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Agro-morphological and Molecular Genetic Diversity, and Cytogenetic Analysis of Ethiopian Potato [*Plectranthus edulis* (Vatke) Agnew] from Ethiopia

By

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the PhD Degree in Biology (Applied Genetics)

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DECLARATION

I declare that the dissertation I hereby submitted for the Degree of Doctor of Philosophy (PhD) in Biology (Applied Genetics) to the School of Graduate Studies, Addis Ababa University, is my own independent work and has not previously been submitted by me or anybody else at Addis Ababa University or elsewhere.

To my knowledge, the materials obtained from other sources have been duly acknowledged in the dissertation.

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ACRONYMS

AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
APPRC	Ambo Plant Protection Research Center
CSA	Central Statistical Agency
DPX	Dibutylphthalate Polystyrene Xylene
EHNRI	Ethiopian Health and Nutrition Research Institute
EST-SSRs	Expressed Sequence Tag-based Simple Sequence Repeats
FAO	Food and Agriculture Organization
GRIN	Germplasm Resource Information Network
HARC	Holeta Agricultural Research Center
IAM	Infinite Allele Mutation model
IPGRI	International Plant Genetic Resources Institute
MCMC	Markov Chain Mont Carol
NCBI	National Center for Biotechnology Information
PCA	Principal Components Analysis
PCoA	Principal Co-ordinate Analysis
PCR	Polymerase Chain Reaction
SLU	Sveriges Lantbruks Universitet (Swedish University of Agricultural Sciences)
SNNPs	South Nations Nationalities and Peoples Region
SMM	Stepwise Mutation Model
SSRs	Simple Sequence Repeats
TPM	Two-phase Mutation model
UPGMA	Unweighted Pair Group with Arithmetic Mean
WFP	World Food Program

ABSTRACT

Agro-morphological and Molecular Genetic Diversity, and Cytogenetic Analysis of Ethiopian Potato [*Plectranthus edulis* (Vatke) Agnew] from Ethiopia

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Ethiopian potato *syno*. Ethiopian dinich [*Plectranthus edulis* (Vatke) Agnew] (Lamiaceae) is one of the ancient annual edible tuber crops, originating in Ethiopia. The crop is commonly cultivated by smallholder farmers around homesteads in the highland and semi-highland areas, usually for household consumption and rarely for marketing. In spite of its' wide economic importances, the crop is neglected by research and development community and currently it is at risk of total extinction.

Hence, this study was conducted with the main aims of assessing agro-morphological and molecular (EST-SSRs) markers-based extents of genetic diversity analysis as well as chromosome number and ploidy level determination using 174 accessions from diverse agro-ecologies in Ethiopia.

For agro-morphological diversity analysis, the experimental samples were tested at Ambo and Holeta agricultural research centres, using an alpha lattice design at the locations and three blocks/replication followed by appropriate management practices. Agronomic and morphological traits-based data were collected on twenty-eight (12 qualitative and 16 quantitative) traits at the right growth stage and analysed using SAS v9.0, MINITAB® v14.13 and FigTree v1.4.3. Cytogenetic characterization was also carried out using very young root tips generated from soil covered stem rings, followed by appropriate pre-treatment, fixation and maceration. For molecular genetic diversity analysis, genomic DNA was extracted form silcagel dried young leaves collected from 287 plants (1-3 plants per accessions) following CTAB protocol. EST-SSRs marker were designed from *Plectranthus barbatus* cDNA sequences deposited in the GenBank, followed by PCR amplification, capillary electrophoresis, peak identification, and scoring. The scored allele size data were analysed for polymorphism, diversity indices and genetic relationship and structure using windows compatible applications.

The agro-morphological traits considered showed varied morphotypes in all of the leaf (four in leaf color, three in leaf arrangement, three in leaf shape), stem (three in stem color, two in each of stem spot and stem spot colors) and tuber (four in tuber skin color, three in each of tuber texture, tuber shape and tuber hair) characterstics. Similarly, the traits revealed a wide range of variability in mean performance (minimum range of 1.39 – 2.13 cm observed in tuber diameter and maximum range of 112.90 – 165.10

days observed in days to 50% flower initiation) and variance components among and within the accessions. Similarly, the mean square of all the traits showed a highly significant (P< 0.001) variation among the tested accessions. Such wide variation suggests the presence of variability which can be exploited through selection. Several of the traits showed a slightly greater or nearly equal phenotypic coefficient of variation (PCV) to that of genotypic coefficients of variation (GCV), suggesting larger contribution of the genotypic effect for phenotypic expression of such characters and hence, phenotypic values-based selection for the traits may be effective. High estimates of GCV (>23%) coupled with high estimate of heritability (Hb%) (>94%) and high genetic advance as a percent of mean (GAM) (>46) were revealed in tuber weight per hill, number of primary branches per plant, number of tubers per hill, and number of plants per hill indicating the importance of such traits for selection in Ethiopian potato improvement programs. The significantly positive phenotypic, and genotypic correlation in tuber weight per hill and number of tubers per hill with each other and several other traits as well as their negative phenotypic correlation with some other traits indicates the direction of selection. Moreover, the significantly higher absolute magnitudes in genotypic correlation compared to their corresponding phenotypic correlation suggest the genetic base of those traits.

Cytogenetic characterization revealed a very smaller sized metaphase chromosome with a count of 2n = c.56 and hepta- or octa-ploidy was speculated on the bases of basic chromosome number reports (x = 8 or 7) for the species of genus *Plectranthus* and other members of the Lamiaceae family. Such chromosome count and ploidy level report could serve as a baseline information in selection and cross-hybridization of Ethiopian potato with other closely related species.

In total, twenty new polymorphic expressed sequence tag based simple sequence repeat (EST-SSRs) markers have successfully been developed and used in genetic diversity analyses. The marker detected a total of 128 alleles (6.4 alleles per locus) over the entire loci and populations with effective number of alleles ranging from 1.06 - 3.17 (an average of 1.67). The marker showed an overall highest (94.17%) percent polymorphism, and extents of PIC in the range of slightly informative to highly informative suggesting the potential of those developed markers as a valuable genetic tool and resource to evaluate the extent of genetic diversity and population structure of not only Ethiopian potato but also various other species within the Lamiaceae family.

The ranges and levels of mean observed heterozygosity (0.33 – 0.429), Shanon's information index (0.523 – 0.663), and Nei's gene diversity (0.307 – 0.384) across loci showed a medium degree of variation among the populations which is a direct reflection of sharing of most of the alleles among the populations that partly resulted from high overall gene flow (Nm=18.29). Comparatively, Wenbera (*Wen)*, Wolaita Sodo (*WSo)*, Hadiya and Kambata-Tembaro (*HKT)* and Southwest Shewa (*SwSh)* populations could be considered as Ethiopian potato diversification and *in-situ* conservation sites.

Hierarchical analysis of molecular variance (AMOVA) showed significant but low population differentiation with at most 3% of the total variation in each of the groupings, such as among the populations, among geographic regions, and among regions of accessions. Likewise, cluster analysis in all the cases and STRUCTURE analyses did not group the populations into sharply distinct clusters, which could be attributed to historical and contemporary gene flow and/or the reproductive biology of the crop.

In conclusion, this study has wider implications in bringing such a 'super-neglected' crop to the scientific agenda and thus, opens up the door to its improvement and conservation. However, it is important to exhaust all areas and regions in the country and more number of SSRs or other up-to-date molecular marker systems to come up with more accurate level of genetic diversity estimates.

Keywords/phrases: Cytogenetics, Expressed sequence based simple sequence repeats, Ethiopian potato, Genetic diversity, Morphological trait, *Plectranthus edulis*, Population structure

1. INTRODUCTION

1.1. Background and Justification

Ethiopia is one of the countries with great genetic diversity and a primary gene centre for several crop plants (Vavilov, 1951) including cultivated food crops (Harlan, 1969; Westphal, 1975). Furthermore, the country is a center of origin and diversity to several root and tuber crops including Ethiopian potato (*Plectrantus edulis*) (Zohary, 1970; Yeshitila Mekbib *et al*., 2007). Such a rich plant/crop diversity is attributed to varied topography, cultural diversity, environmental (agro-ecological) heterogeneity, and diverse farming systems of the country (Ensermu Kelbessa *et al*., 1992; IBCR, 2001). However, food insecurity and malnutrition remained a big challenge in several parts of the country because of the rapidly increasing population pressure, low productivity of the agricultural sector, widespread environmental degradation, and frequent drought (WFP/CSA, 2014).

The food potential of horticultural crops, particularly indigenous roots and tubers, has not been fully exploited despite their significant contributions to the livelihood of subsistence farmers. They are largely overlooked in terms of holistic research on their improvement and conservation. Their production and management system is restricted to the use of local varieties maintained by farmers using local knowledge, unlike cereals such as tef, barley, wheat, maize, and sorghum which are the leading crops in terms of both area coverage, and national research focus (Birhanu Gebremedhin *et al.,* 2001). But, efforts to improve food security through a grain crop-led approach has been less successful, as it could not keep up with population growth and environmental challenges (Schulz *et al*., 2012). Hence, inclusive and focused research and breeding efforts, especially in developing countries such as Ethiopia with diverse agricultural endowments, is important to help exploit indigenous edible root and tuber crops in a way they

can contribute significantly in improving food security (Yeshitila Mekbib and Temesgen Deressa, 2015; Zemede Asfaw, 1997; Amsalu Nebiyu *et al*., 2008).

Ethiopian potato, also called Ethiopian potato (Lamiaceae) is one of the economically important tuber crops, said to have originated and domesticated in Ethiopia (Hedge, 1992; Harlan, 1969) with wide distribution in some parts of Africa, mainly in wild form (Codd, 1975). The plant is known to produce edible stem tubers on stolon below ground. The crop can thrive and set tubers under strong environmental constraints including degraded and poor soil and does not require intensive management practices when compared to other tuber crops (Edwards, 1991).

This edible tuber crop is commonly cultivated by smallholder farmers around homesteads, as pure stand or mixed with other crops such as maize, sorghum, coffee, enset etc. It is cultivated largely for household consumption and rarely for marketing of its tubers. It is one of the most common Ethiopian staple crops (Zemede Asfaw and Mesfin Tadesse, 2001) and usually called 'hunger crop' as it fills the food shortage gap that occurs from August to November, the period before the harvest of cereal crops, in the highland and semi-highland areas (Birhanu Gebremedhin *et al.,* 2001). It has also been widely used as a folk medicine in several parts of Africa including Ethiopia (Abera Hora, 1995). The crop is commonly visited by honeybees for its nectar (Reinhard and Admasu Adi, 1994).

Nowadays, the local gene pool of the crop is under threat of sever genetic erosion as it is completely vanishing from several areas where it used to be widely cultivated and restricted to highly marginalized land and limited to few elderly farmers in some other areas. Introduction of improved tuber crops like Irish potato (*Solanium tuberosum*) to the area, lack of improved planting material, short shelf-life, recurrent drought, environmental degradation and limited awareness among younger farmers have contributed much to the genetic erosion and

marginalization of the crop (Abebe Demisse, 1988; Amsalu Nebiyu and Tesfaye Awas, 2004; Yeshitila Mekbib and Temesgen Deressa, 2015).

Assessing the extent of genetic diversity of crop plants in general and Ethiopian potato in particular is a pre-requisite for efficient conservation and improvement programmes. Agronomic, morphological and molecular marker systems, and cytogenetic analysis are some of the most important genetic tools and resources for attaining such goals (Singh *et al*., 2005; Leal *et al.*, 2010).

Agronomic and morphological traits-based systems are the most common and easily observable markers, usually used by taxonomists, farmers, and breeders for classification and evaluation of yield or other traits of interest (Singh and Parab, 2015; Newbury and Ford-Lloyd, 1997). In addition, it provides good opportunity for effective selection and conservation measures (Singh *et al*, 2005). The process usually demands partitioning the overall variability of genotypes into its heritable and non-heritable components with the use of suitable genetic parameters such as genetic coefficient of variation, heritability, genetic advance and correlation (Akinwale *et al.,* 2010). In addition, multivariate analysis methods are also used especially for accessions/genotypes and agronomic traits that are several in number (Peeters and Martinelli, 1989).

In this regard, there have been very limited studies using only few accessions of Ethiopian potato tested at single location (Wayessa Garedew *et al*., 2009; 2013; Yeshitila Mekbib, 2007). Therefore, it is important to include more accessions from all potential growing areas of the country and testing at multiple locations to clearly understand the extents of the existing genetic diversity for proper conservation and breeding programmes.

However, the agronomic and morphological differences between genotypes are not always absolute and reliable since morphological features are often controlled by multiple genes and subject to varying degrees of environmental modification and interaction (Islam *et al.,* 2002; Falconer, 1986). Therefore, to increase the resolving power and hence, to obtain complementary information, it is important to couple agro-morphological markers with other appropriate marker systems, such as, biochemical (isozymes), cytogenetic, and/or molecular markers (Falconer, 1986).

Molecular marker systems are one of the most important genetic tools for assessing genetic diversity of crop plants. The simple sequence repeats (SSRs) or microsatellite is one of such standard DNA markers used for whole genome characterization, gene mapping and/or crop improvement partly because of its extensive and even distribution throughout the genome (including organellar genome), co-dominant inheritance, multi-allelic nature, chromosome specific location and high reproducibility (Kalia *et al*., 2011).

Expressed sequence tags based simple sequence repeats (EST-SSRs) is one of SSRs markers which is relatively faster and cost effective to enable detect the existing variability and draw population genetic structure (Ellis *et al*., 2006). It is widely used in many plant species because of their advantages over genomic SSRs in being located in the coding region of the genome so that the flanking sequences are found in a well-conserved region over phylogenetically related species and thereby highly transferable among related taxa, and has less susceptibility to null alleles (Uchiyama *et al*., 2013; Saha *et al.,* 2004).

Cross-genera and cross-species molecular marker transferability is highly important, particularly to species with little/no sequence information, such as, Ethiopian dinch. Until recently, only very few transferable EST-SSRs marker are developed for certain genera of the

Lamiaceae (Mehmet *et al*., 2012; Guojie *et al*., 2013) and so far, there is no report on the genus *Plectranthus.*

However, to our knowledge, neither joint morphological and molecular genetic diversity evaluation nor microsatellite based molecular genetic diversity evaluation is in place for Ethiopian potato and this has restricted the production, utilization and development of best performing, highly productive and good quality varieties that, to a larger extent, excels its tuber (food) and medicinal quality. Therefore, it is highly important to develop EST-SSRs marker system and couple it with agro-morphological traits for achieving such goals.

Similarly, investigating the genetic information in general and cytogenetics in particular is a very important aspect for crop plants as it helps for correct plant identification and production of commercial verities in breeding programs. In view of this, the base for cytogenetic studies of the genus *Plectranthus* was laid during the early 1930s, 1940s, and 1950s that reported the basic chromosome numbers (x) for different species to be 6, 7 and 8 (Scheel, 1931, Furdosto, 1940, and Reddy, 1952). Moreover, many species of the genus display a large variation in chromosome morphology, chemistry as well as numbers (2n = 14 to 2n = 84) (Morton, 1962, De Wet, 1958, Lukhoba *et al*., 2006, Alasbahi and Melzig, 2010).

So far, there is no report regarding the cytogenetic analysis of Ethiopian potato. Therefore, it is essential to establish the correct chromosome number, and ploidy level to be coupled with molecular and agro-morphological markers for use in the systematics, breeding, and genetic studies of Ethiopian potato.

1.2. Hypotheses and Objectives of the Study

1.2.1. Research hypotheses

The present study was conducted to test the following hypotheses:

1. No genetic variation exists within and among Ethiopian potato populations on the bases of both agro-morphological and molecular markers;
2. There is a lower genetic variability within population (accessions confined in the same geographic area or administrative zone) than among populations (accessions of different areas or administrative zones);
3. Inter-collection/population genetic distance follows their geographical distribution /location;
4. Ethiopian potato population bears a di-ploidy (2n=2x) somatic chromosome level;

1.2.2. Objective

1.2.2.1. General objective

The main objective of the present study was to assess the pattern and magnitude of agro-morphological and molecular markers-based genetic diversity and population structure, and to determine the somatic chromosome number and ploidy level of Ethiopian potato populations collected from diverse agro-ecologies in Ethiopia.

1.2.2.2. Specific objectives

The specific objectives of the study are to:

1. Assess agro-morphological diversity within and among Ethiopian potato populations
2. Estimate the magnitude of genetic variability, heritability and expected genetic gains for selected and important agronomic traits

3. Determine the somatic chromosome number and ploidy level of the crop
4. Develope efficient and polymorphic EST-SSRs marker and estimate molecular diversity and population structure of Ethiopian potato populations using the developed EST-SSRs marker
5. Identify collection(s)/sites with high genetic diversity as potential sites for further utilization and conservation of the genetic diversity

2. LITERATURE REVIEW

2.1. Ethiopia as a Centre of Biodiversity

Ethiopia is one of the tropical countries, located between 3º and 15º N, 33º and 48º E, characterized by diverse physio-geographic features and wide altitudinal variation, ranging from 210m below sea level at the Danakil Depression (Dalol) in the northeast to over 4500 m above sea level at the Semen Mountains (Ras Dashen) in the north. The highland regions cover the central lava highlands and massifs (the Gondar, Wello and Gojam highlands), the Southwestern plateau (Gamo Gofa, Illu Aba Bora and Wollega), the Southeastern highlands (Arsi, Bale, Hararge) and the Southern highland (Sidamo/parts of Gedeo) (IBC, 2007; FAO, 1984).

However, unlike the tropical countries with typically hot and dry conditions, Ethiopia has varied macro and micro-climatic conditions because of high-altitude ranges that modified mean temperatures and lead to a more moderate Mediterranean type climate in the areas. Such varied climatic conditions have contributed much to the formation of diverse ecosystems, that allowed inhabitation with a great diversity of life forms. The rainfall distribution is seasonal with mean annual patterns ranging from below 350 mm to 2,800 mm with the south-western region receiving the heaviest annual rainfall (which goes up to 2,800 mm in some areas) and the central and northern central regions and the southeastern and northern regions receiving moderate and low rainfall, respectively (FAO, 1984).

Currently, Ethiopia has an estimated population size of more than 100 million with a growth rate of about 2.8% per annum, according to CSA, (2007) projection (CSA, 2016). The settlement pattern of the population is skewed towards the highlands because of environmental factors: altitude, climate, soil fertility and commercial economic activities. As a result, the highlands (>1, 800 masl), which cover 37% of the total area are densely populated (inhabited

by about 77 % of the population), resulted in over-grazing and severe degradation of the vegetations unlike the lowlands which are sparsely populated and highly prone to insufficient rainfall and high temperature. This non-proportional distribution of human population is one of the important factors that negatively affected the productivity of agricultural lands and the conservation and management of biological resources, especially indigenous food crops.

Ethiopia is also characterized by a wide range of agro-climatic conditions that led to diverse cultural and farming practices, the driving engine for the entire socio-economic structure and has a major influence on all other economic sectors and development processes of the country. More than half of the total agricultural land area is used only for major crop production per annum (CSA, 2005; FAO, 2005), implying the less attention given to minor crops.

The country is one of the worlds biodiversity centres, hosting an estimated 6,500 to 7,000 plant species, of which around 12% are considered to be endemic. Moreover, the country is among the largest world's biodiversity centres in terms of crop plants. Diverse agro-ecologies, farming systems, socio-economics, and cultural practises are among the major drivers for the creation of the vast genetic resources. Some of the widely known indigenous crops are coffee (*Coffea arabica* L.), safflower (*Carthamus tinctures* L.), tef (*Eragrostis tef* (Zucc.) Trotter), noug (*Guizotia abyssinica* (*L. f.*) Cass.), cereals, pulses and other oil crops. Moreover, the country is a centre of origin for several root and tuber crops such as Enset (*Ensete ventricosum* (Welw.) Cheesman), Anchote (*Coccinia abyssinica* (Lam.) Cogn.), 'Ethiopian potato' (*Plectranthus edulis* (Vatke) Agnew) and Yam (*Dioscorea* spp.) (IBC, 2007).

However, regardless of being the centre of origin and diversity to several food crops, food insecurity, malnutrition, poor health condition and even hunger and starvation are the greatest and ever worsening challenges in the country. Major underlying causes include rapidly

increasing population pressure, low productivity of the agricultural sector, widespread environmental degradation, and recurrent drought. The country is highly prone to recurrent natural hazards that left and continuously leaving several people especially the pastoral and semi-pastoral community chronically food insecure every year (FAO/WFP, 2012).

In terms of research focus, the country largely concentrates on few cereal crops such as barley, maize, wheat, sorghum, and tef. But, efforts to improve food security through a grain-led approach seems less successful as it is not keeping up with population growth (Schulz, 2012). Hence, horticultural crops, particularly indigenous orphan crops, need to be given especial emphasis in terms of investment in research, extension and training for farmers on the utilization and management, to excel their contribution towards food security, income generation, provision of food energy and conservation (Birhanu Gebremedhin *et al*., 2009).

2.2. **Minor/Orphan Crops**

The term ‘minor/orphan’ broadly includes ‘under-utilized’ and ‘neglected’ species that pinpoints two crucial aspects of the species: the degree of attention paid by users, and the level of research and conservation efforts spent on them (IPGRI, 2002). In this view, ‘neglected’ crops are those that are grown primarily in their centers of origin or centers of diversity by traditional farmers, where they are important for the subsistence of local communities. Moreover, such crops are characterized by contentious maintenance following cultural preferences and traditional practices, and hence remain inadequately characterized, and neglected by researchers and conservation experts. On the other hand, ‘under-utilized’ crops are those which were once, widely grown but are falling into disuse for a number of reasons. The crops are less selected by farmers and consumers because of their less competitive nature compared with other crop species in the same agricultural environment. The down selection of

the crops may eventually erode the genetic base and prevent the use of distinctive useful traits in crop adaptation and improvement (IPGRI, 2002).

In the African context, the sub-Saharan countries including Ethiopia, are experiencing high food and nutrition insecurity, partly due to lack of crop diversification (Bekunda *et al*., 2010). But, the indigenous crops in the broader sense and 'neglected' or 'under-utilized' ones in the narrow sense, are very important not only in the daily and healthy diet for several rural poor but also to the local economy, environmental issues, and traditional medicine of the continent. Moreover, various 'orphan' crops can offer an alternative source of micronutrients, vitamins, as well as health-promoting secondary plant metabolites for low-income households (rural poor) (Aworh, 2015; Muhanji *et al*., 2011; Tumwet *et al*., 2014; Williams and Haq, 2002).

Mainstreaming these crops into the local food systems will, therefore, help in alleviating malnutrition, especially in rural communities, where farming is the main source of food and income. Therefore, agricultural and horticultural research should develop strategies not only to produce more food, but also to improve access to more nutritious food through active engagement on indigenous 'orphan' crops and bringing them to the scientific agenda (Baldermann *et al*., 2016).

Similarly, Ethiopian indigenous root and tuber crops (example, Ethiopian potato) are among those which are deprived of research attention and their production and management system is yet limited to local varieties maintained by farmers using local knowledge. Besides, scientific intervention to improve the production and management system and to bring to the market chain is very limited regardless of its pivotal role in the densely populated highland and semi-highland as well as drought-prone low land areas (Amsalu Nebiyu *et al*., 2008).

