.1. Extents of SSR variability within potato (*S. tubersom*) population

In this study 12 SSR markers were used to detect variability in potato *(S. tuberosum)* genotypes conserved at Amhara Regional Agricultural Research Institute (ARARI) and to evaluate their genetic diversity and relatedness. The 12 SSR markers used in this study amplified a total of 79 polymorphic alleles in the 53 conserved potatoes *(S. tuberosum)* genotypes. This study revealed high mean values for the number of alleles (6.58), gene diversity (0.87) and PIC (0.85). This study showed there is genetic variability among the *in vitro* conserved potato genotypes in Ethiopia. The total number of alleles achieved in this study was 79 with a range of 3 to 10 per marker. This result is in the range of similar studies on potato. Favoretto (*et al.,* 2011*)* reported a total of 46 alleles with an average of 4.6 alleles per marker with a range of 2 to 12 alleles using 10 SSR markers in thirty-eight potato genotypes. Muhinyuz *et al.,* (2015) identified a total of 84 alleles with an average of 6.5 per marker a range of 3 to 10 alleles per marker using 13 SSR primers in 18 potato genotypes. Liao and GUO (2014) reported a total of 304 alleles with an average value of 12 per marker with arrange of 5 to 19 using 24 SSR markers in 85 Yunnan potato varieties. K Tiwari *et al*., (2013) used 24 SSR markers to characterize 77 Andigena potato core collection. They reported a total of 214 SSR alleles with a range of 6 to 14 alleles per marker. Rocha *et al*., (2010) reported a total of 136 polymorphic bands ranged from 2 to 18 with an average value of 6.8 per marker using 20 SSR markers in 16 potato cultivars. The wide range of variation reported by the authors is associated with different SSR markers used in each study.

The mean genetic diversity of the markers observed in the current studied populations was 0.87 with the maximum gene diversity recorded of 0.94 in marker STI0004 and the lowest value of 0.64 for marker STM1104. Biniam *et al.,* (2016) used similar SSR markers and reported the gene diversity value of 0.98 for marker STM0037 with a mean value of 0.89 which is a bit a higher than the current study. In another study done by Kandemir *et* al.,(2010) they reported the mean gene diversity value of 0.53 which is lower than the value obtained in the current study. The high levels of gene diversity of SSR markers observed in this study was probably due to genetic diversity in the conserved potato

genotypes that represented different geographic origins and lineages.

In the current study, the PIC value ranged from 0.57 to 0.93 with a mean value of 0.85. This result was slightly lower than the results reported by Biniam *et al.,* (2016) who found the PIC value ranging from 0 .51 to 0.98 with a mean value of 0.87 using the same SSR markers. The PIC value was also lower than the result reported by Lioa and Gua (2014) who reported a mean PIC value of 0.86. However, the pic value in the current was higher than the PIC values reported by Muhinyuz *et al.,* (2015) and Favorreto *et al.,* (2011) who reported the PIC value ranging from 0.51 to 0.84 with a mean value of 0.71 and PIC value ranging between 0.12 to 0.85 with a mean of 0.54 respectively. The higher PIC value signifies the discriminatory power of the SSR markers between the samples. Moreover, according to Muhinyuza *et al.,* (2015) PIC value effectively demonstrates the power of SSR markers in measuring genetic variation among the cultivars. The value reported by several studies in potato varied depending on the SSR marker used and samples tested.

5.2. Magnitude of genetic diversity among and within population of in vitro conserved potato (*S. tuberosum*) genotypes

Understanding the genetic relationship and divergence of genetic resources is useful in making a choice of parents for breeding and genetic conservation strategies (Muhinyuza *et al.,* 2015). CIP, nationally released varieties, and Exotic populations exhibited higher values of percentage of polymorphism, Observed Heterozygosity and Unbiased Expected Heterozygosity except, the population from Ethiopia local varieties which had relatively lower diversity indexes; the results indicate that the high genetic diversity could be due to their different geographical origins. Moreover, among the four geographic populations, CIP had more genetic diversity as compared to others, on the contrary populations from Ethiopia local varieties showed the least genetic diversity, which could be explained by the effect of population size on genetic diversity because the Ethiopian local Varieties had the lowest population size as compared to the other populations and CIP had the highest population size .CIP populations could have been sourced from different geographical locations of the world that could explain their high genetic diversity.