2.3. Ethiopian Potato

2.3.1. Origin

Ethiopian potato is one of the most economically important tuber crops, said to have originated and domesticated in Ethiopia, particularly in the highland areas where it served as a food source since long time (Zemede Asfaw, 1997; Ryding 2000 as cited in Mulugeta Taye *et al*. 2006). The wild forms are found distributed in Ethiopia and other tropical and warm African countries such as Kenya, Tanzania, Uganda, and the former Zaire (Democratic Republic of the Congo) (Codd, 1975; Lukhoba *et al*., 2006; Alasbahi and Melzigh, 2010).

2.3.2. Taxonomy and vernacular names

Ethiopian potato is a dicotyledonous plant, classified under the family Lamiaceae, subfamily Nepetoideae, tribe Ocimeae (Gurcharan, 2004; GRIN, 2005) and genus *Plectranthus.* The family is comprising around 264 genera including the genus *Plectranthus* that consists of around 300 species. Several of the species in the genus, for example; *P. esculentus* (Livingstone potato; Tindall, 1983; Allemann *et al*., 2004; Allemann and Hammes, 2006), *P. parviflorus* (Sudan potato; Tindall, 1983) and *P. rotundifolius* (Madagaskar potato; Jansen, 1996), and *P. edulis* are tuber-bearing. 'Ethiopian potato' was formerly grouped with *Coleus* (member of the mint) which are perennial, branched herbs and entirely aromatic (Soni and Singhai, 2012).

Vernacular (common) names of organisms are names often used at a given area by some or all members of a society. Unlike scientific names, it is not governed by strict rules and also lack standardization but remain more stable than the scientific names.

Table 1 shows the various vernacular names of Ethiopian potato used in its different growing areas in Ehiopia and this probably shows its indegeniousity (ownership) and wide distribution in the country. Among the common names, 'Dinnicha Oromoo' in Oromo language or 'Ye Oromo dinich' in 'Amharic' is relatively widely used by the community and researchers.

However, as the crop is only locally known and little studied, there is no well-established and nationally agreed upon single common name other than its scientific name (*P. edulis*). As a result, the crop is known by various local names in different literature. Several authors use local names such as 'Dinnicha Oromoo' or 'Oromo dinich' (in a wider case), 'Oromo potato', 'Ethiopian potato', 'Wolaita potato' 'African potato' etc. Therefore, it looks confusing as to which of these common names (Table 1) to use to best address the crop to the scientific and local communities. Since, the name 'dinich' (syn. potato) is common to all the vernacular names used at the different growing areas and ethnic groups, we suggested calling its common name 'Ethiopian potato', which is equivalent of 'Ethiopian dinich'. This name can better indicate the nature of the crop (being potato/dinich) and its endemicity to the country. Thus, in the present study, we use the name Ethiopian potato for this crop.

2.3.3. Cultivar names

There are also various local names for different local cultivars that have been identified by farmers. For example:- 'Lofuwa' (considered as early maturing and having good shelf life and cream tuber color), 'Chanqowa' (considered to be late maturing and red-purple in tuber color), and 'Unnuka' (considered to be interim in maturity and cream white tuber color) are common in Wolaita Zone (Yeshitila Mekbib, 2007 as cited in Mulugeta Taye *et al*., 2012); 'Deleko' (the red, long hairy) and 'Oise' (the whitish) are well known in Gamo Gofa zone particularly at around Chencha and Dita woredas (personal observation during sample collection).

Table 1: Varnacular names, ethnic group(s) using the names, administrative region or area where the names are commonly used, equivalent amharic naming and meaning (description) of the naming's for Ethiopian potato

Vernacular (Common) names	Ethic group	Region/specific area	Equivalent Amharic name	Description	Reference
'Dinnicha Oromoo'	Oromo and others living in Oromia region	Oromia, Yem liyu woreda, Wenbera woreda	'Ye Oromo dinich'	Potato belonging to the Oromo people	Abera Hora, 1995; Abdissa Geleta, 2000) and personal observation
'Debbennoo'	Oromo	Oromia, Dedo woreda			
'Dinnicha Kaka'oo'	Oromo	Orimia, Sokoru Woreda			
'Gamo Dono'	Gamo, Dorze and others living in the area	SNNPs, Gamo Gofa zone, Chencha and Dita woredas	'Ye Gamo dinich'	Potato of the Gamo people	Endale Tsegaye, 1997
'Wolaita Dono/ Laduno'	Wolaita and few Gamo	SNNPs, Wolaita zone	'Ye Wolaita dinich'	Potato of the Wolaita people	Mulugeta Taye *et al*., 2006 and personal observation
'Ajo'	Kefficho	SNNPs, Keffa zone	'Ye Keffa/Kefficho dinich'	Potato of the Keffa/Kefficho people	
'Yem Duna'	Yem	SNNPs, Yem liyu woreda	'Ye Yem dinich'	Potato of the Yem people	
'Dinicho'	Hadiya and neighbouring Kembata	SNNPs, Hadiya and Kembata Tembaro woreda	'Ye Hadiya dinich'	Potato of the Hadiya people	
'Ye Gurage Dinich'	Gurage	SNNPs, Gurage zone	'Ye Gurage dinich'	Potato of the Gurage people	Westphal, 1975
'Bundunke'	Gumuz	Benshangul Gumz, Wenbera woreda and neighbours	'Ye Gumz dinich'	Potato of the Gumuz people	Wolde Miche, 1987 and personal observation
'Doka'	Shinasha	Benshangul Gumz, Wenbera woreda	'Ye Shinasha dinich'	Potato of the Shinasha people	
'Awi Doneza' / 'Ye Agew dinich'	Agew and Amhara living in the zone	Amhara region, Awi zone	'Ye Agew dinich'	Potato of the Agew people	
'Ye hagere dinich'	Amhara	Amhara region	'Ye Amara or local dinich'	Potato of the Amhara/local region	

SNNPs = South Nations, Nationalities and Peoples

2.3.4. Ecology

Ethiopian potato is an ancient, annual tuber crop (Greenway, 1944; Siegenthaler, 1963; Westphal, 1975), that can thrive and produce tubers under strong environmental constraints (marginal conditions) such as moisture deficiency, soil degradation, and it requires little management practises (Yeshitila Mekbib and Jenes, 2012) and it is less susceptible to natural hazards which makes its harvest safe under all conditions and cost as well as labour effective.

The crop usually grows from cooler or marshy to semi-dry areas (mid- to high-altitude ranges). It requires full sunlight and preferably well drained, less to medium fertile soils. Highly fertile soils simply allow the stems grow taller with no associated increase in tuber number and size. In general, southern and south-western parts of the country ranging from 1300-2600 meters above sea level (m.a.s.l) and north-western (Awi zone) and (Wenbera woreda) highlands are relatively the common growing areas because of their high to medium annual rainfall, suitable humidity, soil fertility and other climatic conditions suitable for growing Ethiopian potato (personal observation during sample accessions).

2.3.5. Geographic distribution

Ethiopian potato grows in a relatively wide agro-ecologies in the country and its distribution as a wild plant covers few countries in Africa such as Kenya, Tanzenia, Uganda and Democratic Republic of Gongo (Codd, 1985 as cited in Mulugeta Taye *et al*., 2006). In Ethiopia, the crop is found widely distributed in the Central, Southern, Western, Northwestern and Southwestern parts (Uphof, 1968; Westphal, 1975; Zeven and Zhukovsky, 1975; PGRC/E, 1986; Edward, 1991; Edossa Etissa 1996; GRIN, 2005 Hedberg *et al*., 2006). The important growing areas commonly identified includes Western and Southwestern Shewa, Jimma, lllu Aba Bora, Eastern, Western and Horo Guduru Wollega zones of Oromia region, Yem Liyu

woreda, the previous North Omo (Gamo Gofa and Wolaita Sodo zones), Gedeo, Keffa, Hadiya, Kambata Tambaro and Gurage zones of the South Nations, Nationalities and Peoples (SNNPs) Region, Awi Nationality Administrative Zone of the Amhara Regional State and Wenbera Woreda of Beneshangul Gumuz region. But recently, the crop is being vanished or highly marginalized in some of the above-mentioned zones (for example: Keffa and Gedeo) and limited only to few kebles or localities in other zones (personal observation during sample collection). Hence, the crop is categorized under 'orphan' ('super neglected') species (Mulugeta Taye, 2008). The wild relatives are tuber-less, annual or perennial and weedy, found distributed throughout the country especially on marshy areas and along sewerage lines (Edwards, 1991).

2.3.6. Biology of Ethiopian potato

2.3.6.1. Morphology

The crop is a large, erect, coarse, hairy, aromatic, succulent annual herb, up to one-meter high. Stems hirsute and glandular, decumbent and rooting at the base with swollen nodes, producing edible underground potato-like tubers on slender rhizomes. Leaves vary from dark-green to purplish-green, lanceolate to elliptic-lanceolate, acute, dentate, rounded into a sessile base, more or less glabrous on upper surface, glandular punctate and pubescent on the veins on the lower surface. Inflorescence a terminal spike, a raceme of small flowers which are usually purplish-blue, shuttering at early stage of maturity, elongating and interrupted in flower and fruit; glandular- pubescent with long septate hairs; cymes sessile, pedicels erect, shorter than to equalling the mature calyx. Mature calyx, villose and gland dotted; upper lip ovate apiculate and slightly upcurved, equalling the tube; teeth lanceolate, acuminate, about equalling the lip. Corolla rich purple-blue with small dark spots, tube bent and funnel shaped with a bulge on the upper surface, erect to recurved, broadened above, obtriangular, bilobed and fluted, with a

small lanceolate lobe on either side at the base; lower lip large and deeply concave, bearded and gland-dotted. Stamens included in the lip, and fused. Tubers have different shapes, textures, colour, and size (Hedberg *et al.,* 2006; Abebe Demise, 1998) ***(Appendix 1, Figures 1-5)***.

2.3.6.2. Mode of reproduction

The plant usually reproduces asexually through vegetative propagation of the tubers, suckers or soft-woody stem cuttings. However, sexual mode of reproduction is also a possibility as it produces seeds. The seeds are very small (less than 1mm in size), whitish-brown to black in color (that usually matches the leaf colour), ovoid shaped, fewer (4 per calyx) (Mulugeta Taye *et al*., 2012) ***(Appendix 1, figures 1-5)***. Once planted, it requires little care except earthing up and collecting the rhizomes is done when needed (Mulugeta Taye *et al*., 2006).

Tubers are usually produced as a swelling of the tip or middle parts of the stolons which originate from buds located in the main stems and the lower primary branches found above or below ground. More than two stolons can arise from one node. The first swelling of stolons to tubers seemed to occur at the nodes, whereas later the internodes swell and in older tubers the internodes are more swollen than the nodal parts. Tubers are also produced without stolons from the mother tuber piece (sessile tubers). The number of tubers obtained per hill could be largely determined by the number of stolons which could depend on the extents of earthing up (Mulugeta Taye *et al*., 2012).

2.3.7. Economic importance

2.3.7.1. Food and cultural values

Subsistence farmers in different parts of Ethiopia have been cultivating Ethiopian potato primarily for its edible tuber that is consumed after cooking. According to Siegenthaler (1960),

this crop is considered as one of the highland people's ancient foods. In addition, Ermias Lulekal *et al*. (2011) identified the crop as one of the wild edible root/rhizome crops in Kaffa zone, Southern Ethiopia.

The crop can offer significant amounts of micro- and macro-nutrients with relatively higher food energy than *Solanium tuberosum* when cooked. The fat and calcium contents are almost twice as high as that of *S. tuberosum*. The protein content is similar to that of *S. tuberosum* and is almost twice as high as that of *Ipomoea batatas* when cooked. The cooked tubers have more amounts of energy, fibre and carbohydrate compared to the raw tuber. However, the raw tuber is richer in nitrogen, protein, calcium, phosphorous, iron and niacin than cooked ones (***Table 2***).

Table 2: Nutritional content (per 100g of edible portion) of *P. edulis*, *S. tuberosum* and *I. batatas* tubers in Ethiopia. (Source Ethiopian Health and Nutrition Research Institute, EHNRI 1997)

Composition	*P. edulis*		*S. tuberosum*		*I. batatas*	
	Raw	**Cooked**	**Raw**	**Cooked**	**Raw**	**Cooked**
Food energy (calories)	69.00	100.60	103.70	89.70	136.00	134.20
Moisture (%)	81.90	73.80	73.10	76.80	67.40	65.60
Nitrogen (grams)	0.30	0.24	0.30	0.26	0.30	0.13
Protein (grams)	1.50	1.00	1.30	1.10	1.30	0.50
Fat (grams)	0.20	0.20	0.10	0.10	2.00	0.20
Carbohy. (incl. fiber) (grms)	15.30	23.70	24.40	21.10	28.20	32.60
Fiber (grams)	0.70	1.00	1.40	0.90	1.10	1.50
Ash (grams)	1.10	1.30	1.10	0.90	1.10	1.10
Minerals (milligrams)						
Calcium	29.00	19.00	14.00	9.00	52.00	35.00
Phosphorous	90.00	62.00	57.00	49.00	34.00	54.00
Iron	9.30	1.10	2.30	1.50	3.40	0.90
Vitamins (milligrams)						
Thiamin	-	0.11	0.08	0.05	0.08	0.06
Riboflavin	-	0.32	0.08	0.09	0.05	0.01
Niacin	0.70	0.30	1.00	0.80	0.90	0.40

It is very important in filling the food shortage gap and because of this it is usually called 'the hunger crop'. It is also known as 'Ye Meskel dinich' (potato of the Meskel), because it is usually ready for food around the celebration of Ethiopian Meskel (finding of the true cross) around the end of September and continuous as a source of food till mid-December when cereals and pulses are ready for harvesting, especially in the highland and semi-highland areas (Mulugeta Taye *et la*., 2012).

Apart from the tubers, leaves are also eaten by human as green vegetable in certain regions and the leaf parts and dry stems are used as a feed for animals and cattle fattening, according to Abebe Demisse, (1998). In addition, it has a cultural value by Wolaita people in that it is served with 'Data' (fine ground chile) for distinguished guests and individuals distinguished as 'courageous/heroes' by the local community.

2.3.7.2. Medicinal value, income source and ecological service for honey bee

Ethnobotanically, Ethiopian potato is extremely important in several parts of Africa including Ethiopia. For example, in Kenya, the edible roots or tubers are used to treat abdominal pain (Githinji and Kokwaro, 1993). In Ethiopia and other parts of Africa, the edible tubers and leaves are used for treating people with asthma (IAR, 1980). Moreover, a phytochemical compound called abietane diterpenoids such as edulone A and 16-*O*-acetylcoleon D were reported for their antimicrobial activity to treat infections, fever and inflammation (Buchbauer *et al*., 1978).

In addition to its direct ethnobotanical importance to human beings, the plant is a good source of forage for honey bee because it produces abundant sugary, fragrant and attractive nectar (Reinhard and Admasu Adi, 1994).

Furthermore, the crop is important in generating cash for those subsistence farmers in potential growing areas such as Chencha, Dita, Awi, Wenbera, Dedo, Yem, Sokoru, Wolaita, parts of West Shewa, and Gurage though not comparable with other crops as *S. tubersum*, *I. batatas*, cereals and pulses which are cultivated in large scale (personal observation).

2.3.8. Cultivation, harvesting, and productivity

Ethiopian potato plant is established mainly by planting an intact or broken, seed tuber in one planting hole which is followed by cultural practices such as hand weeding twice or more, tipping (removal of the apical parts of the stem) to decrease vertical growth and hence to get more branches which could produce more stolens that can grow (swell) in to tubers on earthing up. The source of seed tuber for planting is either from selectively maintained tubers of the previous harvest or from the nearby market. Usually, the seed tubers are left on the land, covered with mulching material or the tubers are bulk removed and buried in a hole under a

tree or house shade to protect from direct sunlight. Preparation of land usually begins after the harvest of main crops (around January) for planting to take place from March to April. It is cultivated on small scale bases around home stead or rarely in open field, as a pure stand and/or mixed with other crops like maize, sorghum, coffee, enset (Abebe Demissie, 1998).

Similar to *S. tuberosum, I. batatas* and other tuber crops, Ethiopian potato is largely cultivated for its edible tubers. The tubers are usually harvested from mid-September to early November (following the end of the main rainy season) (Mulugeta Taye *et al.,* 2007). Usually, harvesting is carried out only when the tuber is needed or rarely, in bulk if the land is needed for other cropping or if the tubers are to be stored for the next growing season (Yeshitila Mekbib and Jens, 2012; Mulugeta Taye *et al*., 2012). At some places, the leaves are also harvested for human consumption and animal feed (Westphal, 1975; Zemede Asfaw and Zerihun Woldu, 1997; Mulugeta Taye *et al*., 2007).

At present, there is no report of work done on the productivity of the crop. But according to the works by Wayessa Garedew (2013), Yeshitila Mekbib (2007), and personal observation during sample collection, its productivity may be considered as average compared to that of other tuber crops such as *S. tubersum*.

2.3.9. Ethiopian potato cultivation and associated constraints

Until now, the information towards estimate of Ethiopian potato cultivation is scarce. What is well known is the negligence cultivation and its being under risk of extinction. Short shelf life, especially high susceptibility to temperature is one of the critical problems associated with long term storage of the tubers on the field or in the storage. If exposed to high temperature, the tubers change their colour and eventually completely die within few weeks on the field or in few days in the case of home storage. In addition, mole rat, mouse and porcupine, are the major

problems that hinder extended field storage over months because of the aromatic smell of the crop (personal observation during sample collection). Lack of improved cultivars/varieties with excel tuber yield, is another constraints to the production and productivity of the crop (Yeshitila Mekbib and Jens, 2012). Otherwise, unlike other root crops, there is no report regarding the potential disease and pests of Ethiopian potato which may be due to endemicity of the crop that enabled it to accumulate more resistance and adaptive traits.

2.3.10. Research and development interventions

Until recently, only limited research activities have been conducted on Ethiopian potato. The studies were focusing on local customary use and management (Yeshitila Mekbib, 2007; Yeshitila Mekbib and Jens, 2012), agro-morphological diversity assessment using few accessions tested at single location (Wayessa Garedew *et al*, 2009 and 2013; Yeshitila Mekbib, 2007), ontogeny (Mulugeta Taye *et al.*, 2012), ISSR based molecular diversity study using tubers (Ijara Shiferaw, 2015) and seeds (Medhin Gebrehiwot, 2017), ethnobotany (IAR, 1980), micropropagation and tissue culture (Belete Kebede and Balcha Abera, 2015; Mesfin Tsegaw and Tileye Feyissa, 2014; Mulatu Gebre, 2015), and nutritional analysis (ENHIR, 1997). Moreover, only few accessions have been made by Ethiopian biodiversity institute (EBI) for conservation at Choche Field GenBank. But, the accessions have not been properly conserved, characterized and evaluated, and their attributes remained less known to breeders.

In general, detailed reproductive biology, nutritional analysis, molecular genetic diversity analysis, agro-morphological diversity analysis, joint molecular and phenotypic genetic diversity analysis, and cytogenetics of the crop are very short of what is desirable and this hindered the breeding and conservation measures of the crop.

2.4. Genetic Diversity Assessment and its Importance

Genetic diversity refers to diversity within species, between species or ecosystem diversity, which can be viewed at different geographical scales or levels of analysis (Almekinders and Struik, 2000). It facilitates reliable grouping of accessions and identification of subsets of important accessions/accessions with possible utility for specific breeding purpose, or it is the basic step for further improvement, breeding programs, cultivar release, and conservation management (Haussman *et al*., 2004; Akhtar *et al.,* 2007).

The available genetic diversity (variation) can be assessed using morphological, biochemical and / or molecular markers (Almekinders and Struik, 2000). Choice of the methods depends on the objective of the study, level of resolution required, available resource and time (Mohammedi and Prasanna, 2003).

2.4.1. Agro-morphological traits for diversity study in root and tuber crops including Ethiopian potato

Morphological traits (descriptors) are the most common and easily observable markers, usually used by taxonomists, farmers, and breeders for classification and evaluation of yield or other traits of interest in crop plants in general and fruit, root, or tuber crops and their relatives, in particular (Singh and Parab, 2015; Newbury and Ford-Lloyd, 1997). Morphological characterization of several crop plants, especially main crops, is mainly done by using standard descriptors developed by the International Plant Genetic Resources Institute (IPGRI), available since the first release of descriptor list in 1977 (Biodiversity International, 2007). The magnitude, nature and inter-relations of genotypic and phenotypic variation in the various characters is crucial in determining breeding progress (effectiveness in selection) and conservation measures (Singh, 2005).

The process of determining morphological genetic diversity demands partitioning of the overall phenotypic variability into its heritable and non-heritable components with the use of suitable

genetic parameters such as genetic coefficient of variation, heritability, genetic advance and correlation (Akinwale *et al.,* 2010). Besides, multivariate analysis methods are useful for characterization, evaluation and classification of plant genetic resources provided that many accessions are assessed for many characters of agronomic and physiological importance (Peeters and Martinelli, 1989).

The total variance of a given character is the result of its genotypic and environmental variances (Falconer and Mackay, 1996). The total genetic variance (variance of genotypic value) can be further portioned into additive, dominance and epistatic genetic variances. The additive genetic variance, which is the variance of breeding values, is an important component in determining the observable genetic properties of the population and their response to selection (Dudley and Moll, 1969). The amount of available genetic variation is the most important factor influencing selection gains and general adaptation in traits that are necessary for improved production under specific constraints (Vasal *et al.,* 1997).

In Ethiopia, several studies have been done to estimate genetic variability in different root and tuber crops. Among these, Wayessa Garedew *et al*., (2009) and Yeshitila Mekbib (2007) tried to determine the nature and magnitude of agro-morphological variability on few accessions of Ethiopian potato. Desta Fikadu (2011) also estimated the nature and magnitude of variability in morphological characters among anchote accessions. Both authors observed high (>20%) genotypic and phenotypic coefficients of variations for a number of variables. Engida Tsegaye *et al.* (2007) also reported similar result for such traits like vine length, vine internodes length, leaf length, number of storage roots per plant, individual storage root weight and storage root fresh yield per plant in sweet potato. Teshome Anshebo *et al*. 2004 reported similar result on sweet potato.

Although morphological features are indicative of genotypes, they are often controlled by multiple genes and subject to varying degrees of environmental modification and interaction. Moreover, they can be affected by growth pattern. As a result, the differences between genotypes are not always absolute and reliable (Islam *et al.,* 2002; Falconer, 1986). Consequently, it may be erroneous to completely rely on them (Ullah *et al.,* 2010). To this end, biochemical analysis (isozymes) which depends on gene product, cytogenetic data, and/or molecular analysis needs to be coupled with morphological and agronomic evaluations to obtain complementary information and increase the resolving power of genetic diversity analysis.

2.4.2. Cytogenetic study in the genus *Plectranthus*

The base for cytogenetic studies in the genus *Plectranthus* was laid during the early 1930s, 1940s, and 1950s and different basic somatic chromosome numbers were reported. For example: x= 6, 7 and 8 were reported in different species (Scheel, 1931; Furdosto, 1940; and Reddy, 1952). Since then, several works have been done on the genus regarding the somatic metaphase chromosome and meiotic behavior for use in genetic diversity, evolutionary systematics, and phylogenetic analyses (Darlington and Wylie, 1955; De Wett, 1958; Lukhoba *et al*., 2006; Alasbahi and Melzig, 2010; Reis, 2015).

In this view, many species of the genus *Plectranthus* showed a large variation in morphology, chemistry as well as in the number of chromosomes (2n = 14 to 2n = 84) (Morton, 1962; De Wet, 1958; Lukhoba *et al*., 2006; Alasbahi and Melzig, 2010). The chromosome size of the species of the genus investigated so far is very small, making the detailed study of their morphology (karyotyping) difficult/impossible, and hence the cytogenetic information is restricted to determining the somatic number (2n) and basic numbers (x) or the ploidy level of the species.

2.4.2.1 Polyploidy in the genus *Plectranthus*

Polyploidy or whole-genome duplication is recognized as being present in almost all lineages of higher plants. About half of the plant species were known to be recent polyploids, where multiple whole genomes or sets of chromosomes have come together from close ancestors (Soltis *et al*., 2015). Moreover, it has been suggested that all flowering plants, over their evolutionary time, have gone through at least one polyploidy event in their ancestry, before the divergence of gymnosperms and angiosperms. This is called the ξ (zeta) event (Fawcett *et al*., 2009; Jiao *et al*., 2011; Li *et al*., 2016). The relatively higher diversity of angiosperms thus illustrates the success of polyploidy displacing the diploid ancestors prior to or simultaneously with major evolutionary transitions and adaptive radiation of species which supports the concept that polyploidy plays a predominant role in bringing adaptive speciation beyond the emergence of new gene functions important for novelty.

Two main types of recent polyploidy are recognized. The first one is autopolyploidy which can be defined as duplication of one genome within one species, that results in more than two homologous chromosome sets in the cell and the second is allopolyploidy which could be defined as whole genome duplication associated with the merger of two or more divergent genomes in a single nucleus following interspecific hybridization, that results in homoeologous chromosome sets in the cell. In practice, auto- and allo-polyploidy are not entirely separate, since these terminologies depend on the taxonomic definition of a species and the scope of a designated 'genome'. Higher polyploids may be combinations of the two types of polyploides as a result of the evolutionary histories and includes both auto- and allo-polyploidy events, thus giving the 'auto-allo-polyploid' designation. For ancient whole genome duplication events, where the ancestral diploid species are unknown and DNA sequences are diverged, auto- or allo-polyploidy cannot be distinguished (Alix *et al*., 2017).

According to reports so far, polyploidy is a common occurence in the family Lamiaceae in general and genus *Plectranthus* in particular. Accordingly, several genera of the family as well as species of the genus *Plectranthus* have been reported as being polyploid. In this regard, *P. japonicus* from the tropics of the Old World was found to be tetraploid (2n = 24) with a postulated basic number of x = 6 (Darlington and Wylie, 1955). On the other hand, two cytological races of *P. grallatus* are tetraploid *(*2n = 28, x = 7) and octaploid *(*2n = 56, x = 8), and four hexaploid *(*2n = 42, x = 7) species of *P. grandidentajtus, P. hirtus, P. woodii* and *P. tomentosus* have been reported from South Africa (De Wet, 1958). In addition, *Coleus,* which was previously including *P. edulis* and several related species of the genus *Plectranthus* thus far has been reported to be polyploid. For example, five species of *Coleus* from Asia: *C. blumei (*2n = 24), *C. laciniatus (2n* = 48), *C. rehnel· tialuts (*2n = c.48), *C. forskohlii (*2n = 28) and *C. aromaticus (*2n = 32) were found to be polyploids with suggested basic number (x) of 6, 7 and 8. Similarly, two groups: one group consisting of *C. pentheri, C. comosus* and *C. vagatus,* all with 2n = 32 and x = 8, and a second group having 2n = 24, 48 and 60 with a basic chromosome number apparently of x = 6 or 12 have been reported from South Africa (Scheel, 1931; Furosato, 1940; Reddy, 1952). *Ascocarydion, Iboza, Hemizygia* and *Ocimum* are all closely related genera reported to bear at least few polyploid species with basic numbers of 7 in the first two and 8 in *Ocimum (*Goluhinski, 1938; Vaarama, 1947; de Wet, 1958).

2.4.2.2 Karyotypic characterization

Evolution is basically studied at various biological levels of organization. However, the state at which character states remained constant for longer time (example: change in chromosome number and its fine structures) is highly preferable (Sessions, 1996). Moreover, to study the diversity or identity of a group of species or individuals of a given species, examining the diploid chromosome count and/or ploidy level is the fast, easy to manage, reliable and well-

established method (Kazem, 2010). Chromosome (cytogenetic) characterization for many of closely related plant species has revealed some variation for different cytogenetic parameters (karyotypic formulae): somatic number (2n), total diploid chromosomes length (TCL), length of long arm (LA), length of short arm (SA), total length (TL) which is given by [LA+SA], and arm ratio (AR) computed using [LA/SA]. Most of these parameters are usually used in species having relatively smaller chromosome number and/or larger chromosome sizes that are suitable for karyotyping and hence not ambiguous to measure the arm lengths and ratios, otherwise only the somatic (2n) number report is applicable. The arm ratio is usually used to determine the centromeric position and classify chromosomes as median (m), sub-median (sm), subterminal (st), and terminal (t) (Levan *et al*, 1964).

Karyotype symmetry, which is usually described by intra- and inter-chromosomal asymmetry indices (A1 and A2, respectively), can also be utilized for species separations or identification (Romero–Zarco, 1986). It can be estimated using one or more of the following components: the total form percentage (TF %) given by the formula [(ΣSA/ΣTL)*100]; difference or range of relative length (DRL) that is given by [Max-Min]; intra-chromosomal asymmetry (A1), i.e. [1 Σ(SA/LA)/n]; and/or inter-chromosomal asymmetry index (A2) which is equal to $[\overline{sd}/\overline{X}]$ where n is the number of homologues, $\overline{sd}$ is the average of standard deviation, and $\overline{X}$ is the mean chromosome length (Stebbins, 1971; Romero-Zarco, 1986; Kazem, 2010; Kalvandi *et al*., 2012; Sutar *et al*., 2013). The significant variation of any of these parameters may be the indication of outbreeding (recombination) or recent hybrid formation (Chen *et al*., 1997). As a general rule, the trend of karyotype evolution is towards greater asymmetry in plants (Stebbins, 1974) that might be accounted to somatic chromosome fusion (Huang *et al*., 2009), which consequently reduced the chromosome number. The situation seems the reverse in animals' where the most derived karyotype was symmetric forms (all bi-armed) (Sessions, 1996).

Generating the genetic information in general and cytogenetics in particular is a very important approach in crop plants for correct plant identification and production of improved verities in breeding programs. Nevertheless, to our knowledge, there is no work/report on the cytogenetics of Ethiopian potato that may partly be due to its endemicity and partly due to / it's being rare garden plant ('orphan' crop). Therefore, it is essential to establish the correct ploidy level of chromosomes of the crop, which is useful for its systematics, breeding, and genetic studies.

2.4.3. Molecular markers-based diversity and population structure analyses

Molecular (DNA) markers arise from different classes of DNA mutations (Paterson, 1996a). They are the most widely used and relatively precise types of markers in measuring genetic relationships predominantly due to their abundance and selective neutrality because of their location in the non-coding regions of DNA (Collard *et al*., 2005). They are used to complement the limitations of morphological and cytogenetic approaches and hence, for better characterization of genetic diversity, population structure, and evolutionary studies (Sessions, 1996). In general, they are very important for breeding programs and conservation and management of genetically diverse crop plants (Wen *et al*., 2010; Lu *et al*., 2011). Moreover, they may or may not correlate with phenotypic expression of a genomic trait, stable and are detectable in all tissues regardless of growth, differentiation, development, or defence status of the cell (Mondini *et al.,* 2009) and hence, offer unambiguous estimates of genetic variability of populations. Additionally, they are not confounded by environmental, pleiotropic and epistatic effects (Ullah *et al.,* 2010).

One of the breakthroughs in biological sciences in general and molecular research in particular, is the invention of Polymerase Chain Reaction (PCR) that amplifies DNA *in vitro* (Mullis and Faloona, 1987). Since then, molecular marker techniques were grouped into non-PCR based

and PCR-based, depending on the requirement of PCR or not to produce different DNA fragments.

In this view, the non-PCR markers include RFLP and its modifications while the PCR-based markers include RAPD, AFLP, ISSR, SSR, etc (Botstein *et al*., 1980; Litt and Lutty, 1989; Williams *et al*., 1990; Zietkiewicz *et al*., 1994; Vos *et al*., 1995). Different PCR-based markers are used with different powers for genome sampling and generating polymorphisms in plant genetic diversity analyses (Weising *et al*., 2005).

2.4.3.1 Simple sequence repeats (SSRs) markers

Microsatellites or SSRs are highly variable, usually 2-6 base pairs (di- to hexa-nucleotide repeat units) in length, tandemly repeated motifs of DNA, distributed abundantly throughout the eukaryotic genome (Tautz and Renz, 1984; Temnykh *et al*., 2001). SSRs exist in either of the four categories: perfect, imperfect, interrupted, and composite. The perfect microsatellite is composed of non-interrupted repeat motifs whereas the imperfect SSRs include a base pair that is not part of the repetitive motif. In interrupted SSRs, the repeat motifs include a small sequence that does not match the repeat sequence and in composite microsatellites, the motif contains two adjacent distinctive repeat sequences (Olivera *et al*., 2006).

The repeated motives are believed to be originated from the non-repeat sequences either by insertions or by substitutions. Eventually, they become more abundant and occurring in variant forms by several mechanisms including errors during recombination (unequal crossing-over), and polymerase slippage during DNA replication or repairing. The crossing-over mutation effect is more pronounced than the polymerase slippage since it adds or deletes large number of repeats (de Vienne *et al*., 2003; Olivera *et al*., 2006). Polymorphism between individuals of

the same population can be caused by mismatches at priming sites and the length of repeat motifs (Zietkiewicz *et al*, 1994).

SSRs marker development is achieved through either enrichment procedure (for whose genomic library is already constructed) (Ostrander *et al.,* 1992; Edwards *et al*., 1996) or in silico mining from existing databases (Temnykh *et al*., 2001), where the later approach being cost and time effective (Simko, 2009). The enrichment procedure follows screening the marker by probe, sequencing the positive clones, synthesizing the oligonucleotide primers, and finally testing the primers with samples (Edwards *et al*., 1996). This method is very important in enriching SSRs marker library, which in turn facilitate germplasm genotyping, selection, and genetic mapping. Moreover, this method is an efficient approach, especially for plants that do not have expressed sequence tag (ESTs) or genomic databases (Novelli *et al*., 2013).

SSRs are markers of choice because of their high reproducibility, co-dominant inheritance, rich in information content or high polymorphism, locus-specificity (independent marker or it distinguishes between alleles), ease of automation, and relatively low cost as compared to single nucleotide polymorphism (SNP) marker. The polymorphism in length of each motif can be detected either by agarose gel or by polyacrylamide gel electrophoreses depending on the length between the alleles or more frequently with automatic sequencing methods (de Vienne *et al*., 2003).

2.4.3.2 Origin of microsatellites

In population genetic analysis, there are four commonly used models concerning the origin of microsatellites: the infinite allele model (IAM), stepwise mutation model (SMM), two-phase mutation model (TPM), and the K-allele model.

The IAM considers that each mutation randomly creates new allele and the proximity in terms of number of repeats that does not indicate a greater phylogenetic relationship. This model is called Wright's (1951) model, for which he used F-statistics for differentiation analysis. SMM, in contrast, assumes that each mutation is accompanied by gain or loss of one repeat motif, i.e. two alleles differing by one repeat unit are more closely related than alleles that differ by several repeat units. Slatkin's (1995) RST (that take into account the effect of mutation), and Nei's (1973) GST are genetic differentiation measures proposed for this model. In the investigation of population structure by these measurements, it was evident that the two measurements usually give significantly different values (usually FST/GST is lower than RST) because of the high and variable mutation rates of microsatellites and as a result one can observe a high level of within population heterozygosity. SMM is highly preferable and fits for non-homoplasy markers (alleles identical not by state but by descent) like EST-SSRs, which are sampled from the same loci for all individuals. The third model, TPM- was introduced by Di Reinzo *et al*. (1994) as an extension of SMM briefing the possibility of a rare event by which a larger number of repeats can occur rather than by the most frequent SMM. Crow and Kimura (1970) proposed another model, the K-allele model. This model assumes that if there are exactly k possible alleles per locus then the probability of a given allele to mutate into any one of them is μ/k-1, where μ is mutation rate (Olivera *et al*., 2006).

2.4.3.3 Expressed sequence-based simple sequence repeats (EST-SSRs) marker and its applications

SSRs can occur both in genic (expressed sequence tag-EST-SSRs) and non-genic regions (G-SSRs) of a genome. Relying on EST-SSR marker is more advantageous in that, they are derived from transcripts (cDNAs) where their flanking regions are highly conserved across taxa and hence they serve as a functional marker, inexpensive to develop, and highly transferable between related species and even between related genera (cross species amplification) (Peakall

et al., 1998; Wen *et al.,* 2010; Mao *et al*., 2014). Transferability not only reduces the cost of designing specific primers but also facilitates the isolation of microsatellites from a taxon with low microsatellite frequencies. It is usually hampered by kinship, genome size and complexity (Olivera *et al*., 2006). In addition, using EST-SSR markers reduces sampling error associated with sampling of loci, since the same loci are considered for each accession in EST SSRs amplification (Mohammedi and Prasanna, 2003). Therefore, they are potential candidates for gene tagging and comparative studies of related species (Simko, 2009).

Moreover, EST-SSRs have been preferentially used for a wide range of reliable analyses targeting genetic mapping studies, marker assisted selection (MAS), genetic diversity surveys, quantitative trait loci (QTL) analysis, germplasm maintenance programs, cultivar genome fingerprinting, marker-trait association, and population structure study (Zietkiewicz *et al.*, 1994; Simko, 2009; de Vienne *et al*., 2003; Ferrao *et al*., 2014; Wang *et al*., 2014).

As part of expressed region, EST-SSRs are also important for many biological functions: gene regulation, recombination (especially, di-nucleotides are recombination hot spot), DNA replication, cell cycle and mismatch repair (Olivera *et al*., 2006; Ranade *et al*., 2014).
Even though ESTs-based SSRs are limited in detecting the number of alleles per locus and heterozygosity when compared to G-SSR (Simko, 2009), both are widely used to estimate genetic diversity and population structure (Kim *et al*., 2008).

Assessment of the level of genetic diversity and the proper management of genetic resources are important issues and major concerns in the modern scenario of plant and animal improvement programmes and for proper designing of conservation measures (Farooq and Azam, 2002; Somasundaram and Balaise, 2007). However, the work done so far on Ethiopian potato in this regard is very little of what it should be. Only few works related to inter simple

sequence repeat (ISSRs) markers, on limited number of accessions have been done (Medhin Gebrehiwot, 2017; Ijara Shiferaw, 2015).

3. MATERIALS AND METHODS

The present study encompasses three main studies that include agro-morphological genetic diversity analysis using data collected from two test locations, EST-SSRs based molecular marker development, molecular genetic diversity and population structure analysis, and chromosome count and ploidy level determination for Ethiopian potato populations.

3.1. Plant Material

A total of 174 accessions, representing 12 populations, were randomly collected from diverse agro-ecologies of the four-potential growing regional states of Ethiopia (***Figure 1; Table 3***). The number of accessions collected from Oromia = 74, Amhara = 21, Southern Nations, Nationalities and Peoples Regional state (SNNPs) = 69, and Beneshangul Gumuz = 10. The collection was done from November, 2013 to February, 2014. The sample tubers were collected from farmers fields where it is maintained for the next season sowing. The tuber accessions were collected in net bags and were temporarily maintained for three months on a semi-dry soil filled pot in the glasshouse at College of Natural Sciences, Addis Ababa University (AAU), to inhibit sprouting and damage until the normal field growing season (April).

The accessions were grouped into 12 populations mainly on the bases of administrative zones of collections with the assumption of restricted gene flow between/among the zones. In rare occasions, we merged nearby woredas of adjacent zones that largely share common market and had high population movement together as one population, for example; Hadiya and Kembata-Tembaro zones as *HKT* population. Similarly, we designated woredas that seem isolated from other woredas, for example; Yem Liyu woreda and Wenbera woreda, as a separate population (***Table 3***).

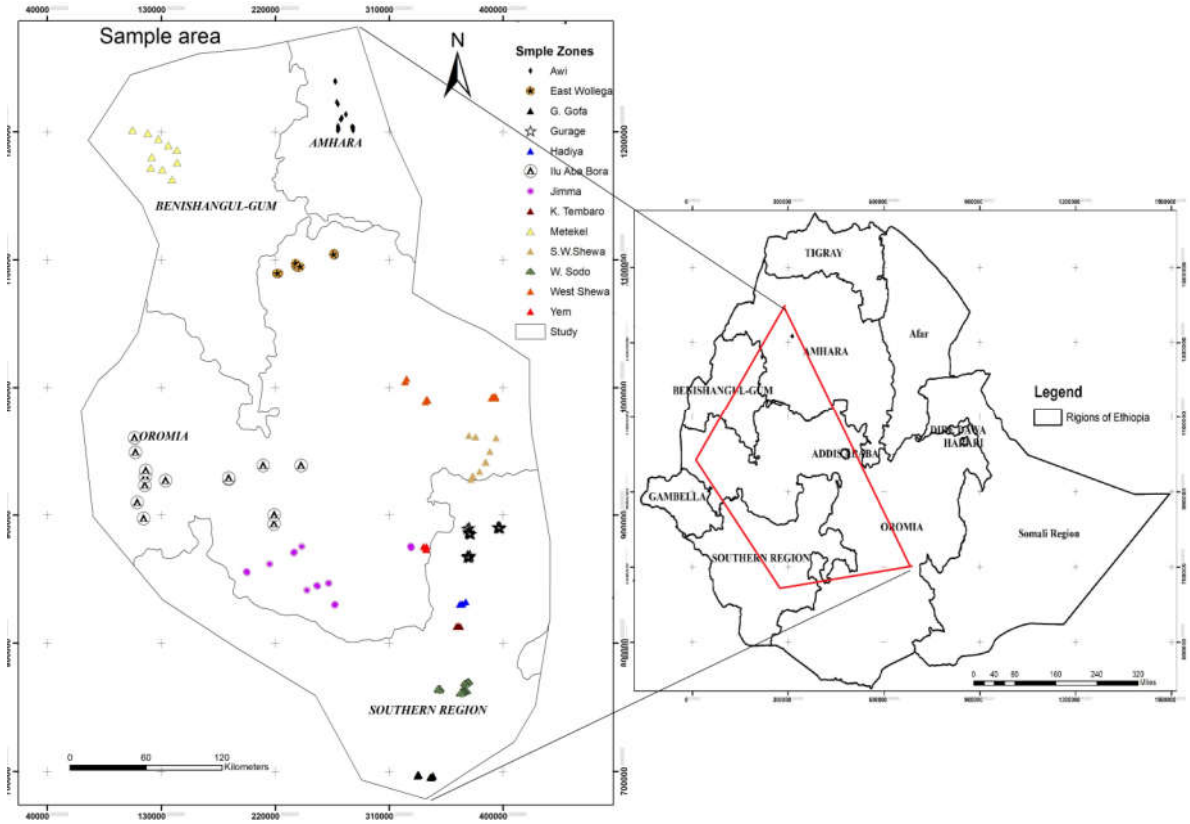

Figure 1: Map of Ethiopia showing its Federal Regions (right) and tuber sample collection sites within four of the Federal Regions (left). The map was original and constructed using geographic coordinates and elevation data gathered from each collection sites using global positioning system (GPS). Sample zones represent the 12 populations where, Hadiya and Kambata-Tembaro were merged together as one population

Table 3: Plant materials used in the present study indicating names and codes of populations, and regonal states, zones, woredas and kebles of collection as well as collection codes and coordinates of 174 accessions. Numbers within each population are nubmer of accessions and number of plants for which leaf samples were collected for the accession. 'ED' is the code for 'Ethiopian potato'; letters: a, b, c next to numbers represent the individual plants used for leaf sample collection per tuber sample; Latitude and Longitude readings are in standard UTM

Population name and size*	Col. Region	Collection zone	Woreda	Kebele	**Coll. Code**	**Altitude**	**Standard UTM**	
							Lat.	**Long.**
Southwest Shewa *(SwSh)*: 13 tuber and 24 leaf samples	Oromia	Southwest Shewa	Goro	Gambela Goro	ED001 **a, b**	1835m	374792	0927865
				Dambal Dildila	ED002 **a, b**	1828m	375795	0930030
				Dambal Dildila	ED003 **a, b**	1823m	376028	0930442
				Dambi Kono	ED004 **a, b**	1817m	376129	0930166
				Dambi Kono	ED005 **a, b**	1825m	376350	0930570
				Leman Abo	ED006 **a, b**	1915m	381653	0934072
			Darian	Kite Wato	ED007 **a, b**	2599m	378554	0961153
				Kite Wato	ED008 **a, b**	2612m	378282	0960973
				Kite Wato	ED009 **a, b**	2631m	373275	0962106
			Woliso	Foduna Gora	ED010 **a**	1972m	386105	0940381
				Foduna Gora	ED011 **a, b**	1991m	386714	0940594
				Foduna Gora	ED012 **a**	2012m	386594	0940930
				Foduna Gora	ED013 **a, b**	2003m	386256	0941347
East Wollega *(EWo)*: 14 tuber and 24 leaf samples		East Wollega	Gida Ayana	Arele Waja	ED014 **a, b**	1991m	238222	1093812
				Arele Waja	ED015 **a**	2060m	236791	1094660
				Arele Waja	ED016 **a, b**	2124m	236064	1095399
				Sirba Wadessa	ED017 **a**	2126m	236158	1097468
				Kersa Albukane	EDO18 **a, b**	2087m	239841	1094692
			Limmu	Warso	ED019 **a, b**	2156m	222300	1089267
				Warso	ED020 **a**	2166m	222863	1098386
				Warso	ED021 **a, b**	2149m	221339	1089443
				Gode	ED022 **a, b**	2151m	221199	1089499
				Gode	ED023 **a**	2142m	221689	1089526
			Kiramu	Babo	EDO24 **a, b**	2172m	266764	1103908
				Babo	ED025 **a, b**	2163m	266545	1104268
				Nole	ED026 **a, b**	2141m	266478	1105086
				Nole	ED027 **a, b**	2151m	266130	1104296

Table 3: Continued …….