Nei's pairwise genetic distance was used to analyze the genetic distance between *in vitro*

conserved potato populations. The genetic distance varies among each pair. Populations from CIP and Exotics showed the least genetic distance to each other with 37.44% similarity whereas; populations from Ethiopian Local varieties and Exotic had higher distance to each other with 17.64% similarity. Exotics and CIP population could have had the same origin of parental lines yet Ethiopian local and exotic population could have had divergent origin of parental lines. The

Ethiopian local and exotic populations can be used to select parental line to facilitate potato breeding and improvement strategy. This is because crossing genetically similar or closely related genotypes leads to genetic depression and reduce genetic variation in their progenies (Muhinyuza *et al.*, 2015). A similar study conducted by Biniam *et al.*, (2016) showed a narrow genetic distance among five studied populations. Each of five populations may have consisted of genotypes with shared parental lines as most of the African breeding program parental lines have been imported from (Peru) CIP.

AMOVA was used to compare the source of variation in the *in vitro* conserved potato populations. In this study, AMOVA revealed higher genetic diversity within populations than among populations. These higher AMOVA value within populations could be associated with the nature of the breeding and conservation scheme of the country (Ethiopia). Since Ethiopia is not the center of origin for potato introducing potato accessions from abroad is mandatory and has been exercised since 1973 when potato breeding program started in Ethiopia (Baye and Gebremedhin, 2013). The use of potato materials from CIP and other sources increase the genetic variability of the individuals within a population but on the other hand, it reduces the diversity among populations.

In a previous study on genetic diversity using SSR markers in Potato populations from Eritrea (Biniam *et al.*, 2016), from Kenya (Muthoni *et al.*, 2014), from Rwanda (Muhinyuza *et al.*, 2015), from Canada (FU *et al.*, 2009) and from Argentina (Ispizu´a *et al.*, 2007), they all reported that there was moderate to high genetic diversity of Potato in their respective countries; similar to the findings in the current study.

5.3. Population structure and pattern of genetic differentiation on *in vitro* conserved potato *(S. tuberosum)* genotypes

Knowledge about the patterns of population structure is essential for efficient germplasm management (Tesfamichael *et al.*, 2014). In order to visualize the genetic structure and relationship among all studied *in vitro* conserved potato from different origins; Neighbor-joining tree, PCoA analysis, and STRUCTURE analysis were employed. The neighbor-joining tree generated three clusters of potato samples with cluster I containing samples from CIP, Exotics and one Ethiopian nationally released variety. Cluster II consisted of samples from CIP, Exotics, Ethiopian nationally released and all Ethiopian local varieties while cluster III contained all samples from CIP, this indicates these genotypes share the same parental lines. These results are supported by the PCoA analysis.

The CIP genotypes which were observed clustering in cluster III in the Neighbor-joining tree were also clustered together in the second coordinate in PCoA analysis. Cluster I was mainly dominated by the CIP populations, Cluster II mainly grouped samples from all populations. The relatively low unbiased Nei genetic distance between the Exotic and CIP populations could be attributed to having the similar linage of genotypes. This result was supported by PCoA analysis with genotypes from CIP and Exotics clustering together (mixed with each other and also appear in the same coordinates).

In the current study, we detected duplication between the two Ethiopian local varieties which were registered in different local names i.e. Enate legoda (EL) and Abatanahe (AB). But this two local varieties were morphologically different as reported by Semagn *et al*.,(2016) AB had tall plants, red flowers, late maturing, round tubers but EB had Short stature, dense foliage, many stems per plant, small oval-to-oblong tubers this shows that the sharp similarity obtained from this study could be a miss labelling of genotype. This kind of miss-labeling of germplasmes in the collection is a very common which should be given emphasis and reported by different studies. In similar studies reported by Ispizua *et al.*, (2007) in the northwestern Argentina potato landrace collections, they reported that more than one genotype was found under the same name but belonged to different genotypes. In another study by Kandemir *et al*., (2010) there were three landraces with

different local names but belonged to the same genotype, this shows that there were duplications in the conserved potato genotypes because the conservation management only used the information about the origins of the genotypes which was not supported by genetic diversity information. We also found that there was a 94% similarity between the two Ethiopian nationally released varieties Jalenle and Gussa, this could be due to the fact that the two varieties are from CIP 384321.19 and CIP 384321.9 respectively, implying that the two varieties are from the same parental line This result was also reported by Tesfaye *et al*., (2013) who conducted the morphological characterization of cultivated potatoes they reported that these two varieties were morphologically similar they concluded that the two varieties are siblings. This result is also supported Semagn *et al*., (2016) results who reported two varieties are almost identical.