Pop & Size	Region	Zone	Woreda	Kebele	Coll code	Alt.	Lat.	Long.
Awi nationality *(Awn)*: 21 tuber and 25 leaf samples	Amhara	Awi nationality	Banja	Bida Jogla	ED028 **a, b**	2554m	271766	1209820
				Bida Jogla	ED029 **a**	2555m	271844	1209745
				Basa Enguana	ED030 **a**	2610m	275782	1213661
				Basa Enguana	ED031 **a**	2643m	275760	1213723
			Koso Ber Zur	Kebele 02	ED032 **a**	2580m	272444	1210394
				Kebele 02	ED033 **a**	2541m	272580	1211074
			Dangla	Birntu Georgis	ED034 **a**	2167m	267632	1239283
				Birntu Georgis	ED035 **a, b**	2175m	267210	1239531
			Fagta Lekoma	Gafara	ED036 **a**	2555m	269860	1223160
				Gafara	ED037 **a**	2590m	269618	1221656
			Ankesha Goagsa	Sinisa	ED038 **a**	2344m	269504	1201176
				Ateta	ED039 **a, b**	2380m	269983	1202769
				Ateta	ED040 **a, b**	2385m	269889	1202998
				Birta	ED041 **a**	2402m	269625	1203898
				Birta	ED042 **a**	2427m	269710	1204516
				Sinisa	ED043 **a**	2373m	270095	1201499
			Tilili	Jubaita Michael	ED044 **a**	2460m	281614	1201344
				Jubaita Michael	ED045 **a**	2461m	281419	1201272
				Jubaita Michael	ED046 **a**	2488m	281030	1202594
				Agza	ED047 **a**	2486m	281621	1203576
				Agza	ED048 **a**	2500m	280702	1203809
Gurage (*Gur)*: 18 tuber and 24 leaf samples	SNNPs	Gurage	Endegagn	Shawura	ED049 **a, b**	2261m	371729	0867217
				Wolicho	ED050 **a**	2445m	373024	0867647
				Wolicho	ED051 **a**	2253m	372006	0867995
				Jeda	ED052 **a, b**	2382m	373924	0868624
				Jeda	ED053 **a**	2379m	374010	0868572
				Jeda	ED054 **a**	2350m	371908	0868375
			Enemorna Ener	Gesabdi	ED055 **a, b**	2126m	372560	0886833
				Gesabdi	ED056 **a**	2131m	372968	0886340
				Amogera	ED057 **a**	2155m	373160	0885715
				Amogera	ED058 **a**	2176m	374031	0885462
				Okochira	ED059 **a**	2069m	371621	0889342
				Kasaye	ED060 **a**	2099m	373406	0889929
				Kasaye	ED061 **a**	2087m	373025	0889992
			Gumer	Isenina Dangeso	ED062 **a**	2863m	395550	0879979
				Isenina Dangeso	ED063 **a**	2878m	396749	0880412
				Bordana Damber	ED064 **a**	2929m	397159	0880130
				Bordana Damber	ED065 **a**	2906m	397588	0879765
				Harekit	ED066 **a, b, c**	2895m	397269	0879266

Table 3: Continued …….

Pop & Size	Region	Zone	Woreda	Kebele	Coll code	Alt.	Lat.	Long.
Hadiya, Kambata and Tembaro *(HKT):* 9 tuber and 23 leaf samples	SNNPs	Hadiya and Kembata Tembaro	Lemo	Jawe	ED067 **a, b**	2121m	365839	0830439
				Jawe	ED068 **a, b**	2161m	367119	0830378
				Jawe	ED069 **a, b, c**	2178m	368204	0830733
			Sesh Duna	Sesh Duna	ED070 **a, b, c**	2195m	370720	0832265
				Sesh Duna	ED071 **a, b, c**	2192m	370744	0832296
			Doyu Gena	Wagebeta Eba	ED072 **a, b**	2293m	363184	0813164
				Wagebeta Eba	ED073 **a, b, c**	2456m	364593	0813637
				Ancho Sadicho	ED074 **a, b**	2562m	366420	0813142
				Ancho Sadicho	ED075 **a, b, c**	2556m	366447	0813134
Illu Aba Bora (*IAB):* 14 tuber and 25 leaf samples	Oromia	Ilu Aba Bora	Alle	Yubo Mari	ED076 **a, b**	1794m	781347	0900009
				Yubo Meri	ED077 **a, b**	1851m	781044	0901224
			Metu	Metu Zuriya	ED078 **a**	1694m	784997	0916658
				Metu Zuriya	ED079 **a**	1696m	784937	0916755
				Metu Zuriya	ED080 **a**	1714m	784744	0916731
			Darimu	Wale	ED081 **a, b**	1650m	775026	0943622
				Tulema	ED082 **a, b**	1664m	775453	0949107
			Kumbabe	Guji	ED083 **a, b**	1923m	183276	0927973
				Humbe	ED084 **a, b**	1919m	133324	0927001
			Bedele	Digaja	ED085 **a, b**	1947m	183233	0928938
				Urgessa	ED086 **a, b**	1901m	210424	0938681
			Didessa	Sobo	ED087 **a, b**	1915m	240425	0938683
				Sida	ED088 **a, b**	1879m	219020	0893136
				Yembero	ED089 **a, b**	2096m	219193	0900059
Jimma *(Jim:* 16 tuber and 24 leaf samples		Jimma	Goma	Balto	ED090 **a**	1605m	234630	0870448
				Bore	ED091 **a**	1621m	235207	0871151
				Sadi	ED092 **a**	1972m	215625	0861689
				Andode Mari	ED093 **a**	1848m	240990	0875481
			Gera	Gure Ganji	ED094 **a, b**	1975m	197673	0855091
				Nasu	ED095 **a, b**	1979m	197291	0855900
			Seka Chekorsa	Gibe Bosu	ED096 **a, b**	1806m	245128	0841259
				Buyo Kechema	ED097 **a, b**	2161m	253103	0844760
			Dedo	Keta Kedida	ED098 **a, b**	2228m	267135	0829838
				Keta Kedida	ED099 **a, b**	2181m	267348	0830162
			Jimma	Kofe	ED100 **a, b**	1835m	253193	0844702
				Kofe	ED101 **a, b**	1741m	262245	0846733
			Sokoru	Yero Sokru	ED102 **a**	1719m	262234	0846752
				Wengesho	ED103 **a**	1987m	327451	0874811
				Wengesho	ED104 **a**	2010m	327491	0874631
				Yero Sokru	ED105 **a**	1927m	327078	0875685

Table 3: Continued …….

Pop & Size	Region	Zone	Woreda	Kebele	Coll code	Alt.	Lat.	Long.
Gamo Gofa (*GGo)*: 13 tuber and 24 leaf samples	SNNPs	Gamo Gofa	Chencha	Boyina Tupa	ED106 **a, b**	2655m	343464	0691930
				Mafuna Zola	ED107 **a**	2666m	343368	0692737
				Mafuna Zola	ED108 **a**	2678m	343158	0692308
				Gendo Gembela	ED109 **a, b**	2705m	342768	0692333
				Losha	ED110 **a**	2696m	341154	0690582
				Losha	ED111 **a**	2744m	341322	0690946
				Doko Shaye	ED112 **a, b**	2733m	340431	0691145
				Dorze	ED113 **a, b**	2522m	333111	0697778
			Dita	Gessa	ED114 **a, b**	2746m	333765	0697306
				Gessa	ED115 **a, b**	2625m	333653	0696840
				Ache	ED116 **a, b**	2602m	332711	0696733
				Ache	ED117 **a, b, c**	2623m	332363	0696507
				Egra	ED118 **a, b, c**	2513m	332638	0697599
Wolaita Sodo (*WSo)*: 18 tuber and 23 leaf samples		Wolaita Sodo	Damot Gale	Sibaye Korke	ED119 **a, b**	1979m	372876	0770184
				Sibaye Korke	ED120 **a**	2012m	372090	0769633
				Wendara Gale	ED121 **a**	2027m	370174	0768940
				Wandera Gale	ED122 **a**	2034m	370457	0768351
				Wandera Buluso	ED123 **a**	2059m	369715	0763470
				Shasha Gale	ED124 **a, b**	2159m	369409	0764688
			Sodo Zuriya	Kokate Mare Chare	ED125 **a, b**	2215m	366537	0760650
				Delbo Atwaro	ED126 **a**	2188m	369768	0762925
				Delbo Atwaro	ED127 **a**	2140m	371214	0762931
				Delbo Wegene	ED128 **a**	2189m	369255	0762293
				Delbo Wegene	ED129 **a**	2221m	368812	0762302
				Kokate Mare Chare	ED130 **a**	2204m	367925	0761477
				Kokate Mare Chare	ED131 **a**	2232m	367138	0761189
			Damot Sore	Bolola Chew Kare	ED132 **a**	2086m	350087	0764579
				Bolola Chew Kare	ED133 **a**	2099m	349721	0763493
				Doge Meshido	ED134 **a, b**	2094m	349417	0763944
				Doge Meshido	ED135 **a**	2109m	349724	0764974
				Doge Meshido	ED136 **a, b**	2092m	349195	0764333

Table 3: Continued……

Pop & Size	Region	Zone	Woreda	Kebele	Coll code	Alt.	Lat.	Long.
Wenbera *(Wen)*: 10 tuber and 26 leaf samples	Beneshangul Gumuz	Metekel	Wenbera	Babo	ED137 **a, b, c**	2503m	793823	1174693
				Babo	ED138 **a, b, c**	2517m	793350	1174332
				Heto Shimo	ED139 **a, b, c**	2513m	793061	1174586
				Heto Shimo	ED140 **a, b**	2530m	792685	1174041
				Heto Shimo	ED141 **a, b**	2476m	792191	1174686
				Mana Sibu	ED142 **a, b**	2481m	790497	1174919
				Mana Sibu	ED143 **a, b, c**	2504m	789924	1175011
				Mana Sibu	ED144 **a, b, c**	2483m	789551	1174520
				Mana Sibu	ED145 **a, b, c**	2509m	790030	1175300
				Hambifata	ED146 **a, b**	2501m	789433	1174471
West Shewa (*WSh)*: 17 tuber and 23 leaf samples	Oromia	West Shewa	Cheliya	Ale Hula Dabi	ED147 **a**	2924m	324020	1007305
				Ale Hula Dabi	ED148 **a**	2898m	322544	1004624
				Ale Hula Dabi	ED149 **a, b**	2918m	322850	1005236
				Liben Gamo	ED150 **a, b**	2333m	339287	0990498
				Liben Gamo	ED151 **a, b**	2304m	338799	0989176
			Toke Kutaye	Toke Kombolcha	ED152 **a, b**	2249m	340335	0990271
				Toke Kombolcha	ED153 **a**	2252m	340402	0990196
				Toke Kombolcha	ED154 **a**	2286m	340673	0990343
				Toke Irensa	ED155 **a**	2249m	340132	0991042
			Dandi	Golole Bolo	ED156 **a**	2469m	390882	0991969
				Golole Bolo	ED157 **a, b**	2467m	391246	0992051
				Sumbela Shiko	ED158 **a, b**	2441m	391789	0992633
				Shumbela Shiko	ED159 **a**	2446m	392269	0992869
				Shumbela Shiko	ED160 **a**	2445m	392667	0992596
				Gatira Lafto	ED161 **a**	2449m	393235	0992986
				Gatira Lafto	ED162 **a**	2456m	394228	0993156
				Gatira Lafto	ED163 **a**	2460m	394336	0992132
Yem Liyu (*YeL)*: 11 tuber and 22 leaf samples	SNNPs	Yem Liyu Woreda	Yem Liyu	Oya Keruwa	ED164 **a, b**	2589m	339188	0873819
				Oya Keruwa	ED165 **a, b**	2540m	339439	0873081
				Oya Keruwa	ED166 **a, b**	2547m	339275	0873365
				Oya Kepo	ED167 **a, b**	2474m	339716	0875351
				Oya Kepo	ED168 **a, b**	2470M	339720	0875350
				Oya Kepo	ED169 **a, b**	2466m	339722	0875348
				Oya Ereto	ED170 **a, b**	2560m	338923	0874054
				Oya Ereto	ED171 **a, b**	2570m	338941	0874058
				Oya Ereto	ED172 **a, b**	2558m	338927	0874051
				Deri	ED173 **a, b**	2479m	337472	0875661
				Deri	ED174 **a, b**	2474m	337470	0875666

3.2. Agro-morphological Genetic Diversity Study

3.2.1. Experimental sites

Morphological and agronomic characterization of the germplasms were conducted at research fields of Holeta Agricultural Research Center (HARC) and Ambo Plant Protection Research Centre (APPRC). HARC is located 44 Kms southwest of Addis Ababa at an altitude of 2400 meters above sea level, and 09° 04'N, 38° 29'E. It receives average annual rainfall of 1037.70 mm, with mean minimum and maximum temperatures of 5.19^{0}C and 23.4^{0}C, respectively. The soil is predominantly nitosol and vertisol types (source: HARC). APPRC is located 115kms west of Addis Ababa, at 80° 57'N, 38° 00'E and altitude of 2185 meters above sea level. It has an annual rainfall of 1100mm with mean annual maximum and minimum temperatures of 26°C and 11°C, respectively. Agro-ecologically, it is a temperate (intermediate highland) area with vertisol soil type (source: APPRC).

3.2.2 Experimental design

The experiment was laid out in an incomplete random block design called alpha lattice with two replications at each site and three blocks per replication per site. A total of 60m X 36.5m plot of land was used at each site. The position of each accession within the block was randomized and grown in a single row. A total of eight tubers (plants) per plot with a spacing 60cm between plants and 1m between rows were used. At both sites, planting was done at the beginning of the rainy season (end of April) on a well-prepared soil. Three kg of farmyard manure per plot (8.571 t/ha) was applied along the rows as recommended by Wayessa Garedew *et al*., (2009). One month later after planting the tubers, when the crop was well established, two times earthing up (with loose soil) was undertaken to loosen the soil. Hand weeding was done to keep the plots free of weeds.

Planting was done for two seasons at HARC (2014 and 2015 growing seasons) and, due to tuber shortage, plants were grown at APPRC for only one season (2014 growing season). Thus, for analysis, we used the three seasons as three experimental environments (HARC season 1 as environment one, season 2 as environment two, and APPRC season one as environment three).

3.2.3 Traits scored/studied

So far, there are no standard descriptors developed for Ethiopian potato. Therefore, the descriptor lists developed by IPGRI for other root and tuber crops like *Solanium tubersum*, and the descriptors used by Yeshitila Mekbib (2007) and Woyessa Garedew *et al*., (2009) for Ethiopian potato were used in the present study.

In total, 28 agro-morphometric (12 qualitative and 16 quantitative) traits were recorded at the correct growth stages, though most of the data were collected at 50% flowering stage (***Table 4***). For most of the quantitative agro-morphological traits, data were collected on sample of five selected and tagged middle plants from each collection per plot, per replication, per site. Two traits, days to flower initiation and days to 50 percent flowering that were recorded on plot basis. For qualitative morphological traits, recording was done at only one experimental site (HARC), on plot bases.

Tuber yield (YTPH) was calculated based on the average fresh weight of tubers from the five sampled plants per plot. Similarly, tuber dry matter content (TDM) was calculated using the formula, TDM = (Fresh weight -oven dry weight/fresh weight) x100.

Table 4: List of qualitative and quantitative agro-morphological traits recorded, their codes, descriptions and scores

A. Qualitative Traits			
Trait	**Code**	**Description**	**Scores**
Leaf colour	LC	Colour of the leaves at initiation of flowering	Deep Purple (1), Pale Purp(2), Green(3), Deep Green (4)
Leaf arrangement	LA	Positioning of leaf at 50% flowering	Opposite(1), Alternate(2), Whorl(3)
Leaf shape	LSh	Shape of leaves during harvest	Ovate(1), Triangular(2), Round(3)
Stem colour	StC	Colour of the stems at initiation of flowering	Deep Purple(1), Pale Purple(2), Green(3)
Stem hairiness	Sthr	Presence or absence of hair on stems at 50% flowering	Absent(1), Sparse(2), Dense(3)
Spot on stem	SpOnSt	Presence or absence of spots on stems at 50% flowering	Present(1), Absent(2)
Spot colour	SpC	Colour of spots on stems at 50% flowering	Purple(1), Black(2)
Stem growth habit	StGH	Type of growth at 50 % of flowering	Open(1), Erect(2), Ambiguous(3)
Tuber skin colour	TSC	Types of tuber colours during harvest	Cream(1), Cream White(2), Cream Purple(3), Purple(4)
Tuber texture	Tt	Texture of the tubers during harvest	High. Ringed(1), Med. Ringed(2), Less Ringed(3)
Tuber shape	Tsh	Tuber shape during harvest	Elongated(1), Round(2), Oval(3)
Tuber hair	Th	Presence or absence and extent of tubers hair at harvest	High(1), Medium(2), Low(3)
B. Quantitative Traits			
Traits	**code**	**Description**	**Score**
Plant height (cm)	PH	The height measured from the mounding to the tip of the plant at maturity	5 plants/plot/rep/location
Stem girth (cm)	SG	The diam. of the main stem at the 4th internode from the mound at maturity	5 plants/plot/rep/location
No of primary branches/hill (cou.)	NPB	No of primary branches on the main stem counted at crop maturity	5 plants/plot/rep/location
No of stems/plants/tuber (cou.)	NPPH	No of stems at crop maturity per tuber	5 plants/plot/rep/location
Leaf length (cm)	LL	Length of the leaf on the main stem at the fourth node below inflorescence	5 plants/plot/rep/location
Leaf diameter (cm)	LD	Width of the leaf on the main stem at the fourth node below inflorescence	5 plants/plot/rep/location
Days to flower initiation (cou.)	DFI	No of days from planting to the appearance of the first flower	on plot bases
Days to 50% flowering (cou.)	DF	No of days from planting to 50% flowering	on plot bases
Flower (Inflourecence) length (cm)	FL	Length of flowers measured at 50% flowering	5 plants/plot/rep/location
Tuber number per hill	TN	The total count of the number of tubers at harvest	5 plants/plot/rep/location
Tuber length (cm)	TL	The average length of five tubers per hill measured at harvest	5 plants/plot/rep/location
Tuber diameter (cm)	TD	The average diameter measured per hill at harvest using Vernier calliper	5 plants/plot/rep/location
Internodes length (cm)	IL	The length of nodes on the main stem measured at 50% flowering	5 plants/plot/rep/location
Tuber weight per hill (kg)	TW	The total weight of tubers per hill (tuber yield per hill or plant)	5 plants/plot/rep/location
Tuber dry matter content (%)	TDM	The estimate of dried tuber in a forced air circulation oven at 700°C for 72 h	Expressed in % of the total tuber weight
Tuber yield per hectare	YTPH	The yield calculated from tuber weight per plot in terms of hectare	Expressed in t/ht of land

3.2.4. Data analysis

For qualitative traits, percentage of each cladistic (trait) observed in different accessions showing phenotypic deficiency or richness (number of phenotype per trait) and evenness (frequency of each phenotype) across accessions, were determined using MINITAB® Release 14.13 (Minitab, 1998).

The quanntitative data were subjected to statistical analysis using Statistical Analysis Computer Software (SAS version 9.0), Genstat (Rayne *et al*., 2012) and MINITAB® Release 14.13 (Minitab, 1998). Mean separation was made at 1% and 5% probability level using the LSD method.

3.2.4.1. Analysis of variance (ANOVA)

After teste of normality using Kolmogorov-Smirnov test, determination of the magnitude of reduction in experimental error by using alpha lattice design over RCBD design was computed employing relative efficiency estimation from both design and the computed relative efficiency indicated nearly similar precision (R.E = 1.02). Therefore, for the sake of simplicity, analysis of variance (ANOVA) which is used for partitioning the total variation, was done based on RCBD design using the following linear additive model following the general linear model (GLM) procedure of the SAS software:-

$P_{ijk}=\mu + \tau_i + \beta_{k(j)} + \pi_j + \xi_{ijk}$ Where,

$\mathbf{P_{ijk}}$= Phenotypic value of i^{th} treatment under j^{th} replication and k^{th} incomplete block

$\boldsymbol{\mu}$=Grand mean

$\boldsymbol{\tau_i}$= The effect of i^{th} treatments

$\boldsymbol{\beta_{k(j)}}$= The effect of incomplete block k within replication

$\boldsymbol{\pi_j}$= The effect of replication j

ξ= experimental error (pooled error)

Table 5 **:** ANOVA model for individual locations

Source of Variation	DF	SS	MS	Expected Mean
Replication	r-1	SSr	MSr	$\sigma^2_e + g\sigma^2 r$
Block within replication	r(b-1)	SSb	MSb	$\sigma^2_e + b\sigma^2 r$
Treatment	t-1	SSt	MSt	$\sigma^2_e + r\sigma^2_g$
Residual	r(t-b)-t+1	SSe	MSe	σ^2_e
Total	tr-1	-	-	-

Where, r = Number of replication; b = number of block; g = number of genotypes (treatment); SSr = Sum square of replication, SSt = sum square of treatment; SSe = sum square of error; MSr = mean square due to replication; MSt = mean square due to treatment; σ^2_e = environmental variance; σ^2_g = genotypic variance

Before computing the combined analysis, error variance homogeneity test was performed using Hartley`s test (F-max test) (Gomez and Gomez, 1984). There were only three error variances per traits (three locations) and hence, error variance for the test location were found to be homogeneous (F-ratio less than or equal to three times the error variances). In the combined location analysis of variance, locations were considered as random variable and genotypes were considered as fixed variables.

The combined analysis of quantitative trait data was done by using the following linear additive model: -

$$P_{ijks}=\mu + \tau_i + \beta_{k(j)(s)} + \pi_{j(s)} + L_s + (\tau xl)_{is}+\xi_{ijks}$$ Where,

$\mathbf{P_{ijks}}$ = Phenotypic value of i^{th} treatment (collection) under j^{th} replication at s^{th} location and k^{th} incomplete block within replication j and location s; $\boldsymbol{\mu}$ = Grand mean; $\boldsymbol{\tau}_i$ = the effect of i^{th} treatment; $\boldsymbol{\beta}_{k(j)(s)}$ = the effect of incomplete block k within replication j and location s; $\boldsymbol{\pi}_{j(s)}$ = the effect of replication j within location s; $\mathbf{L}_s$ = the effect of location; $(\boldsymbol{\tau xl})_{is}$ = the interaction effects between treatment and location; and $\boldsymbol{\xi}_{ijks}$ = pooled error.

Table 6 : ANOVA model for combined data over locations

Source of Variation	DF	SS	MS	Expected Mean
Location	l-1	SSl	MSl	$\sigma^2_e + g\sigma^2$ l
Replication within location	l(r-1)	SSrl	MSrl	$\sigma^2_e + gl\sigma^2$ r
Genotype	g-1	SSg	MSg	σ^2e + rσ^2g*l* + r*l*σ^2g
Genotype x Location	(*l*-1)(g-1)	SSgl	MSgl	σ^2_e+ rσ^2_{gl}
Residuals	l(g-1)(r-1)	SSE	MSe	σ^2_e
Total	gr*l*-1	-	-	-

Where, **r** = number of replication; **b** = number of blocks; **g** = number of genotype (treatment); **l** = location; **SSl** = Sum square of location; **SSg** = sum square of genotype; **SSe** = sum square of error; **MSl** = mean square due to location; **MSg** = mean square due to genotype; **MSgl** = mean square due to genotype by location; **MSe** = mean square due to error; $\boldsymbol{\sigma^2_e}$ = environmental variance; $\boldsymbol{\sigma^2_g}$ = genotypic variance; $\boldsymbol{\sigma^2_{gl}}$ =Variance for treatment by location interaction

3.2.4.2. Estimating phenotypic and genotypic variances

Estimation of environmental, genotypic and phenotypic variance components and their coefficients of variation per location and combined over locations were done based on the methods by Singh and Chaudhary (1985), and Allard (1960). Accordingly,

Phenotypic variance (δ^2p) per location = δ^2g + δ^2e where,

δ^2p = phenotypic variance; δ^2g = genotypic variance and

δ^2e = the environmental variance = error variance

Genotypic variance (δ^2g) per location = (MSg – MSe)/r

where, MSg = mean square of genotype,

MSe is mean square of error and r is the number of replication

Phenotypic Coefficient of Variation (PCV) per location = ($\sqrt{\delta^2}$p/m) x 100

where, PCV = phenotypic coefficient of variation;

m= population mean for the trait considered

Genotypic Coefficient of Variation (GCV) per location = ($\sqrt{\ }$ δ^2g/m) x 100

where, GCV = genotypic coefficient of variation

Genotypic variance (δ^2g) combined over location = (MSg – MSgl)/rl

Where, MSg = mean square of genotype,

MSgl is mean square due to genotype by environment interaction,

l=number of locations; r = number of replications

G x E interaction variance (δ^2gl**) combined over location= (**MSgl-MSe)/r

Where, MSgl = mean square due to genotype by environment interaction,

MSe = combined error means square (δ^2e)

Phenotypic variance (δ^2p) combined over location = δ^2g + (δ^2gl/l) + (δ^2e/rl)

Phenotypic coefficient of variance (PCV) **combined over location** = ($\sqrt{\delta^2}$p/m) x 100

Where, PCV = phenotypic coefficient of variation; δ^2p = phenotypic variance and

m= population mean for the trait considered

Genotypic coefficient of variation (GCV) combined over locations = ($\sqrt{\delta^2}$g/m) x 100

where,

GCV = genotypic coefficient of variation; δ^2g = genotypic variance

m = population mean for the trait considered

G x E interaction coefficient of variation (GECV) = ($\sqrt{\delta^2}$gl/m) x 100

Where,

δ^2gl = genotypic x environment variance; m = population mean for the trait considered

Broad sense heritability per location were estimated according to Allard (1960) as

Heritability per location (H^2) = (δ^2g/δ^2p) x 100 Where,

δ^2g = genotypic variance and; δ^2p = phenotypic variance.

Similarly, broad sense heritability combined over locations were estimated according to Allard (1960) as:

H^2 = (δ^2g/δ^2p) x 100 Where, δ^2p = δ^2g + (δ^2gl/l) + (δ^2e/rl)

Expected genetic advance under selection assuming the selection intensity at 5% was also computed following Allard (1960) as:

GA = (K) (δp) (H^2) where,

GA = expected genetic advance; K= selection differential that varies depending up on the selection intensity and stands at 2.056 for selecting 5% of the genotypes.

δp = phenotypic standard deviation and, H^2= heritability in broad sense

Genetic advance as percent of mean was obtained as;

GA (% of mean) = (GA/m) x 100 Where, GA= genetic advance; m = population mean for the trait considered

3.2.4.3. Estimating phenotypic and genotypic correlation coefficients

Correlation coefficient measures the mutual relationship between various traits and determines the component character on which selection can be based for improvement in yield (Singh and Chaudhary, 1985). The phenotypic and genotypic coefficients of correlation for the traits measured in the study were estimated using Pearson Simple Correlation which deals with association between any two traits. Phenotypic correlation is the observable correlation between two variables, which include both genotypic and environmental effects, and genotypic correlation is the inherent association between two variables.

Phenotypic and genotypic correlation coefficients between two traits were determined by using PROC CANDISC procedure of SAS software following the variance and covariance components (Singh and Chaudhary, 1985; Sharma, 1998) .

$$r_{P.xy} = \frac{COVp(X,y)}{\sqrt{(\sigma 2px\ \sigma 2py}}$$ where;

$COV_{p\,(x,y)}$ = Phenotypic covariance between traits X and Y

$r_{P.xy}$ = Phenotypic correlation coefficient between traits X and Y

$\sigma^2 p_x$ = Phenotypic variance of trait X; $\sigma^2 p_y$ = Phenotypic variance of trait Y

$$r\,g_{xy} = \frac{Covg(x,y)}{\sqrt{(\sigma 2gx\ \ \sigma 2gy}} \qquad \text{where,}$$

$COV_{g\,(x,y)}$ =Genotypic covariance between traits X and Y

rg_{xy} = Genotypic correlation coefficient between traits X and Y

$\sigma^2 g_x$ = Genotypic variance of trait X; $\sigma^2 g_y$ = Genotypic variance of trait Y

Phenotypic and genotypic correlation coefficients were tested for their significance using the formula suggested by Robertson (1959), using the t- table at (g-2) degrees of freedom at 5% and 1% level of significance; g is the number of genotypes (treatments) used in the study.

$$t = \frac{rpxy}{SEpxy} \qquad \text{and} \quad t = \frac{rgxy}{SEgxy} \qquad \text{respectively, where}$$

SEpxy and SE_{gxy} = Standard error for phenotypic and genotypic correlation respectively

$$SEpxy = \sqrt{\frac{(1-rpxy)2}{2Hx\ \ Hy}} \qquad \text{and} \quad SE_{gxy} = \sqrt{\frac{(1-rgxy)2}{2Hx\ \ Hy}}$$

Where, H_x and H_y are heritability estimate for traits x and y

3.2.4.4. Cluster analysis

Clustering was performed to group samples according to woreda of collections and populations into homogenous classes by average linkage methods and number of cluster was chosen by examining Pseudo F statistics and Pseudo t^2 statistics using SAS software version 9.0. Genetic distance between clusters was calculated using the generalized Mahalanobis's D^2 statistics:

$$D^2_p = (X_i - X_j)^1\ S^{-1}\ (X_i - X_j)$$

where, D^2_p = Total generalized distance based on p characters, X_i and X_j are the p mean vectors of accessions i and j, respectively. S = p x p pooled error variance- covariance matrix (Mahalanobis, 1936).

D^2 value for pairs of clusters was considered as the calculated value of Chi-square (X^2) and was tested for significance at the required level of probability against the tabulated values of X^2 for p degrees of freedom, where p is the number of characters considered (Singh and Chaudhary, 1985). SAS software version 9.0 was used.

Principal components analysis (PCA) and factor analysis was conducted for combined and standardized woreda mean using MINITAB® Release 14.13 statistical software (Minitab, 1998).

3.3 Molecular Genetic Diversity Assessment

3.3.1 Leaf sample collection and DNA extraction

In total, young leaf tissue from 287 individual plants (1–3 individual plants per accession) representing the 12 populations (***Table 3***) were collected and silica gel dried in a separate zip-lock bag. Individual plants resampling was done depending up on morphological heterogeneity and abundance of the samples in the way to balance the number of samples in each population. The dried leaf samples were transported to the Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden, for genetic analysis. Genomic DNA extraction was made using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol as described in Mulatu Geleta *et al*. (2012). The quality and quantity of DNA extracts were determined using agarose gel electrophoresis (1%) (***Figure 2***) and NanoDrop® ND-1000 Spectrophotometer (Saveen Warner, Sweden), respectively.

3.3.2 EST-SSR primers design and screening

A total of 3,263 *Plectranthus barbatus* EST sequences, retrieved from the National Centre for Biotechnology Information (NCBI) public database (http://www.ncbi nlm.nih. gov /nucest /?

term=Plectranthus+barbatus) were used for mining SSRs using WebSat (Martins *et al*., 2009; http://purl.oclc.org/NET/websat/). After excluding redundant, overlapping and short sequences, about 300 sequences containing two to six SSR motives were identified. However, due to time and cost constraints, only 40 of the sequences were selected for use in designing 40 pairs of primers using Primer3 primer designing program (Koressaar and Remm, 2007; Untergasser *et al*. 2012; http://bioinfo.ut.ee/primer3-0.4.0/).

The designed primer-pairs were tested for their amplification of the target genomic regions on 36 representative DNA samples and the polymerase chain reaction (PCR) products were electrophoresed on 1.5% agarose gel containing gel-red and visualized using Saveen Werner AB UV camera equipped with SSM930CE Sony Black and white Monitor. The size of amplified products was estimated by loading GeneRuler 50 bp DNA ladder together with the samples on separate lanes. Under optimized PCR conditions, 20 of the 40 primer pairs consistently amplified their targets and hence were selected for use on the whole samples (***Table 16***).

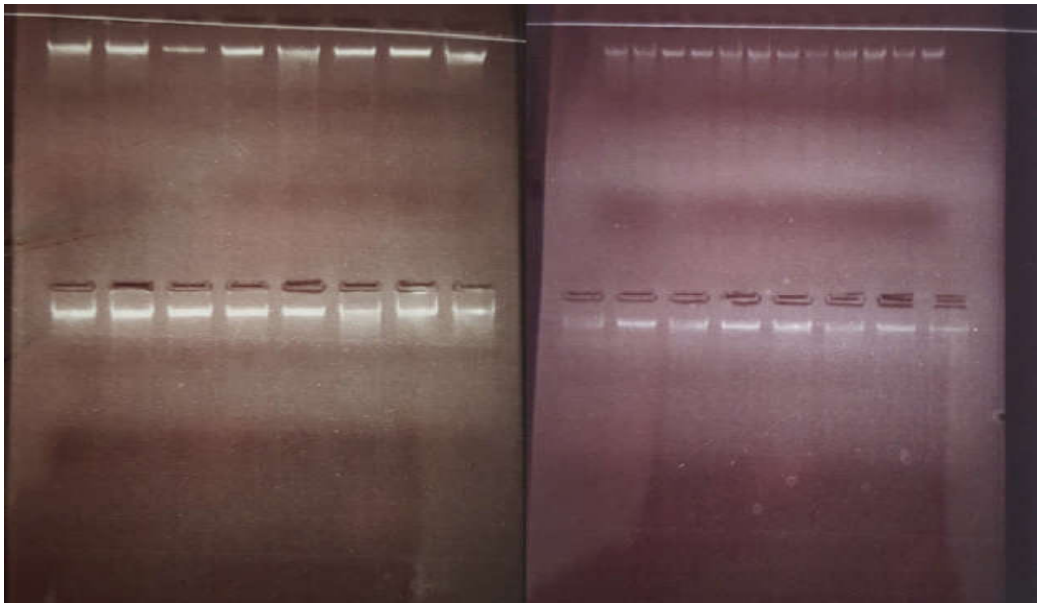

Figure 2: Extracted genomic DNA samples run on 1% agarose gel for quality checking

3.3.3 Pre-amplification and modification of the primers

To be cost effective and to improve the quality of amplified products, the sequences of the 20 primer-pairs were modified as follows: (1)- a 18-bp universal M13 primer sequence (5′-TGTAAAACGACGGCCAGT-3′) was added as a common tail to the 5′ end of all forward primers following Oetting *et al* (1995); and (2)- a PIG-tail sequence of 5′-GCTTCT-3′ was added to 5′ end of the reverse primers to promote full adenylation (Brownstein *et al*., 1996). A 5′ fluorescently labeled universal M13 primer, with HEX™ or 6-FAM™ was used as third primer in each PCR amplification.

3.3.4 PCR amplification

PCR was carried out using 96-well plates with 25 µl reaction volume [1 X reaction buffer, 1.5mM $MgCl_2$, 0.3mM dNTPs, 0.08µM M13-tailed forward primer, 0.3µM pig-tailed reverse primer, 0.3µM 6-FAM or HEX labeled M13 primer, 1U Dream Taq DNA Polymerase, and 25 ng template DNA]. A mix of all the components, except genomic DNA, was included as a negative control. Amplification was performed using GeneAMP PCR 9700 thermo cycler (Applied Biosystems Inc. USA) according to the following touch down (TD) protocol: initial 15min denaturation at 95°C, followed by 35 cycles of denaturation for 30sec at 94°C, annealing for 30sec at optimized annealing temperature for each primer-pair, primer extension for 30sec at 72°C. This was followed by 8 cycles that contain denaturation at 94°C for 30sec, annealing at 53°C for 45sec, and primer extension at 72°C for 45sec. A 10min additional primer extension at 72°C and final extension at 60°C for 30 min were the last steps of each PCR reaction. The amplified products were kept at 4°C until electrophoresis.

3.3.5 Capillary electrophoresis and fragment size scoring

The PCR products were multiplexed into panels based on fragment sizes of the SSRs and the florescence label of the M-13, and diluted 25x using Millipore water. Finally, 0.7µl of multiplexed and diluted PCR products, 1.9µl Hi-Di formamide and 0.3µl size standard (GenScan$_{Tm}$ 600 LIZ® size standard from Life Technologies) were mixed and used for capillary electrophoresis using Genetic Analyzer 3500 (Applied Biosystems) at SLU, Department of Plant Breeding, Alnarp, Sweden.

Finally, peak identification and fragment sizing was conducted with GeneMarker® V2.6.0 software (SoftwareGenetics®) depending on the internal size standard, GenScan^{Tm} 600 LIZ® size std. Default settings, with 200 threshold intensities, were used for identification and the final acceptance of the peaks was made after checking bin sharpness of each band (***Figure 3, b***). Finally, the fragment sizes of each allele at each locus were exported to excel for statistical analysis.

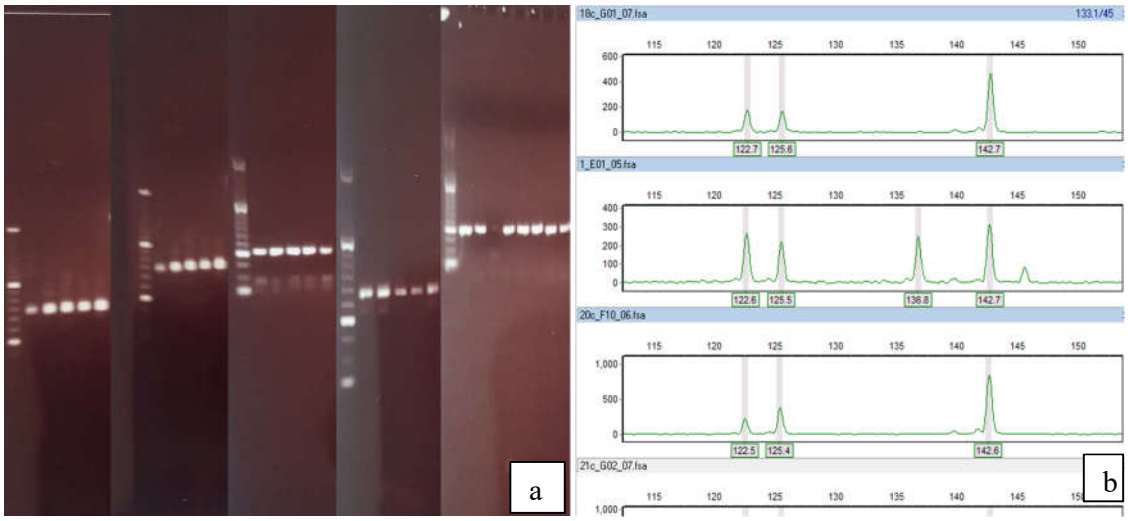

Figure 3 : A sample picture representing primer screening (**a**) and peak scoring (**b)**

3.3.6 Statistical analysis for molecular data

3.3.6.1 Population genetic diversity indices

Locus based diversity indices such as major allele frequency (MAF), number of alleles (NA), number of genotypes (NG), gene diversity (GD), and polymorphic information content (PIC) were determined using PowerMarker ver. 3.25 (Liu and Muse, 2005). PIC was used to estimate the discriminatory power of the microsatellite loci and computed on the bases of the formula, $PIC = 1 - \Sigma P^2_i$, where P^2_i represents the sum of the i^{th} allelic frequency of each microsatellite locus for the genotypes (Botstein *et al*, 1980 and Anderson *et al*, 1983). The number of different alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) (Weir, 1996), Shannon's Information Index (I), and estimate of deviation from Hardy-Weinberg equilibrium (HWE) over the entire population, and population genetic diversity indices: Na, Ne, percentage of polymorphic loci (PPL), Ho, He, I, Nei's gene diversity (uHe) over the entire loci were computed using GenAIEx ver. 6.501 (Peakall and Smouse, 2012).

To detect the effects of null alleles and other genotyping errors such as stuttering and large allele dropout for each locus and population, we used Micro-Checker ver. 2.2.3 (van Oosterhout, 2004) at 95% confidence interval, 1000 iterations and maximum expected allele size of 350bps and the frequencies (using EM algorithm as described by Dempster *et al*. (1977)) were determined using FreeNA programe (Chapuis & Estoup, 2007). ML-NULLFREQ (Kalinowski & Taper, 2006), a maximum likelihood estimator of null allele frequency, were used to cross check the results of FreeNA and to test the HW-equilibrium in terms of heterozygote deficiency.

To control the correlation between observed allelic diversity and sample size of populations, rarified allelic richness (Ar) and private rarified allelic richness (Ap) were estimated using HP-Rare 1.1 (Kalinowski, 2005). Pairwise linkage disequilibrium for all pairs of loci, was computed

assuming HW-equilibrium and collapsing less frequent alleles (Lewis and Zaykin, 2001), using Genetic Diversity Analyzer (GDA) Ver 1.0 (d16c).

3.3.6.2 Analysis of molecular variance and population differentiation

To evaluate population genetic structure, population average pairwise differences: average number of pairwise differences between (**PiXY**) and within (**PiX**) population, and corrected average (**PiXY-(PiX+PiY)/2**) were computed using Arlequine 3.5 (Excoffier *et al.,* 2005). Nei's pairwise genetic distance (Nei, 1972) over the entire loci were computed using PopGene ver. 1.31 (Frances, 1999).

To analyze the distribution of gene diversity and estimate the components of variances of the populations, hierarchical analysis of molecular variance (AMOVA) was computed using Arlequin 3.5 (Excoffier *et al.,* 2005). Similarly, population differentiation tests: Wrights, (1989) fixation indices (F_{IS}, F_{ST} and F_{IT}) and pairwise F_{ST} were computed using GenAIEx ver. 6.501 (Peakall and Smouse, 2012), and significance was tested based on 1000 bootstraps. To minimize the high frequencies of null alleles for some loci and establish a more accurate estimation of F_{ST}, F_{ST}* in this case (using ENA correction method), we used FreeNA programe (Chapuis & Estoup, 2007).

3.3.6.3 Cluster analysis

Individual plants unweighted neighbour joining cluster analysis (Sokal & Michener, 1958) and bootstrapping was generated using Dissimilarity Analysis and Representation for windows (DARwin) 6.0.13 (Perrier and Jacquemoud-Collet, 2006). The resulting tree was viewed using FigTree v1.4.3 (Andrew, 2016). To have a better picture of the clustering pattern, Nei's standard genetic distance ($D_{ST,}$ corrected) (Nei, 1972) based populations UPGMA (Sneath and Sokal, 1973) and NJ (Saitou and Nei, 1978) trees were drawn using POPTREE2 (Takezaki *et al*., 2010)

and significance was tested based on 1000 bootstraps (Felsenstein, 1985). The resulting tree was displayed using TreeView (Win 32) 1.6.6 program (Page, 2001). Gene flow (Nm) among populations was estimated using the formula, Nm = 0.25(1 Fst)/Fst (Slatkin and Barton). Principal co-ordinate analysis (PCoA) was performed using GenAIEx 6.05 program (Peakall and Smouse, 2012) to show the pattern of genetic differentiation of the populations.

3.3.6.4 Analysis of population structure and bottleneck

The pattern of population structure and detection of admixture were inferred using a Bayesian model-based clustering algorithm implemented in STRUCTURE ver. 2.3.4 (Pritchard *et al*, 2000; Falush *et al* 2003). To determine the most appropriate number of populations (K), a burn-in period of 50,000 was used in each run, and data were collected over 500,000 Markov Chain Monte Carlo (MCMC) replications for K=1 to K=12 using 20 iterations for each K. The optimum K value was predicted following the simulation method of Evanno *et al*. (Evanno *et al* ,2005) using the web-based STRUCTURE HARVESTER ver. 0.6.92 (Earl and vonHoldt, 2012) (http://www.taylor0.biology.ucla.edu/structureHarvester/). Bar plot for the optimum K was determined using Clumpak beta version (http://www.clumpak.tau.ac.il/) (Kopelman *et al*., 2015).

Since our material is one of the highly neglected crops, we tried to assesses the potential reduction in population size (bottleneck effect) using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996). The three excess heterozygosity tests: sign, standardized differences and Wilcoxon sign-rank tests (Cornuet and Luikart, 1996) were used to determine recent reduction of effective population size (sampling locations with significant heterozygote excess) under the three possible mutation models: the infinite allele (IA), stepwise mutation (SM) and two-phased mutational (TP) models. Significance of heterozygosity excess as well as mode-shift indicator tests (Luikart *et al*., 1998) were conducted using 1000 iterations. Two-tailed significance

estimation of heterozygosity excess was used for Wilcoxon test under all mutation models. Variance among multiple steps of 12 and a proportion of multi-step mutation (IAM) of 10% (single step mutation of 90%), as suggested by Cornuet and Luikart (1996) were employed.

3.4. Cytogenetic Analysis

3.4.1. Generation of young roots and collection of root tips

Representative random seeds and tuber samples were planted in soil filled pots in the glasshouse at College of Natural Sciences, Addis Ababa University, Ethiopia. Young stems were layed horizontally, covered with moist soil and regularly watered to generate new roots from the stem nodes. Young clean roots were collected into a 5ml tube for next steps.

3.4.2. Mitotic metaphase chromosome preparation

Root tips pretreatment, fixation, and maceration were performed following the method of Singh (2003) with minor modifications. Pretreatment step was used to arrest chromosomes at metaphase stage such that one could easily observe and study the chromosome morphology. With this concept, the collected root tips were pre-treated with ice-cold water for 24 hours, or alternatively treated with 8-hyrdroxyqulinone (0.003M), at room temperature for 5 hours. Pre-treatments in each method was followed by fixation in Carnoy's I solution/fixative (3-parts absolute ethanol **:** 1-part glacial acetic acid). The fixative was prepared afresh for every batch of the trial. The fixed roots were kept at 4 C for 24 h or longer before enzyme maceration. After rinsing for 2-3 times with distilled water at five-minutes intervals, the roots were macerated with 4% cellulase and 4% pectinase solution at 37 C for about 5 hrs so as to make the root tips soft. After maceration, the enzyme was removed; root tips rinsed in distilled water, and two to three root tips were pipetted onto clean glass slide and mashed in one or two drops of fresh fixative. To avoid clumping of cells and hence to get best spread of cells, the slides were held

inclined at 45 orientation, and air was blown over the slides. The slides were then allowed to air dry and stored until needed for staining.

Staining was done by immersing the slides in staining jar containing Giemsa stain (in phosphate buffer, pH 6.8) for 15-30 minutes or more until satisfactory staining was obtained. The slides were then rinsed in distilled water, air-dried, and mounted under a 22x55mm cover slip in Dibutylphthalate Polystyrene Xylene (DPX) mounting medium (Kifle Dagne, and Heneen, 1992).

3.4.3. Chromosome counting and determination of ploidy level

Cells with good chromosome spreads were selected and photomicrographs were taken under x100 objective of a cannon camera fitted microscope. Chromosome counts were made for a number of cells with well spread chromosomes.

The ploidy level was estimated by comparing the count we obtained to the basic numbers reported so far in the literature for some members/species of the genus *Plectranthus*.

4 RESULTS

The main results obtained regarding agronomic and non-agronomic morphological traits based, and microsatellites (EST-SSRs) markers system, as well as cytogenetic analysis of Ethiopian potato accessions from varied agro-ecologies in Ethiopia are presented below.

4.1. Agro-morphological Traits-based Patterns of Variations

Qualitative and quantitative agronomic and non-agronomic morphological traits vaiations in 174 Ethiopian potato accessions (samples) were investigated during the main growing seasons of the crop (May – October) in 2014 and 2015. The main results of the study are presented below under separate headings.