The PCoA analysis showed that there was no relationship between the samples and their geographic origins. The samples were clustered in the mixed pattern. Genotypes from CIP, Exotics and Ethiopian national released varieties were mixed in the second and third coordinate. This observation could be ascribed to the fact that most samples are labeled differently even if they are from the same source. This is in agreement with Biniam *et al*., (2016) who reported that samples were not grouped based on their sources of origin. This also could due to poor control of germplasm materials in Ethiopia and Eritrea. This is contrast with findings of Solano *et al.*, (2013) who reported that samples were clustered in accordance to their geographical origin in Chile.

The results obtained from the Neighbor-joining tree, PCoA analysis, and the STRUCTURE analysis confirmed that the members of each sub-population were from different sources of origin. Hence, this study confirmed that there is no strong correlation between the given population name and genetic relatedness of potato genotypes. The grouping obtained through Neighbor-joining tree was found to be comparable to the clusters obtained by STRUCTURE analysis. This result was also supported by AMOVA which revealed high diversity within the population.

5.4. Cluster analysis based on the Morphological characteristics

The dendrogarm generated five clusters based on the genotypes morphological characteristics .The first cluster(CI) which had 3 genotypes all from exotic(Granola, Atlantic and DRN) Atlantic and Granola had white to yellow tuber skin color but DRN had Red tuber skin color. Granola and DRN were oval to round tuber shape, while Atlantic had round tuber shape. Granola and DRN had similar tuber flesh color which was white but Atlantic had white cream tuber flash color. The three genotypes had similar tuber eye depth which was shallow (Table 4.1).

The second cluster (CII) with 5 genotypes was identified by the following morphological characteristics. The three Ethiopian local varieties (AB, Dull had round tuber shape while EL had oval to round tuber shape. CIP 399048.39 and AB had red tuber skin color.AKO7 and CIP 399048.39 had shallow tuber eye depth while AB had deep tuber eye. The four Ethiopian local varieties also clustered together in the Neighbor-joining analysis in this study based on SSR markers analysis in which they were grouped in the second cluster. In the genetic analysis results there was duplication of the Ethiopian local varieties AB and EL which was not captured in the morphological analysis. This could be due to the fact that the two Ethiopian local varieties were morphologically different as observed by semagn et *al.*, (2016) who reported AB had tall plants, red flowers, late maturing, round tubers while EL had Short stature, dense foliage, many stems per plant, and small oval-to-oblong tubers. This shows that the sharp similarity obtained from SSR analysis could be a miss labelling of genotype.

Cluster three which was the biggest cluster with five subcluster had genotypes from three population but the majority were from the CIP populations. The seven genotypes which were grouped in Cluster three sub-cluster one (SC1) had genotypes all from CIP (395037.107, 395169.4, 396009.239, 399064.12, 396009.258, 395112.36 and 396012.266) All of these genotypes had Pale yellow tuner flash color and oblong tuber shape. Except for the two genotypes, 395112.36 and 396012.266 all of them had white-cream tuber skin color. Five of the genotypes from this sub-cluster had Shallow tuber eye depth but the two genotypes (396009.239 and 396009.258) had slightly deep tuber eye depth.

Sub-cluster two (SC2) had six genotypes from CIP and two from Ethiopian Nationally released varieties (396026.103, 395015.6, 395017.12, 396027.111, 395169.17, 396031.108 Dagme and Belete). These genotypes had oblong tuber shape, cream tuber flash colour, and shallow tuber eye depth..Six of these genotypes from this cluster had white-cream tuber skin color except the two(396026.103, and 395015.6) which had red tuber skin colour.

Sub-cluster three (SC3) was the smallest subcluster with two CIP germplasms (399051.1 and 396037.215) which had similar oblong tuber shape ,yellow tuber flash color and shallow tuber eye depth but different tuber skin color which was red for 399051.1 and white- cream for 396037.215). These genotypes clustered together due to the high morphological similarity between them.