4.1.1. Qualitative traits distribution

Several morphotypes were observed for a number of qualitative agronomic and non-agronomic morphological traits including leaf, stem, and tuber characteristics (***Appendix 1, Figures 1-5***). Percentage of each cladistics (trait) observed in different accessions is summarized in the cross-tabulation, showing phenotypic deficiency or richness (number of phenotype per trait) and evenness (frequency of each phenotype) across accessions (***Table 7***). In general, most of the morphotypes are proportionally distributed but some are rare. Details are presented below.

4.1.1.1. Leaf characteristics

Three leaf related characteristics that include leaf color, leaf arrangement and leaf shape, were recorded. Regarding leaf color, pale purple was the most frequent (58.65 %,) phenetic character followed by green (28.85 %,), deep green (9.13%) and deep purple (3.40%). Of the tested accessions, 90.63% had opposite, 7.29 % alternate, and 2.08% whorl leaf arrangement patterns.

Regarding leaf shape, three phenotypic classes, triangular, ovate and round, were observed in the frequency of 69.00 %, 27.00 %, and 4.00 %, respectively (***Table 7***).

4.1.1.2. Stem characteristics

A total of five stem related characteristics that included stem color, stem hairiness, stem spot, stem spot color and stem growth habit were observed. Accessions with pale purple stems were the most frequent (71.71 %) followed by green (24.39 %) and deep purple (3.90 %). Accessions with sparsely distributed stem hairs were dominant in frequency (66.99%) followed by accessions with dense stem hairs (28.23%). Accessions with no stem hair were rare (4.78%). It has been observed that 88.87% of the total accessions have spots on stem, with the majority of them colored black (85.29%) and few (14.71%) colored purple. For stem growth habit, three phenotypic classes (erect, open and intermediate) growth patterns were recorded. Most accessions (73.11%) exhibited erected (upright and longer) stems while the rest showed open (stunted and horizontal) (19.81%) and intermediate (7.08%) growth patterns (***Table 7***).

4.1.1.3. Tuber characteristics

Four tuber related phenetic characters, tubers skin color, tubers texture, tubers shape, and tubers hair, were recorded. Regarding tuber skin color, accessions with creamy purple were the dominant (63.30%) followed by creamy white (26.61%), creamy (5.96%), and purplish red (4.13%). Moreover, most of the accessions (54.87%) had moderately ringed (rough) tuber texture followed by highly ringed (too rough) (41.18%) and smooth to low ringed (4.87%). With regards to tuber shape, most of the accessions were elongate type (68.12%). Of the remaining, 30.13% and 1.75% had round and oval shapes, respectively. Similarly, 56.79% of the total accessions had tubers with medium tuber hair followed by sparsely hairy (31.28%) and densely hairy (11.93%) types (***Table 7***).

Table 7: Groups of traits, specific qualitative morphological traits, their scores and equivalent phenetic characters, and frequency and percent coverage in each score during the analysis of 174 Ethiopian potato accessions from Ethiopia

Group character	Individual trait	Score	Phenetic characters	Number of accessions (frequency)	Relative % of each phenotype	% of accessions possessing a phenotype
Leaf characteristics	Leaf colour	1	Deep purple	7	3.40	4.02
		2	Pale purple	122	58.65	70.11
		3	Green	60	28.85	34.48
		4	Deep green	19	9.13	10.92
	Leaf arrangement	1	Opposite	174	90.63	100
		2	Alternate	14	7.29	8.05
		3	Whorl	4	2.08	2.30
	Leaf shape	1	Ovate	54	27.00	31.03
		2	Triangular	138	69.00	79.31
		3	Round	8	4.00	4.60
Stem characteristics	Stem colour	1	Deep purple	8	3.90	4.60
		2	Pale purple	147	71.71	84.50
		3	Green	50	24.39	28.74
	Stem spot	1	Present	174	88.78	100
		2	Absent	22	11.22	12.64
	Stem spot colour	1	Purple	30	14.71	17.24
		2	Black	174	85.29	100
	Stem hair	1	Absent	10	4.78	5.75
		2	Sparse	140	66.99	80.50
		3	Dense	59	28.23	33.91
	Stem growth habit	1	Open	42	19.81	24.14
		2	Erect	155	73.11	89.08
		3	Ambiguous	15	7.08	8.62
Tuber characteristics	Tuber skin colour	1	Cream	13	5.96	7.47
		2	Creamy white	58	26.61	33.33
		3	Creamy purple	138	63.30	79.31
		4	Purple	9	4.13	5.17
	Tuber texture	1	Highly ringed	91	41.18	52.30
		2	Moderately ringed	124	54.87	71.26
		3	Little ringed	11	4.87	6.32
	Tuber shape	1	Elongated	156	68.12	89.66
		2	Round	69	30.13	39.66
		3	Oval	4	1.75	2.30
	Tuber hair	1	High (dense)	29	11.93	16.67
		2	Medium	138	56.79	79.31
		3	Low (sparse)	76	31.28	43.68

The traits were recorded on plot (accession) bases

4.1.2. Variation in quantitative agro-morphological traits

4.1.2.1. Mean, range and analysis of variance (ANOVA)

Summary of the ranges and the means together with their standard errors, obtained on the basis of quantitative traits data combined over the experimental locations, are shown in ***Table 9***. In general, the Ethiopian potato accessions considered in this study showed wide ranges of variability and wide ranges between the maximum and minimum mean values in most of the 16 quantitative traits considered. Accordingly, days to 50% flower initiation (DFI) revealed the widest range (112.90 - 165.10), about seventy folds of the minimum range, with average mean performance value of 148.18 days, followed by plant height (PH) (23.50 - 58.50), number of tubers per hill (TN) (12.11 - 18.37), days to 50% flowering (DF) (140.44 - 174.69), and flower length (FL) (8.06 - 28.45), each with 42.03 cm, 26.71 tubers, 160.75 days, and 11.75 cm in mean performance, in that order. On the other hand, tuber diameter (TD) showed the least range (1.39 - 2.13cm), with a mean performance value of 1.79 cm, followed by tuber weight per hill (TW) (0.07 - 2.07), stem girth (SG) (1.78 - 4.46), and number pf plant per hill (NPPH) (1.12 - 3.89) with mean trait values of 1.01 kg, 3.4 cm, 2.18 plants, in that order. Tuber yield per hectare of land (YTPH) revealed a moderate range (0.14t/ha to 4.14t/ha), about five-folds of the minimum range, with average yield of around 2.01t/ha.

ANOVA, computed using data combined over experimental locations, is presented in ***Table 8***. Mean squares of all the traits considered showed a highly significant ($P< 0.001$) variation among the accessions. Similarly, all the traits, except tuber number per hill and tuber dry matter contents per hill, showed a significant variation among the accessions per location (***Appendix 5, Tables 1-3***). However, all the tested traits, except flower length, showed a non-significant variation for treatment-location interactions. A reasonably high coefficient of genetic

determination (R^2) was recorded for half of the traits, including tuber yield per hectare of land and yield contributing traits, which presented values greater than 0.90. The coefficient of variations (CV) seems higher but is within acceptable range. Tuber yield per hectare of land and days to 50% flower initiation showed the highest and lowest CV, respectively.

4.1.2.2. Analysis of components of variance

Both phenotypic (δ^2_p) and genotypic (δ^2_g) variance estimates showed a wide range of variation (0.01 in tuber diameter to 62.52 or 62.25 in days to 50% flower initiation). However, estimate of variance due to environment (δ^2_e) revealed a narrow range (0.02 in tuber diameter and tuber weight per hill to 8.48 in number of tubers per hill) and lower level of variation. Similarly, all the quantitative traits, except flower length (13.86), showed negligible (nearly zero) variation due to genotype environment interaction (δ^2_{ge}). Six traits, including number of primary branches per plant, number of plants per hill, flower length, number of tubers per hill, tuber weight per hill and yield of tubers per hectare of land scored high phenotypic (PCV) and genotypic (GCV) coefficients of variation. The remaining traits exhibited medium to low estimates.

In general, estimates of both PCV and GCV showed a wide range of variations (4.10% and 3.4%, respectively in tuber dry matter contents to 43.56% and 43.16%, respectively in yield of tubers per hectare of land). In addition, PCV estimate was slightly higher than or equal to their corresponding GCV values in all the traits, except flower length, which exhibited a considerably higher PCV than GCV. In the same way as in variance due to genotype-environment interaction, all the traits except, flower length (31.77%) showed a negligible variation concerning genotype environment coefficients of variation (GECV) (***Table 9***).

4.1.2.3. Estimates of heritability in broad sense and genetic advance

Among the traits studied, estimate of heritability in broad sense (Hb%) revealed a wide range of variation (16.55% in flower length to 99.52% in days to 50% flower initiation). Moreover, more than half of the traits (68.75%) exhibited high estimate (>80%) of such parameter and all the remaining traits showed medium to moderately high estimates except, flower length which showed a lower estimate (***Table 9***).

Similarly, estimates of genetic advance (GA) revealed a wider variation (0.18 in tuber diameter to 16.56 in leaf diameter) among the traits with eventually wider ranges in genetic advance as a percent of traits mean (GA as % mean) (5.80 in tuber dry matter contents to 87.96 in tuber weight per hill) (***Table 9).***

Table 8 : Analysis of Variance (ANOVA) computed using the 16 quantitative traits data combined over the three experimental locations

Variables	Loc (***2***)	Rep(Loc) (***3***)	Trt (***173***)	Loc*Trt (***346***)	MSE (***519***)	CV(%)	R^2
NPB	1.83*	0.60	13.15***	0.32	0.48	13.36	0.93
SG	0.54*	1.27***	0.90***	0.07	0.12	10.21	0.80
NPPH	0.46*	0.41*	1.26***	0.06	0.12	16.02	0.84
IL	3.17**	2.29**	1.75***	0.23	0.46	12.80	0.69
PH	502.6***	344.88***	113.44***	4.78	9.31	7.28	0.87
LL	1110.71***	514.38***	5.74***	1.89	2.65	13.01	0.85
LD	162.09***	72.72***	0.55***	0.19	0.28	13.20	0.87
DFI	19.59**	3.05	309.00***	1.48	2.21	1.00	0.98
DF	13.34*	3.02	225.34***	1.68	3.36	1.14	0.97
FL	41.88***	10.25**	31.79***	20.31***	1.88	11.68	0.93
TL	12.22**	1.41	7.96***	1.35	2.32	9.97	0.68
TD	0.13**	0.03	0.09***	0.02	0.03	9.41	0.68
TN	0.10	1.88	220.68***	6.29	10.38	12.05	0.91
TW	0.19**	0.02	0.91***	0.01	0.03	17.81	0.93
TDM	0.12	1.34	4.31***	1.07	1.86	6.26	0.59
YTPH	0.49*	0.07	3.64***	0.06	0.13	17.87	0.93

*** (significant at p<0.05) ** (highly significant at p<0.01) *** (highly significant at p<0.001)**

NPB (number of primary branches per plant), SG (stem girth), NPPH (number of plants per hill), IL (internode length), PH (plant height), LL (leaf length), LD (leaf diameter), DFI (days to flower initiation), DF (days to 50% flowering), FL (flower length), TL (tuber length), TD (tuber diameter), TN (number of tubers per hill), TW (tuber weight per hill), TDM (tuber dry matter contents), YTPH (tuber yield per hectare of land), numbers in parenthesis represent degree of freedom

Loc = Location; Rep = Replication within location; Trt = Treatment (Accessions); Loc*Trt = Location Treatment interaction; MSE = Mean square error; CV = Coefficient of Variation; R^2 = coefficient of genetic determination

Table 9: Estimates of trait mean, range, variance components, heritability, and genetic advance computed using data from the 16 quantitative traits combined over the three experimental locations

Traits	Combined Trait Mean	Range	δ^2e	δ^2g	δ^2ge	δ^2p	GCV (%)	PCV (%)	GECV(%)	Hb(%)	GA	GAM
NPB	5.19 ± 1.64	1.57 - 9.57	0.41	2.60	0.00	2.67	31.07	31.47	0.00	97.44	3.27	63.05
SG	3.40 ± 0.46	1.78 - 4.46	0.10	0.18	0.00	0.20	12.48	13.04	0.00	91.53	0.83	24.54
NPPH	2.18 ± 0.52	1.12 - 3.89	0.09	0.25	0.00	0.27	23.04	23.72	0.00	94.34	1.00	46.01
IL	5.32± 0.60	3.68 - 6.83	0.36	0.28	0.00	0.34	9.97	10.98	0.00	82.35	0.99	18.59
PH	42.03± 5.01	23.50 - 58.50	7.24	22.15	0.00	23.36	11.23	11.53	0.00	94.83	9.42	22.48
LL	12.56 ± 1.34	5.00 - 16.40	2.30	0.69	0.00	1.07	6.64	8.28	0.00	64.29	1.37	10.95
LD	4.05 ± 0.43	1.89 - 5.55	0.24	0.06	0.00	0.10	6.09	7.87	0.00	60.00	0.39	9.70
DFI	148.18 ± 8.11	112.90 - 165.10	1.88	65.21	0.00	65.52	5.44	5.45	0.00	99.52	16.56	11.16
DF	160.75 ± 6.70	140.44 - 174.69	2.60	44.31	0.00	44.74	4.14	4.16	0.00	99.03	13.62	8.47
FL	11.75 ± 1.95	8.06 - 28.45	1.98	1.44	13.86	8.70	10.24	25.17	31.77	16.55	1.00	8.56
TL	15.28 ± 1.31	12.11 - 18.37	1.88	1.28	0.00	1.59	7.40	8.26	0.00	80.33	2.08	13.64
TD	1.79 ± 0.14	1.39 - 2.13	0.02	0.01	0.00	0.01	5.59	6.45	0.00	75.00	0.18	9.95
TN	26.71 ± 6.72	9.96 - 44.54	8.48	43.28	0.00	44.69	24.61	25.01	0.00	96.84	13.31	49.80
TW	1.01 ± 0.44	0.07 - 2.07	0.02	0.19	0.00	0.19	43.16	43.53	0.00	98.28	0.89	87.96
TDM	21.80 ± 0.94	19.06 - 24.24	1.50	0.55	0.00	0.80	3.40	4.10	0.00	68.75	1.26	5.80
YTPH	2.01 ± 0.88	0.14 - 4.14	0.10	0.75	0.00	0.77	43.09	43.56	0.00	97.83	1.76	87.62

δ^2e = environmental variance; δ^2g= genotypic variance; δ^2ge = variance due to genotype environment interaction; δ^2p= phenotypic variance; GCV (%)= Genotypic coefficients of variation; PCV (%) = phenotypic coefficients of variation; GECV (%) = genotype environment interaction coefficients of variation; Hb(%) = Broad sense Heritability; GA = Genetic Advance; GAM = Genetic advance as percent of mean

4.1.2.4. Analysis of correlation coefficients

Analysis of correlation coefficients was performed to find out the inter-relationship between the studied pairs of traits. In this regard, genotypic and phenotypic correlation coefficients between yield of tubers per hectare of land and all the remaining traits are presented below:

Accordingly, yield of tubers per hectare of land showed a highly significant ($p<0.001$) and positive genotypic correlation with number of primary branches per plant (0.69), tuber length per hill (0.31), number of tubers per hill (0.81), tuber weight per hill (1.00), and significant ($p<0.05$) positive correlation with tuber dry matter contents (0.19), whereas, the correlation with all the remaining traits were non-significant. In addition, the extents of correlation sound higher ($r>0.5$) for tuber weight per hill, number of tubers per hill, and number of primary branches per plant, in that order (***Table 10***).

Likewise, tuber yield per hectare showed a highly significant positive phenotypic correlation with tuber weight per hill (0.99), number of tubers per hill (0.76), number of primary branches per plant (0.66), tuber length (0.26) ($p<0.001$), number of plants per hill (0.08), tuber diameter (0.11), tuber dry matter contents (0.12) ($p<0.01$) and a negative but significant correlation ($p<0.05$) with days to 50% flowering (-0.08) (***Table 10***). Moreover, the first three traits, in the order of magnitude, revealed a strong correlation ($r>0.5$).

Table 10: Estimates of genotypic (*below diagonal*) and phenotypic (*above diagonal*) correlation coefficients computed using data from the 16 quantitative traits combined over the three experimental locations

Traits	NPB	SG	NPPH	IL	PH	LL	LD	DFI	DF	FL	TL	TD	TN	TW	TDM	YTPH
NPB	1	**0.15*** **	**0.05**	**0.09** **	**0.09** **	**0.04**	**-0.005**	**-0.06**	**-0.16*** **	**0.02**	**-0.002**	**-0.12** **	**0.87*** **	**0.66*** **	**0.32*** **	**0.66*** **
SG	0.18*	1	**0.00**	**0.15*** **	**0.31*** **	**0.21*** **	**0.11** **	**0.09** **	**0.05**	**0.09***	**-0.10** **	**-0.03**	**0.05**	**0.01** **	**0.06**	**0.004**
NPPH	0.05	-0.05	1	**0.16*** **	**0.05**	**-0.001**	**-0.04**	**-0.05**	**-0.02**	**-0.04**	**0.10** **	**0.07***	**0.06**	**0.08**	**-0.03**	**0.08** **
IL	0.13	0.11	0.12	1	**0.37*** **	**0.23*** **	**0.17*** **	**-0.18*** **	**-0.16*** **	**0.07***	**0.02**	**0.04**	**0.10** **	**0.06**	**-0.01**	**0.06**
PH	0.12	0.29**	0.02	0.37***	1	**0.44*** **	**0.37*** **	**-0.04**	**-0.04**	**0.09** **	**0.01**	**0.02**	**0.04**	**0.05**	**-0.002**	**0.06**
LL	0.08	0.31***	0.02	0.27**	0.42***	1	**0.89*** **	**-0.04**	**-0.03**	**0.04**	**-0.05**	**-0.05**	**0.01**	**-0.03**	**0.02**	**-0.02**
LD	0.002	0.18*	-0.05	0.12	0.3***	0.76	1	**-0.05**	**-0.04**	**-0.001**	**-0.06**	**-0.09** **	**0.001**	**-0.02**	**0.03**	**-0.01**
DFI	-0.06	0.12	-0.06	-0.22**	-0.03	-0.05	-0.1	1	**0.80*** **	**0.06**	**-0.03**	**0.07***	**-0.08***	**-0.02**	**-0.04**	**-0.02**
DF	-0.17*	0.07	-0.02	-0.22**	-0.04	-0.07	-0.08	0.82***	1	**0.05**	**-0.03**	**0.09** **	**-0.16*** **	**-0.08***	**-0.08***	**-0.08***
FL	0.04	0.13	-0.06	0.16*	0.16*	0.11	0.06	0.09	0.09	1	**-0.03**	**-0.01**	**0.01**	**0.03**	**0.05**	**0.03**
TL	0.01	-0.18*	0.13	0.08	0.05	0.04	0.05	-0.03	-0.03	-0.02	1	**0.40*** **	**0.04**	**0.27*** **	**-0.38*** **	**0.26*** **
TD	-0.13	-0.07	0.07	0.07	0.06	-0.03	-0.09	0.10	0.12	0.03	0.43***	1	**-0.14*** **	**0.11** **	**-0.54*** **	**0.11** **
TN	0.88***	0.06	0.06	0.13	0.06	-0.003	-0.01	-0.08	-0.17*	0.02	0.04	-0.15*	1	**0.76*** **	**0.34*** **	**0.76*** **
TW	0.69***	-0.01	0.08	0.09	0.08	-0.03	0.003	-0.02	-0.09	0.06	0.31***	0.12	0.81***	1	**0.12** **	**0.99*** **
TDM	0.41***	0.12	-0.02	-0.04	-0.02	-0.07	-0.02	-0.05	-0.12	0.06	-0.42***	-0.57***	0.44***	0.19**	1	**0.12** **
YTPH	0.69***	-0.01	0.07	0.09	0.08	-0.03	0.004	-0.02	-0.09	0.06	0.31***	0.11	0.81***	1.00***	0.19*	1

**** significant at p<0.05; ** Highly significant at p<0.01; *** Highly significant at p<0.001***

4.1.2.5. Principal components analysis (PCA)

PC analysis, conducted using the 15 mathematically unrelated quantitative traits revealed that the first six principal axes (eigenvalue ≥ 0.98**)** accounted for 76% of the total variation (***Table 11***). The first principal component (PC1) accounted for 20% of the total variance and had high contributing factor loadings from number of tubers per hill (-0.53), number of primary branches per plant (-0.52), tuber weight per hill (-0.44), and tuber dry matter contents (-0.34), in that order. The second PC axis accounted for 17.0% of the total variation and differentiated among accessions on the bases of leaf length (0.54), leaf diameter (0.50), plant height (0.41) and stem girth (-0.28). The third PC axis contributed 14.0% of the total variation and had high contributing factor loadings from tuber length (-0.52), and tuber diameter (-0.44). The fourth PC axis accounted for 12.0% of the total variation and differentiated accessions on the bases of days to 50% flower initiation (-0.56) and days to 50% flowering (-0.54). The fifth and sixth PC axes each accounted for 7.0% of the total variation and differentiated largely on the bases of leaf diameter (0.44), flower length (-0.56) (fifth), and number of plants per hill (-0.85) (sixth) (***Table 11***).

PCA loading plot again showed strong correlation among traits such as number of tubers per hill, tuber weight per hill, number of plants per hill, tuber dry matter contents and number of primary branches per plant and hence their contribution to the observed variation. Similarly, flower length, stem girth, internode length, plant height, leaf length and leaf diameter showed a close correlation and contribution. The remaining traits, tuber length, days to 50% flower initiation, days to 50% flowering and tuber diameter revealed a positive association and contribution to the variation (***Figure 4)***.