Sub-cluster four (SC4) contained six genotypes all from CIP populations (396240.23, 395096.2, 395114.5, 399062.119, 399053.11 and 399064.3).Genotypes in this subcluster were characterized by long oblong tuber shape. Three of them (399062.119, 399053.11 and 399064.3 had red tuber skin color while the rest three (396240.23, 395096.2 and 395114.5) had white-cream tuber skin color. Four of the genotypes had pale yellow tuber flash color except the two (395096.2 and 395114.5) which had yellow and cream tuber flash color. Except for the two (399062.119 and 399064.3) germplasms which had slightly deep tuber eye depth the rest had shallow tuber eye depth.In this sub-cluster there was high similarity between 399062.119 and 399053.11 because they had the same red tuber skin color, long- oblong tuber shape, pale-yellow tuber flash color but these two genotypes were not clustered together in the SSR analysis which implies that though they are morphologically similar they are genetically different.

Sub-cluster five (SC5) had 3 genotypes two from CIP and one exotic (395112.19, 395112.32 and Kennebec).These genotypes were characterized by their shallow tuber eye depth. The two genotypes (395112.19 and 395112.32) had pink tuber skin color and cream tuber flash colour but different tuber shape round and long-oblong respectively. Kennebec had white tuber skin and flash colour.

Cluster five (CIV) had seven genotypes all from Ethiopian nationally released varieties (Gudene Jalena Gera, Guassa, Shenkolla, Zengena and Gorbela) which were identified by the following

characteristics all had white tuber flash color. Gudena had yellow tuber skin color, Gera had white skin color and Gorbela had purplish skin color. In this cluster there was similarity between the two Ethiopian nationally released varieties Jalena and Guassa. This result was also observed Nebor-joining tree of the current study based on SSR markers .This high similarity was also reported by other similar potato genetic diversity studies done by Tesfaye *et al*., (2013) who used morphological analysis and that reported Guasa and Jalene were sister line varieties derived from the same parents. In another similar study done by Semagn *et al*., (2016) they reported that the two nationally relased varieties Jalena and Guassa were found to be similar based on both SNP and Morphological analysis. These two cultivars could have originated from the same CIP cross. Genetic diversity is essential to study the taxonomic relationships present among germplasms and also to identify the sources of genes for a particular trait from the existing germplasms (Arslanoglu *et al*., 2011). Therefore, knowledge of genetic diversity present within existing genotypes is crucial for effective utilization of genetic resources by plant breeders (Martins *et al*., 2006).

CHAPTER SIX: Conclusions and Recommendations

6.1. Conclusion

The study unveiled that there is molecular and morphological genetic diversity in the *In vitro* conserved potato germplasms in ARARI Ethiopia. CIP population was more genetically diverse than Exotic, Ethiopian local and nationally released populations, and can, therefore, be exploited for further potato breeding program in Ethiopia. The study also divulged that there was duplication between two Ethiopian local varieties (Abatanahe and Enate legoda) and a high (94%) similarity between two Ethiopian Nationally released varieties (Jalene and Gussa). The 12 M 13 tailed fluorescently labeled SSR markers used in this study provided a clear genetic diversity assessment among *in vitro* conserved potato genotypes from Ethiopia. The neighbor joining, and the PCoA analysis disclosed that the *in vitro* conserved potato germplasms are clustered without regard to their geographical origin. The Structure analysis revealed that the *in vitro* conserved potato germplasms clustered in three subpopulations. The molecular characterization of *in vitro* conserved potato genotypes generated not only essential information for managing the potato collection but also provided a useful guide for selecting specific germplasms with distinct genetic background for diversifying potato breeding program in Ethiopia.

6.2. Recommendation

The information generated in this study should be used to facilitate potato breeding program for guiding to choose divergent potential parental line selection. The duplicated germplasms in the Ethiopian local varieties should be removed from the Amhara Agricultural Research Institute Ethiopia plant tissue culture laboratory conservation system. The SSR markers used in this study could be used for other potato genetic diversity studies as they were able to reveal genetic diversity even among closely related genotypes. Eventually, based on the results of the current study, it is strongly recommended that conservation should be done based on genotypes genetic diversity information. This will avoid duplication and minimize the cost of *in vitro* preservation in the tissue culture laboratory .Maximum care should be taken when labeling germplasms .The morphological characteristics of the 11 Exotic potato germplasms (NYIZY/1015, PMPR#2, R157IY, M182/21, W5015-12, RD14-20, H, HG, S176/1, M171 and Algeria) should be done.

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APPENDICES

Appendix 1

Qiagen Dneasy Plant Mini Kit Extraction procedure

Notes before starting

- Perform all centrifuge steps at room temperature (15-25^{0}c).
- If necessary re dissolve any precipitates in Buffer AP1 and Buffer AW1 Concentrates.
- Add ethanol to Buffer AW1 and Buffer AW2 Concentrates.
- Preheat a water bath or a heating Block to 65^{0}c.