Table 11: Eigen values and extent of variation for corresponding 15 components of mathematically unrelated quantitative characters in 174 Ethiopian potato accessions

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15
NPB	-0.52	0.09	-0.02	-0.13	0.09	-0.01	0.08	-0.07	-0.15	0.06	-0.18	0.52	0.21	-0.27	0.49
SG	-0.12	-0.28	0.20	-0.20	-0.23	-0.13	0.47	-0.64	0.00	-0.33	0.04	-0.15	-0.01	-0.01	-0.04
NPPH	-0.02	0.04	-0.21	0.05	-0.26	-0.85	-0.33	-0.14	-0.01	0.11	-0.08	-0.01	-0.06	-0.04	0.00
IL	-0.14	-0.28	-0.25	-0.01	-0.44	-0.02	0.15	0.57	-0.36	-0.39	0.10	-0.08	-0.02	-0.07	0.05
PH	-0.12	-0.41	-0.09	-0.18	-0.19	-0.02	0.27	0.24	0.59	0.50	-0.11	-0.01	0.00	-0.05	-0.02
LL	-0.05	-0.54	-0.01	-0.14	0.29	-0.08	-0.20	-0.01	-0.14	-0.05	0.04	0.36	-0.03	0.64	-0.05
LD	-0.03	-0.50	0.01	-0.07	0.44	-0.02	-0.31	-0.03	-0.09	-0.02	0.07	-0.31	0.00	-0.59	0.02
DFI	0.10	0.16	0.30	-0.56	0.04	-0.11	0.00	0.20	0.01	-0.09	0.04	0.23	-0.64	-0.17	-0.09
DF	0.18	0.12	0.28	-0.54	0.02	-0.16	-0.07	0.21	0.00	-0.06	-0.01	-0.22	0.65	0.12	0.09
FL	-0.06	-0.10	0.04	-0.22	-0.56	0.44	-0.60	-0.25	-0.02	0.07	-0.11	0.03	0.00	-0.02	-0.02
TL	0.04	0.11	-0.52	-0.18	0.11	0.05	-0.13	-0.07	0.54	-0.53	0.17	0.18	0.10	-0.07	-0.04
TD	0.19	0.06	-0.44	-0.31	-0.02	0.07	0.17	-0.20	-0.35	0.43	0.53	0.08	0.06	-0.07	-0.07
TN	-0.53	0.15	-0.05	-0.11	0.10	-0.01	0.00	0.04	-0.11	0.03	-0.13	-0.01	0.14	-0.04	-0.79
TW	-0.44	0.19	-0.22	-0.23	0.14	0.06	-0.06	-0.02	0.02	0.05	0.01	-0.58	-0.29	0.34	0.33
TDM	-0.34	0.03	0.39	0.20	-0.11	-0.07	-0.15	0.08	0.21	0.01	0.77	0.05	0.05	0.01	0.03
Eigenvalue	**2.97**	**2.48**	**2.02**	**1.86**	**1.11**	**0.98**	**0.83**	**0.72**	**0.55**	**0.51**	**0.34**	**0.23**	**0.17**	**0.15**	**0.08**
Proportion	**0.20**	**0.17**	**0.14**	**0.12**	**0.07**	**0.07**	**0.06**	**0.05**	**0.04**	**0.03**	**0.02**	**0.02**	**0.01**	**0.01**	**0.01**
Cumulative	**0.20**	**0.36**	**0.50**	**0.62**	**0.70**	**0.76**	**0.82**	**0.86**	**0.90**	**0.94**	**0.96**	**0.97**	**0.99**	**1.00**	**1.00**

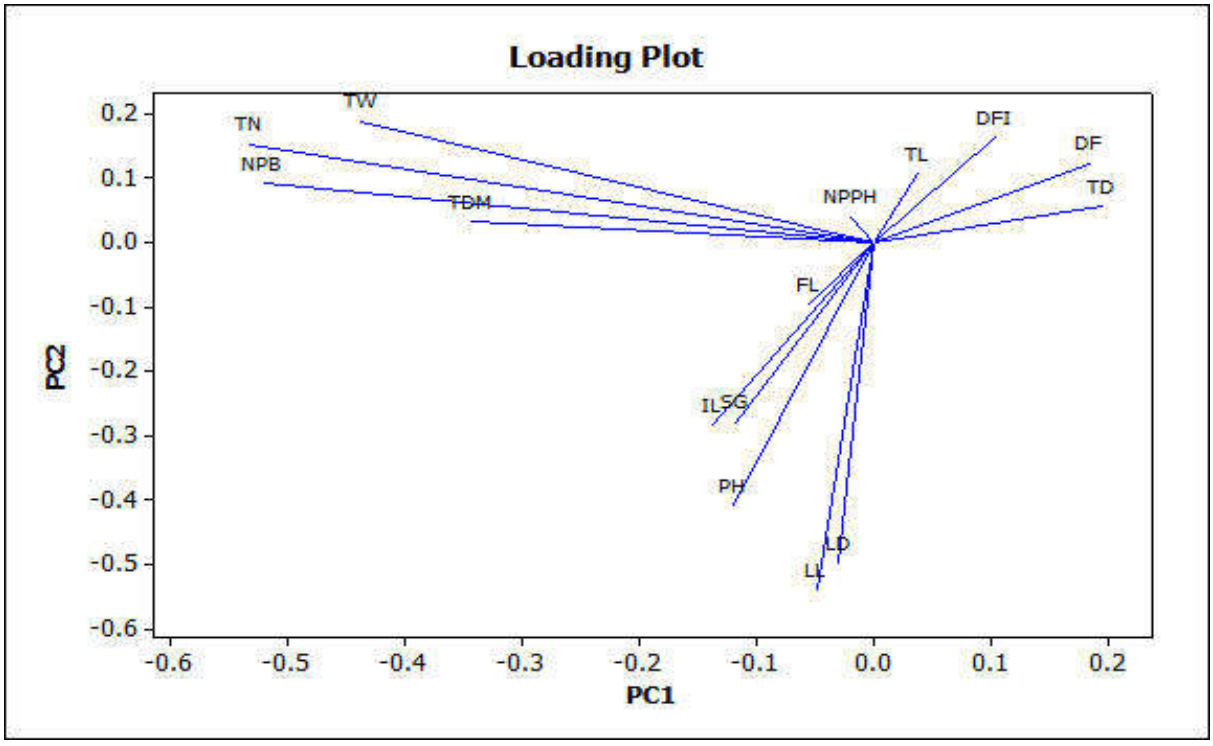

Figure 4 : PCA loading plot of the 15 quantitative traits used in its commutation

4.1.2.6. Factor analysis

Factor analysis, conducted based on PCA, further showed the contribution of traits towards divergence of accessions. Accordingly, factor 1 revealed that number of tubers per hill (-0.916), number of primary branches per plant (-0.897), tuber weight per hill (-0.592) and tuber dry matter contents (-0.592) were major characters that contributed much to the genetic divergence of the accessions. Factor 2 showed that leaf length (-0.851), leaf diameter (-0.784), plant height (0.643), internode length (-0.447), and stem girth (-0.440) were the traits that contributed most to the genetic divergence of the accessions. Similarly, factor 3 represented tuber length (-0.743), tuber diameter (-0.627), days to 50% flower initiation (0.427), and days to 50% flowering (0.401) as the characters that contributed most to the genetic divergence of the accessions. In general, the communality indicated four traits; tuber weight per hill (0.757), number of tubers

per hill (0.902), number of primary branches per plant (0.825) and leaf length (0.731) contributed much to the divergence of the accessions (***Table 12***).

Table 12: Latent vectors (factor analysis) for the 15 principal components characters, computed on the bases of PCA

Variable	Factor1	Factor2	Factor3	**Communality**
NPB	**-0.897**	0.145	-0.022	0.825
SG	-0.204	**-0.440**	0.29	0.320
NPPH	-0.033	0.062	-0.297	0.093
IL	-0.239	**-0.447**	-0.357	0.384
PH	-0.208	**-0.643**	-0.124	0.472
LL	-0.085	**-0.851**	-0.016	0.731
LD	-0.053	**-0.784**	0.021	0.618
DFI	0.178	0.257	**0.427**	0.280
DF	0.315	0.193	**0.401**	0.297
FL	-0.097	-0.152	0.061	0.036
TL	0.065	0.168	**-0.743**	0.585
TD	0.333	0.089	**-0.627**	0.513
TN	**-0.916**	0.24	-0.073	0.902
TW	**-0.754**	0.297	-0.318	0.757
TDM	**-0.592**	0.05	0.552	0.657
Variance	**2.9685**	**2.4818**	**2.0204**	**7.4708**
% Var	**0.198**	**0.165**	**0.135**	**0.498**

4.1.2.7. Cluster analysis

In order to have a good picture of genetic association between or among the accessions, cluster analysis has been conducted without grouping the accessions as well as by grouping them into woreda (administrative woreda of collection sites), and zone or woredas (administrative zones and/or special and isolated woredas of collection sites) of accessions, using standardized data.

Accordingly, cluster analysis of individual accessions grouped the entire accessions into eleven clusters in which a large number of accessions (93%) was grouped under the first three clusters with gradually decreasing number in the subsequent clusters. In general, the apparent grouping pattern coincided with that observed in PCA (data not shown). Moreover, the clustering pattern

seems poor in revealing geographic regions of origin and hence, accessions from different populations and geographic regions were found intermingled and vice versa (***Appendix 3***, ***Figure 1 and Tabe 1)***.

However, cluster analysis made on the bases of grouping the accessions into administrative woredas (collection area) resulted in eight clusters, and the grouping seemingly coincided with the geographic region of origin, but not distinctively woredas of a given region all together. Cluster 1 was found to include the largest number of collection woredas (15), (Sokoru, Yem Liyu, Alle, Seka Chekorsa, Didessa, Kumbabe, Goma, Jimma, Dedo, Gera, Bedele, Darimu, Metu, Damot Sore, and Doyu Gena) representing parts of South and Southwestern Ethiopia. Accessions under this cluster earned second rank in cluster mean for stem girth (3.53), plant height (42.9) and flower length (11.87) and the lowest cluster mean for leaf diameter (3.93).

Cluster 2 contained accessions from 11 woredas (Darian, Goro, Endegagn, Enemor and Ener, Woliso, Damot Gale, Lemo, Gumer, Tokke Kutaye, Sesh Duna, and Sodo Zuriya), all except Tokke Kutaye, representing South and Southwestern Ethiopia. This cluster leads in cluster mean values for leaf diameter (4.00 cm) and tuber diameter (2 cm). Cluster 3 composed of accessions from 5 woredas *viz*. Ankesh Guagsa, Banja, Dangla, Fagta Lekoma, and Wenbera, all from Northwestern Ethiopia, where cluster mean was highest for number of primary branches per plant (6.4 cm), stem girth (3.6 cm), leaf length (13 cm), leaf diameter (4 cm) and tuber diameter (2 cm). Cluster 4 was established by accessions from Chencha and Dita woredas representing Gamo Gofa population (South Ethiopia), where the cluster mean leads for leaf diameter (4 cm), days to 50% flower initiation (153 days), and days to 50% flowering (166.50days). Cluster 5 contained accessions from Kosober Zuriya and Tillili woredas, members of Awi population (Northwest Ethiopia), where the cluster mean leads only for leaf diameter and tuber diameter. Cluster 6 contained accessions from Gida Ayana, Limmu and

Cheliya woredas, (West Ethiopia) where, the cluster mean leads for leaf diameter, tuber diameter, number of tubers per hill (33), and tuber weight per hill (1.33kg). Clusters 7 and 8 were formed by accessions from Kiramu and Dandi woredas (West Ethiopia), respectively. Cluster 7 was highest for number of plants per hill (3), internode length (6 cm), plant height (43 cm), leaf diameter (4 cm), flower length (19 cm), tuber length (16 cm), and tuber diameter (2 cm) cluster mean values while cluster 8 leads only in internode length (6 cm), leaf diameter (4 cm), and tuber diameter (2 cm) (***Tables 13, 14 A & B; Figure 5***).

On the other hand, clustering on the bases of administrative zones of collection and/or special and isolated woredas, that represented study populations, revealed five major groups with a similar trend of grouping as revealed in woredas clustering (***Figure 6***).

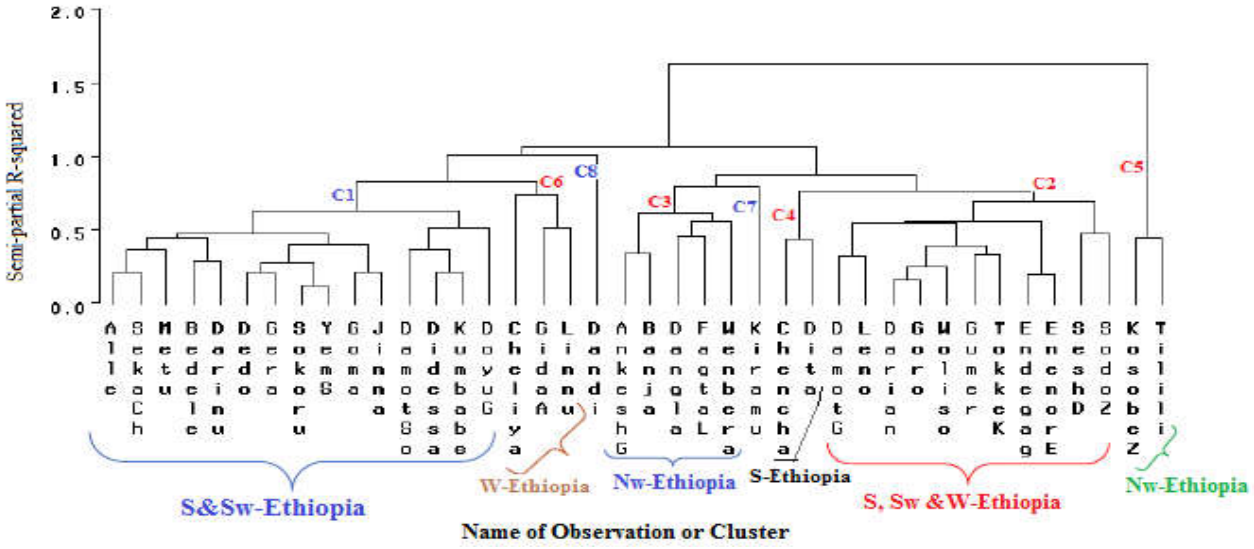

Figure 5 : Clustering of Ethiopian potato accessions, on the bases of adminisrative woredas of accessions; letters arranged in vertical position represents woreda names

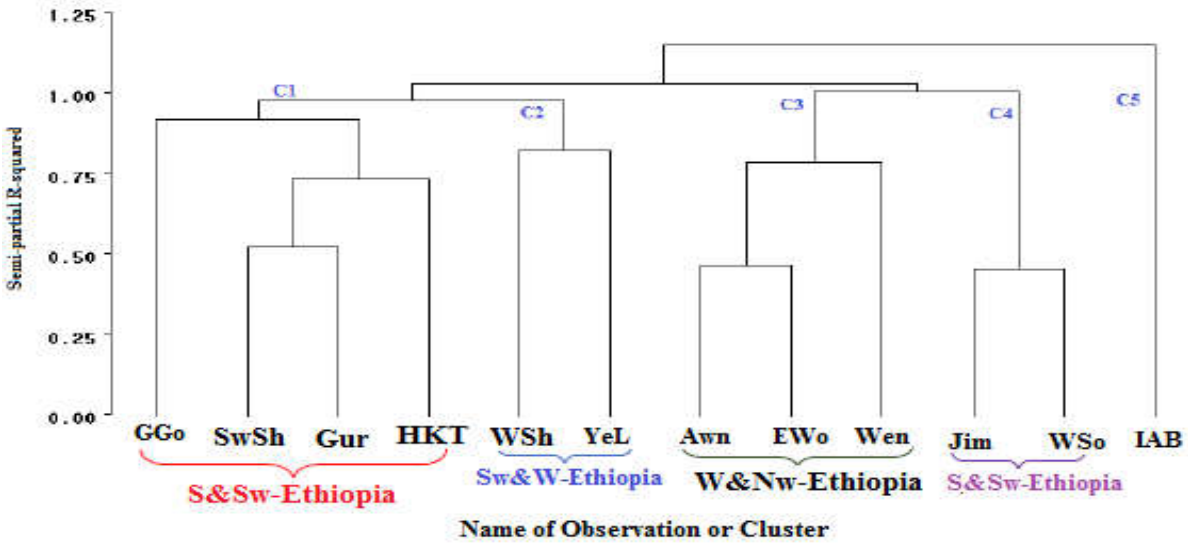

Figure 6: Clustering of Ethiopian potato accessions on the bases of administrative zones of accessions or isolated (pocketed) woredas, each representing study population, where, *GGo*=Gamo Gofa; *SwSh*=Southwest Shewa; *Gur*=Gurage; *HKT*=Hadiya and Kambata-Tembaro; *WSh*=West Shew; *YeL*=Yem Liyu Woreda; *Awn*=Awi nationality; *EWo*=East Wollega; *Wen*=Wenbera; *Jim*=Jimma; *WSo*=Wolaita Sodo; *IAB*=Illu Aba Bora

Table 13: List of woreda(s) under each cluster and respective cluster score of the traits contributed to each woreda and cluster

Cluster	Woreda	NPB	SG	NPPH	IL	PH	LL	LD	DFI	DF	FL	TL	TD	TN	TW	TDM
	Sokoru	5	3	2	6	41	12	4	148	160	11	16	2	27	1	21
	YemL*	5	4	2	5	42	13	4	148	160	11	16	2	28	1	21
	Alle	6	3	2	5	41	12	4	146	157	13	15	2	29	1	22
	SekaCh*	6	3	2	5	38	12	4	146	158	13	15	2	30	1	22
1	Didessa	6	3	2	5	41	13	4	144	158	12	15	2	26	1	21
	Kumbabe	5	4	2	5	41	13	4	145	157	12	16	2	24	1	22
	Goma	5	3	2	5	42	12	4	148	159	12	14	2	25	1	22
	Jimma	6	4	2	5	42	13	4	148	159	12	14	2	27	1	22
	Dedo	6	4	2	5	42	12	4	146	158	12	16	2	31	1	22
	Gera	7	3	2	5	44	13	4	149	159	12	15	2	31	1	22
	Bedele	6	3	3	5	41	11	3	144	156	11	15	2	31	1	22
	Darimu	5	3	2	6	41	12	4	148	160	11	16	2	27	1	21
	Metu	5	4	2	5	42	13	4	148	160	11	16	2	28	1	21
	DamotS*	6	3	2	5	41	12	4	146	157	13	15	2	29	1	22
	DoyuG*	6	3	2	5	38	12	4	146	158	13	15	2	30	1	22
	Darian	5	3	2	5	40	12	4	151	163	11	15	2	26	1	22
	Goro	5	3	2	5	40	11	4	152	163	12	15	2	27	1	22
	Endegagn	4	3	2	5	43	13	4	150	162	13	15	2	24	1	21
	EnemorE*	4	4	2	5	43	14	4	150	161	12	15	2	22	1	21
2	Woliso	5	3	2	5	40	12	4	153	165	10	15	2	26	1	23
	DamotG*	5	3	2	5	38	12	4	150	159	10	15	2	25	1	22
	Lemo	5	3	2	5	37	12	4	151	162	12	15	2	26	1	22
	Gumer	4	3	2	5	39	12	4	155	168	12	15	2	26	1	22
	TokkeK*	6	4	2	5	42	14	4	154	166	12	16	2	25	1	21
	SeshD*	4	3	2	4	43	12	4	154	170	10	16	2	25	1	21
	SodoZ*	4	3	3	5	45	12	4	149	168	11	17	2	25	1	21

Tale 13, continued……..

Cluster	Woreda	NPB	SG	NPPH	IL	PH	LL	LD	DFI	DF	FL	TL	TD	TN	TW	TDM
	AnkeshG*	6	3	3	5	38	12	4	152	165	10	16	2	32	1	22
	Banja	6	4	3	6	39	13	4	150	167	13	14	2	34	1	23
3	Dangla	7	4	2	6	44	13	4	156	165	11	15	2	34	1	22
	FagtaL*	7	4	2	6	44	14	4	153	163	12	14	2	29	1	22
	Wenbera	6	3	2	6	44	13	4	149	165	13	16	2	33	1	22
4	Chencha	3	4	2	6	42	12	4	152	168	11	14	2	16	1	22
	Dita	3	3	2	5	40	12	4	154	165	11	16	2	19	1	21
5	KosobeZ*	3	3	3	6	43	11	4	130	155	10	15	2	21	1	21
	Tilili	3	2	2	5	42	13	4	134	157	10	15	2	24	1	21
	GidaA*	4	3	2	5	42	13	4	141	154	10	15	2	34	1	22
6	Limmu	5	3	2	5	41	12	4	139	154	11	16	2	29	1	22
	Cheliya	6	3	2	6	45	13	4	145	157	12	15	2	36	2	23
7	Kiramu	5	3	3	6	43	12	4	150	163	19	16	2	28	1	22
8	Dandi	6	3	2	6	33	11	4	143	153	10	14	2	31	1	23

YemL (Yem Liyu); SekaCh (Seka Chekorsa); DamotS (Damot Sore); DoyuG (Doyu Gena); EnemorE (Enemor ena Ener); DamotG (Damot Gale); TokkeK (Toke Kutaye); SeshD (Sesh Duna); SodoZ (Sodo Zuriya); AnkeshaG (Ankesha Guagsa); FagtaL (Fagta Lekoma); KosobeZ (Kosober Zuriya); GidaA (Gida Ayana) woredas

Table 14: Clusters, cluster mean and total mean values (A), and difference percentage from total mean (B) contribution in the 15 quantitative traits used for Woreda cluster analysis

A

Cluster/Traits	NPB	SG	NPPH	IL	PH	LL	LD	DFI	DF	FL	TL	TD	TN	TW	TDM
1	5.60	3.53	2.07	5.20	42.93	12.53	3.93	146.47	158.13	11.87	15.07	2.00	27.67	1.00	21.80
2	4.64	3.18	2.09	4.91	40.91	12.36	4.00	151.73	164.27	11.36	15.36	2.00	25.18	1.00	21.64
3	6.40	3.60	2.40	5.80	41.80	13.00	4.00	152.00	165.00	11.80	15.00	2.00	32.40	1.00	22.20
4	3.00	3.50	2.00	5.50	41.00	12.00	4.00	153.00	166.50	11.00	15.00	2.00	17.50	1.00	21.50
5	3.00	2.50	2.50	5.50	42.50	12.00	4.00	132.00	156.00	10.00	15.00	2.00	22.50	1.00	21.00
6	5.00	3.00	2.00	5.33	42.67	12.67	4.00	141.67	155.00	11.00	15.33	2.00	33.00	1.33	22.33
7	5.00	3.00	3.00	6.00	43.00	12.00	4.00	150.00	163.00	19.00	16.00	2.00	28.00	1.00	22.00
8	6.00	3.00	2.00	6.00	33.00	11.00	4.00	143.00	153.00	10.00	14.00	2.00	31.00	1.00	23.00
Total Mean	**4.83**	**3.16**	**2.26**	**5.53**	**40.98**	**12.20**	**3.99**	**146.23**	**160.11**	**12.00**	**15.10**	**2.00**	**27.16**	**1.04**	**21.93**

B

Cluster/Traits	NPB	SG	NPPH	IL	PH	LL	LD	DFI	DF	FL	TL	TD	TN	TW	TDM
1	15.94	11.71	-8.41	-5.97	4.76	2.70	-1.50	0.16	-1.24	-1.08	-0.20	0.00	1.88	-3.85	-0.59
2	-3.93	0.63	-7.52	-11.21	-0.17	1.31	0.25	3.76	2.60	-5.33	1.72	0.00	-7.29	-3.85	-1.32
3	32.51	13.92	6.19	4.88	2.00	6.56	0.25	3.95	3.05	-1.67	-0.66	0.00	19.29	-3.85	1.23
4	-37.89	10.76	-11.50	-0.54	0.05	-1.64	0.25	4.63	3.99	-8.33	-0.66	0.00	-35.57	-3.85	-1.96
5	-37.89	-20.89	10.62	-0.54	3.71	-1.64	0.25	-9.73	-2.57	-16.67	-0.66	0.00	-17.16	-3.85	-4.24
6	3.52	-5.06	-11.50	-3.62	4.12	3.85	0.25	-3.12	-3.19	-8.33	1.52	0.00	21.50	27.88	1.82
7	3.52	-5.06	32.74	8.50	4.93	-1.64	0.25	2.58	1.81	58.33	5.96	0.00	3.09	-3.85	0.32
8	24.22	-5.06	-11.50	8.50	-19.47	-9.84	0.25	-2.21	-4.44	-16.67	-7.28	0.00	14.14	-3.85	4.88

4.1.2.7.1. Distances between the woreda-based clusters

Pairwise generalized square distance estimates between the woredas clusters revealed the largest distance between clusters 5 and 7 (594.24), followed by clusters 7 and 8 (458.08), 6 and 7 (431.46). The least cluster distance was observed between clusters 1 and 2 (26.97). In line with the mean distance estimate, cluster 7 is the most distant from all other clusters (344.18), followed by clusters 5 (232.93), 7 (155.67) and 8 (154.5), in the order of magnitude. Cluster 1 is the least distant (92.90) from all other clusters (***Table 15)***.