1. Disrupt samples (< 100mg wet weight or <20mg lyophilized tissue) using the Tissue rupter^R, the tissue Lyser ll, or a mortar and pestle.

2. Add 400ul Buffer AP1 and 4ul RNase A. Vortex and incubate for 10 min at 65^{0}c.Invert the tubes2-3 times during incubation.

Note: Do not mix Buffer AP1 and RNase A before use.

3. Add 130ul buffer P3.Mix and incubate for 5 min on ice.

4. Recommended: Centrifuge the lysate for 5 min at 20,000x g (14,000 rpm).

5. Pipet the lysate into a QLAshredder spin column placed in a 2ml collection tube centrifuge for 2 min at 20,000xg.

6. Transfer the flow-through into a new tube without disturbing the pellet if present. Add 1.5 volumes of Buffer AW1, and mix by pipetting.

7. Transfer 650 ul of the mixture into a Dneasy Mini spin column placed in a 2 ml collection tube. Centrifuge for 1 min at > 6000xg (>8000rpm).Discard the flow-through .Repeat this step with the remaining samples.

8. Place the spin column into a new 2 ml collection tube. Add 500ul Buffer AW2, and centrifuge for 1 min at >6000xg.Discard the flow-through.

9. Add another 500ul Buffer AW2.Centrifuge for 2 min at 20,000xg.

Note: Remove the spin column from the collection tube carefully so that the column does not

come in to contact with the flow-through.

10 Transfer the spin column to a new 1.5ml or 2 ml micro centrifuge tube.

11. Add 100 ul Buffer AE for elution. Incubate for 5 min at room temperature (15-25^{0}c).Centrifuge for 1 min at >6000xg

12. Repeat step 11.

Appendix 2

The Nano drop Readings of Genomic DNA.

Sample ID	*ng/ul*	gDNA	Water
1	32.73	31	69
2	24.34	41	59
3	20.61	49	51
4	24.24	41	59
5	23.76	42	58
6	12.72	79	21
7	19.62	51	49
8	19.6	51	49
9	15.46	65	35
10	70.1	14	86
11	24	42	58
12	19.96	50	50
13	32.28	31	69
14	24.35	41	59
15	18.84	53	47
16	21.23	47	53
17	21.62	46	54
18	27.68	36	64
19	22.07	45	55
20	30.36	33	67
21	18.33	55	45
22	27.85	36	64
23	13.45	74	26
24	41.32	24	76
25	19.81	50	50
26	33.46	30	70
27	26.07	38	62
28	30.06	33	67
29	40.08	25	75
30	12.45	80	20
31	48.34	21	79
32	16.31	61	39
33	30.19	33	67
34	44.29	23	77
35	19.83	50	50
36	65.07	15	85
37	25.43	39	61
38	123.44	8	92

39	90.46	11	89
40	32.57	31	69
41	79.44	13	87
42	38.16	26	74
43	74.29	13	87
44	36.52	27	73
45	37.86	26	74
46	25.14	40	60
47	51.15	20	80
48	44.9	22	78
49	39.65	25	75
50	35.14	28	72
51	43.77	23	77
52	23.63	42	58
53	21.89	46	54

Appendix 2

Capillary electrophoresis Procedure

1. Dilute PCR product in triple distilled water
2. Up to 4 separate PCR reactions can be pooled into one ABI sample if each PCR uses a different ABI. Pooling is not necessary but greatly increases ABI throughput and lowers costs.
3. Prepare the formamide-standard (FS) mix on ice by adding 12 ul GS500 LIZ (stored at 4°C) to 1 ml Hi-Di Formamide (stored in aliquots of 1 ml at -20°C) per 96 samples and mix well by vortexing.
4. Pipette 9 ul of the FS mix into each well of a new ABI PCR plate generating the FS plate.
5. Add 2.0 ul of pooled PCR products to the FS plate and mix well by vortexing. Once the sample is mixed with formamide, it is best to run the plate on the ABI within 48 hours.
6. Denature the FS plate at 95°C for 5 minutes, place on ice for 5 minutes.
7. Place the processed samples in the customer fridge at SEGOLI unit.
8. Prepare sample sheet and mail it to SEGOLIP unit at SEGOLILAB@CGIA