Table 15: Pairwise Generalized Square Distance (D^2) between woreda clusters assuming 'zero' intra-cluster distance

CLS	1	2	3	4	5	6	7	8	Mean
1	0								**92.90**
2	26.97*	0							**105.97**
3	24.89*	32.98**	0						**110.70**
4	99.76**	35.83**	114.9**	0					**181.17**
5	140.03**	211.87**	186.06**	308.63**	0				**232.93**
6	51.72**	127.07**	79.55**	257.18**	92.92**	0			**155.67**
7	247.03**	187.53**	267.32**	223.57**	594.24**	431.46**	0		**344.18**
8	59.93**	119.52**	69.19**	228.29**	96.75**	49.77**	458.08**	0	**154.50**

* significant at 0.05 probability; ** Significant at 0.01 probability level

4.2. Molecular Genetic Diversity Analysis

4.2.1. Validation of the EST-SSR markers and evaluation of their levels of polymorphism

In the present study, twenty new EST-SSR markers were successfully developed from *Plectranthus barbatus* cDNA sequences, deposited in the GenBank (***Table 16***). The markers were predominantly trinucleotides and dinucleotides. Trinucleotide SSRs accounted for 55% of the loci with the number of repeats ranging from four to ten whereas dinucleotide SSRs accounted for 35% of the loci with the number of repeats ranging from six to ten. The remaining two loci (10%) were pentanucleotde and hexanucleotide repeats with four and five number of repeats, in that order.

All the 20 loci were polymorphic and produced a total of 128 alleles (an average of 6.4 alleles per locus) (***Table 17***), out of which 62 (48.4%) were rare (frequency < 0.01) and 22 (17.2%) were scarce (frequency between 0.01 and 0.05). The frequency of seven alleles (5.5%) was between 0.05 and 0.10 (moderate) whereas 37 alleles (28.9%) had a frequency of 0.1 or higher frequent/abundant alleles (***Table 18***). The top four frequent alleles were recorded at PE_13 (0.97), PE_24 (0.96), PE_15 (0.95) and PE_06 (0.91) in that order. Similarly, the largest percentage of the total rare alleles (88.89%) were recorded at PE_13 (***Table 18***).

The maximum and minimum number of alleles detected per locus was 12 (PE_31) and 2 (PE_06), respectively, and constituted a total of 194 (3 to 19) multi-locus microsatellite genotypes (NG) (***Table 17***).

The least major allele frequency (MAF; 0.46), and largest effective number of alleles (Ne) (3.17), allelic richness (4.97), Nei's gene diversity (GD) (0.70), polymorphic information contents (PIC) (0.66) and Shannon information index (I) (1.33) were recorded for PE_12. The

highest MAF (0.97), private allelic richness (0.46), and the least Ne (1.06), I (0.12), GD (0.05) and PIC (0.05) were recorded for PE_13. In terms of the overall PIC, one SSR locus (PE_06) was found to be highly informative (PIC ≥ 0.5), 12 loci (60%) were moderately informative (0.5< PIC ≥ 0.25), and the remaining seven were less informative (PIC < 0.25). The highest observed heterozygosity (Ho) (0.97), the lowest fixation index (F) (-0.75) and the highest value for gene flow (Nm) (83.08) were observed for PE_02 (***Table 17***). Ten of the twenty loci showed a highly significant deviation from HW-equilibrium over the entire populations (***Table 17***). Assuming Hardy-Weinberg equilibrium and on collapsing the less frequent alleles, PE_23 showed a significant linkage disequilibrium with all the remaining loci (***Appendix 8, Table 1***).

Table 16 : Characteristic features of the 20 polymorphic SSR markers developed for genetic diversity analyses of Ethiopian potato populations

Primer /SSR loci name	GenBank ANSS*	SSR motif (5' to 3')	Fw-primer (5' to 3')	Rv-primer (5' to 3')	Exp. allele size (bp)	Obs. Alle. size range (bp)	Ta C
PE_01	GB|JZ730850.1	(TC)9	TCACCGCAGTTTTCAGTCTCTA	ATGAGATTCGCCATAGGTTTGT	102	122-146	59
PE_02	GB|JZ730431.1	(AAG)6	TGAATCTGCAAAGACATCTGCT	AACTGAGACAACTCCATTGACG	114	108-126	57
PE_05	GB|JZ730426.1	(TA)10	GAGACGACGACCAATGTTGTTA	CTCTCTATCACTCCTGACGGCT	125	106-142	56
PE_06	GB|JZ732709.1	(ACACCC)5	GGGGAATTAGAGATGGAAAGATAGA	TTGAGGGTGTGAAGTGGTACAG	131	137-143	57
PE_09	GB|JZ732789.1	(TG)10	GCCGTATCTCCATTTGTTGATT	TCCATGCTCCTCACACATTATC	141	140-168	57
PE_12	GB|JZ731502.1	(AC)6	GCTTACGCCAAGAACTGAAACT	AATAGCAATCTCTTTCCCTCCC	164	162-182	55
PE_13	GB|JZ730947.1	(CTC)4	GTTGGACGACTGGGTTTTATGT	GAATGACGCTAGTTTGCTGTTG	314	319-340	55
PE_15	GB|JZ731328.1	(GCT)6	CCAACAGCAATCCATATTACCA	ATTTCTCAAGTCAGTCCGAGGT	172	162-189	57
PE_16	GB|JZ732633.1	(CAG)6	AACCAAATGACAGGAGCATCTT	CAATTTCTTCATACTGGGTGGC	184	180-206	55
PE_17	GB|JZ730151.1	(CTT)5	GGGTAACGATTTGAATAATGCG	GTGAAGCGGGATCTACACTGA	188	176-200	52
PE_22	GB|JZ730075.1	(AGAGA)4	GTCAGCCTTTCTCTCTCGTCTC	AGGGGAGTGTGTTATCAAATGG	223	249-264	56
PE_23	GB|JZ730223.1	(AGC)6	GACCATCGGTAAGGAGAACTTG	GGATATGAGCTGGATAGCTGGT	226	213-243	55
PE_24	GB|JZ731331.1	(CAC)5	GCAAAAGTTCTACCAGCGTTTC	GGTTTGTTGATCCCAACGTAAT	229	242-251	57
PE_25	GB|JZ731120.1	(GAA)10	AATTACTTTCATGTCCAACGCC	TTTTCATCACTACCATCCCAGTC	229	228-243	55
PE_30	GB|JZ731840.1	(TGT)5	AGTTGAGATTGTACTCCACGTTGT	CTACTTCAGTTCCGGCCTCTTA	260	233-260	57
PE_31	GB|JZ732555.1	(AC)9	GGCGATATTCAAGAAAGCAACT	TCTTTTCACGCTTCCATCTCTT	355	319-339	57
PE_33	GB|JZ729809.1	(GA)8	GGGGCTTTTCTTGTTTGAAGAT	ATTGGAGGCAACTCATCAGAAT	361	370-382	57
PE_36	GB|JZ732589.1	(GA)9	ATGAAGAGTGAAGAGGCTGGAG	AGGAGCAAGCAAATAGAAATCG	306	304-322	57
PE_37	GB|JZ732378.1	(TGC)5	GTGTACATGCCATAGAATGAGT	GACTTCATCATATACGGCGGTT	167	180-192	57
PE_38	GB|JZ730154.1	(TGC)5	TACTTTATGGCAGAGAATGCGA	CTTGAGAGCCCTGAGACTTCAT	178	200-227	57

*****ANSS as "Accession Number of Source Sequenc"; Ta=annealing temperature; Fw=Forward; Rev=Reverse; PE = Plectranthus edulis*

Table 17 : Informativeness and levels of different diversity indices of the SSR loci across populations

SSR loci	MAF	NA	Ne	GD	Ar	Arp	Ho	He	PIC	I	P_{HWE}[a]	F
PE_01	0.87	7	1.35	0.24	2.91	0.15	0.22	0.23	0.23	0.45	0.530[ns]	0.11
PE_02	0.49	6	2.24	0.55	3.05	0.13	0.97	0.55	0.45	0.87	0.000*	-0.75
PE_05	0.87	8	1.34	0.25	3.32	0.06	0.19	0.23	0.24	0.49	0.000*	0.23
PE_06	0.91	2	1.19	0.16	2.02	0.07	0.18	0.16	0.15	0.28	0.112[ns]	-0.07
PE_09	0.79	10	1.56	0.36	3.56	0.18	0.27	0.35	0.34	0.66	0.000*	0.27
PE_12	0.46	9	3.17	0.70	4.97	0.08	0.86	0.68	0.66	1.32	0.000*	-0.22
PE_13	0.97	9	1.06	0.05	1.72	0.46	0.01	0.05	0.05	0.12	0.783[ns]	0.73
PE_15	0.96	5	1.09	0.08	1.68	0.20	0.07	0.08	0.08	0.15	0.115[ns]	0.18
PE_16	0.68	6	2.04	0.50	4.28	0.08	0.38	0.49	0.47	0.95	0.000*	0.25
PE_17	0.72	5	1.70	0.42	2.43	0.11	0.54	0.4	0.34	0.62	0.000*	-0.29
PE_22	0.77	6	1.66	0.38	3.11	0.16	0.39	0.37	0.35	0.68	0.335[ns]	-0.01
PE_23	0.76	7	1.65	0.39	3.40	0.17	0.29	0.37	0.36	0.70	0.000*	0.26
PE_24	0.96	4	1.09	0.08	2.00	0.09	0.04	0.08	0.07	0.17	0.542[ns]	0.53
PE_25	0.81	4	1.44	0.30	2.24	0.07	0.30	0.28	0.26	0.46	0.965[ns]	0.04
PE_30	0.90	5	1.24	0.19	2.66	0.08	0.13	0.18	0.18	0.36	0.876[ns]	0.30
PE_31	0.70	12	1.8	0.46	4.03	0.30	0.49	0.43	0.42	0.81	0.115[ns]	-0.06
PE_33	0.63	6	1.99	0.51	2.90	0.06	0.57	0.48	0.44	0.78	0.000*	-0.1
PE_36	0.68	8	1.96	0.49	3.40	0.26	0.43	0.48	0.44	0.85	0.128[ns]	0.11
PE_37	0.53	5	2.12	0.53	2.87	0.07	0.88	0.53	0.43	0.82	0.000*	-0.65
PE_38	0.72	4	1.65	0.41	2.22	0.07	0.54	0.38	0.33	0.59	0.000*	-0.33
Mean	**0.76**	**6.45**	**1.67**	**0.35**	**2.94**	**0.14**	**0.39**	**0.34**	**0.32**	**0.61**		**-0.09**

MAF = major allele frequency; NA = Number of alleles; NG = number of genotypes, Na = number of different alleles; NE = number of effective alleles; GD = gene diversity; Ar = Allelic richness; Arp = Private allelic richness; H_O = observed heterozygosity; H_e = expected heterozygosity; PIC = polymorphic information content; I = Shannon's Information Index; F = Fixation Index; P_{HWE}[a] = P-value for deviation from Hardy Weinberg equilibrium; ns = not significant, * = $P < 0.0001$ and hence highly significant.

Table 18 : Allele frequency distribution and overall percentage of rare alleles ($f \leq 0.01$, where f = allele frequency) across populations

Allele/Locus	**PE_01**	**PE_02**	**PE_05**	**PE_06**	**PE_09**	**PE_12**	**PE_13**	**PE_15**	**PE_16**	**PE_17**	**PE_22**	**PE_23**	**PE_24**
Allele A	0.047	0.011	0.029	0.088	0.007	0.006	0.005	0.002	0.004	0.004	0.007	0.138	0.004
Allele B	0.052	0.002	0.027	0.912	0.013	0.009	0.005	0.035	0.013	0.006	0.063	0.004	0.960
Allele C	0.869	0.002	0.006	-	0.004	0.464	0.002	0.002	0.680	0.267	0.002	0.081	0.031
Allele D	0.002	0.044	0.004	-	0.005	0.128	0.004	0.958	0.115	0.716	0.767	0.763	0.005
Allele E	0.002	0.452	0.053	-	0.004	0.197	0.973	0.004	0.126	0.007	0.007	0.009	-
Allele F	0.024	0.489	0.866	-	0.788	0.002	0.004	-	0.062	-	0.153	0.004	-
Allele G	0.004	-	0.011	-	0.121	0.007	0.004	-	-	-	-	0.002	-
Allele H	-	-	0.006	-	0.053	0.162	0.002	-	-	-	-	-	-
Allele I	-	-	-	-	0.002	0.026	0.002	-	-	-	-	-	-
Allele J	-	-	-	-	0.004	-	-	-	-	-	-	-	-
Overall % of rare	***42.85***	***33.33***	***37.50***	***0.00***	***60.00***	***44.44***	***88.89***	***60.00***	***16.67***	***60.00***	***50.00***	***57.14***	***50.00***

Allele/Locus	**PE_25**	**PE_30**	**PE_31**	**PE_33**	**PE_36**	**PE_37**	**PE_38**
Allele A	0.002	0.028	0.040	0.002	0.006	0.028	0.007
Allele B	0.815	0.035	0.004	0.014	0.106	0.011	0.722
Allele C	0.178	0.035	0.004	0.042	0.004	0.002	0.269
Allele D	0.005	0.004	0.209	0.625	0.201	0.533	0.002
Allele E	-	0.898	0.006	0.303	0.676	0.427	-
Allele F	-	-	0.702	0.014	0.004	-	-
Allele G	-	-	0.009	-	0.002	-	-
Allele H	-	-	0.002	-	0.002	-	-
Allele I	-	-	0.004	-	-	-	-
Allele J	-	-	0.013	-	-	-	-
Allele K	-	-	0.002	-	-	-	-
Allele L	-	-	0.007	-	-	-	-
Overall % of rare	***50.00***	***20.00***	***66.67***	***16.67***	***62.50***	***20.00***	***50.00***

4.2.2. Genetic variation within and among populations

The important results of populations specific genetic diversity indices were presented below: Among the 12 populations studied, no much differences were observed in terms of a number of genetic diversity parameters, such as effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He), gene diversity (GD) and Shannon diversity index (I). However, *Wen*, *WSo* and *Awn* populations scored higher values in Ne, GD and I while *SwSh* and *HKT* populations scored a higher Ho as compared to the other populations. Five populations: *Awn*, *Gur*, *EWo*, *WSh*, and *YeL*, in the order of magnitude, scored slightly less than the mean Ho whereas *IAB*, *Jim*, and *GGo* populations had a mean Ho value. Although Ho value is the lowest for *Gur* population, it comes first in terms of allelic richness (Ar) including richness in private alleles (Arp) with Ar of 3.33 and Arp of 0.28 ***(Table 19)***. *Jim* and *WSh* populations ranked second and third in terms of overall Arp. Analysis of percentage of polymorphic loci (PPL) showed that at least 90% of the loci studied were polymorphic in each population studied, with a mean PPL of 94.2% (***Table 19***). Though the overall is small, *YeL* population had two private alleles at PE_31 (***Table 19, Appendix 7, Table 1***). *EWo* population showed the least I (0.523), Ar (2.56) and Arp (0.01) (***Table 19***). Tests of deviation from Hardy-Weinberg equilibrium revealed that all the populations showed a significant deviation at PE_02 and PE_37 and a non-significant deviation at PE_06.

Micro-Checker result, tested at 95% confidence interval and simulated over 1000 iterations showed no evidence for large allele dropout (short allele dominance) and genotyping error due to stutter peaks across all the loci and populations. However, twelve of the loci showed a sign of null allele for one or more population(s) (***Appendix 10, Table 1***). Accordingly, FreeNA estimate using EM algorithm (Dempster *et al*., 1977), showed that Awn (PE_01), *WSo* (PE_31) and *Wen* (PE_33) populations showed a large ($r \geq 0.2$) null allele frequencies. In general, three

populations (*Gur*, *Wen*, and *Awn* in that order) and eight loci (PE_23, PE_13, PE_09, PE_16, PE_25, PE_30, PE_31, PE_36) had shown moderate ($0.2 > r \geq 0.05$) overall null allele frequencies (***Table 20***).

Table 19 : Summary of different population diversity indices averaged over the 20 loci

Pop[b]	Ar	Arp	Ne	PPL	Ho	He	GD	I
SwSh	2.78	0.12	1.6	95	0.43	0.33	0.34	0.59
EWo	2.56	0.01	1.6	90	0.37	0.30	0.31	0.52
Awn	2.93	0.14	1.8	95	0.35	0.36	0.36	0.64
Gur	3.33	0.28	1.6	95	0.33	0.35	0.35	0.64
HKT	2.80	0.04	1.7	95	0.43	0.34	0.35	0.60
IAB	2.80	0.09	1.7	90	0.39	0.34	0.34	0.59
Jim	3.18	0.25	1.6	95	0.39	0.34	0.35	0.62
GGo	2.99	0.17	1.6	95	0.39	0.34	0.35	0.61
WSo	3.17	0.14	1.8	95	0.41	0.37	0.37	0.66
Wen	2.74	0.13	1.8	95	0.42	0.38	0.39	0.65
WSh	3.01	0.22	1.6	90	0.37	0.31	0.32	0.57
YeL	2.96	0.15	1.6	100	0.37	0.32	0.33	0.57
Mean	2.94	0.15	1.7	94.2	0.39	0.34	0.35	0.61

Pop[b] *SwSh* = Southwest Shewa; *EWo* = East Wollega; *Awn* = Awi; *Gur* = Gurage; *HKT* = Hadiya, Kembata and Tembaro; *IAB* = Illu Aba Bora; *Jim* = Jimma; *GGo* = Gamo Gofa; *WSo* = Wollaita Sodo; *Wen* = Wenbera; *WSh* = West Shewa; *YeL* = Yem Liyu woreda. Ar = Allelic richness; Arp = Private allelic richness; Ne = effective number of alleles; PPL = percentage of polymorphic loci; H_O = observed heterozygosity; H_e = expected heterozygosity; GD = gene diversity; I = Shannon diversity index

Table 20 : Free NA based null allele frequency estimates across the 12 populations and 20 SSR loci used

Locus/Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*	**Locus Mean**
PE_01	0.08	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.02**
PE_02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
PE_05	0.00	0.00	0.02	0.16	0.00	0.11	0.00	0.10	0.00	0.05	0.00	0.00	**0.04**
PE_06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
PE_09	0.00	0.06	0.15	0.19	0.09	0.11	0.00	0.07	0.05	0.19	0.06	0.00	**0.08**
PE_12	0.00	0.00	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	**0.01**
PE_13	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.14	0.14	0.12	0.00	0.00	**0.05**
PE_15	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.14	0.00	**0.02**
PE_16	0.00	0.15	0.18	0.00	0.13	0.14	0.08	0.03	0.09	0.12	0.08	0.00	**0.08**
PE_17	0.00	0.00	0.13	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
PE_22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	**0.00**
PE_23	0.09	0.00	0.09	0.09	0.10	0.10	0.13	0.15	0.13	0.05	0.06	0.19	**0.10**
PE_24	0.12	0.00	0.00	0.14	0.00	0.14	0.00	0.00	0.12	0.00	0.00	0.00	**0.04**
PE_25	0.00	0.00	0.00	0.17	0.00	0.00	0.15	0.00	0.00	0.10	0.10	0.10	**0.05**
PE_30	0.00	0.00	0.00	0.17	0.00	0.00	0.14	0.13	0.00	0.18	0.00	0.00	**0.05**
PE_31	0.00	0.00	0.04	0.10	0.14	0.02	0.00	0.00	0.23	0.00	0.09	0.00	**0.05**
PE_33	0.02	0.02	0.18	0.00	0.00	0.02	0.00	0.00	0.00	0.22	0.00	0.00	**0.04**
PE_36	0.01	0.05	0.11	0.04	0.00	0.12	0.13	0.00	0.06	0.10	0.00	0.17	**0.06**
PE_37	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.02	**0.00**
PE_38	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	**0.01**
Pop Mean	**0.02**	**0.01**	**0.06**	**0.07**	**0.02**	**0.04**	**0.04**	**0.04**	**0.04**	**0.06**	**0.03**	**0.02**	

4.2.3. Population genetic differentiation and gene flow

The hierarchical AMOVA was conducted without grouping the populations as well as by grouping the populations according to administrative regions (AR) and geographical regions (GR). In all cases, variation within individuals accounted for at least 97% of the total variation. The variation among populations, AR and GR accounted for only 3%, 2% and 2% of the total variation, respectively. Hence, the vast majority of the within population variation is due to the heterozygosity of the individuals within each population (***Table 21***).

Similarly, Nei's heterozygosity (Nei, 1978) revealed a weak population sub-structuring where the overall Fst (0.03) and pairwise population differentiation test (0.02 – 0.039) values were very small. Moreover, negative overall within population (Fis = -0.12) and total (Fit = -0.09) genetic differentiation values have also been evidenced as revealing heterozygote excess within the populations. In terms of the loci studied, PE_12 (0.694) and PE_38 (0.019), in that order, revealed the highest gene diversity within populations and between sub populations, respectively. On the other hand, 50% of the loci showed a negative Fis and Fit values. Large overall gene flow (Nm = 18.3) was also evident from ***Table 22***.

To detect over estimation of population differentiation because of null alleles, Weir (1996) Fst estimation with ENA (**E**xclusion of **N**ull **A**lleles) correction method, as suggested by Chapuis and Estoup (2007), (Fst* in this case, ***Table 22***), has been computed and hence, provided a slightly higher and non-significant estimate (bias) in only the loci having negligible to medium overall null allele frequencies. Likewise, Weir (1996) pairwise Fst using ENA correction method (***Table 23***) showed the same trend and hence, populations with maximum and minimum pairwise Fst tests attained maximum and minimum values, respectively, except the extent of bias which seems relatively higher for population pairs, *Wen* and *YeL*, *WSo* and *Wen*, *HKT* and *Wen*, having maximum pairwise Fst, and eventually lower gene flow and vice versa.

Overall HW-equilibrium tests using Fit showed a significant deviation in half of the loci and vice versa (***Table 22***).

Table 21: Analysis of molecular variance (AMOVA) for the 12 populations at different hierarchical levels based on data from the 20 loci

Source of Variation	Df	SS	Variance components	%Variation	Fixation index	P value
Among populations	11	61.94	0.072 Va	2.55	F_{ST}: 0.03	Va and F_{ST} = 0.000
Among individuals within populations	275	603.72	-0.56 Vb	-19.66	F_{IS}: -0.20	Vb and F_{IS} = 1.000
Within individuals	287	948.50	3.31 Vc	117.11	F_{IT}: -0.17	Vc and F_{IT} = 1.000
Total	573	1614.17	2.82			
Among AR	3	26.19	0.05 Va	1.85	F_{ST}: 0.02	Va and F_{ST} = 0.000
Among individuals within AR	283	639.48	-0.52 Vb	-18.44	F_{IS}: -0.19	Vb and F_{IS} = 1.000
Within individuals	287	948.50	3.31 Vc	116.59	F_{IT}: -0.17	Vc and F_{IT} = 1.000
Total	573	1614.17	2.84			
Among GR	3	24.42	0.04 Va	1.53	F_{ST}: 0.02	Va and F_{ST} = 0.000
Among individuals within GR	283	641.25	0.52 Vb	-18.37	F_{IS}: -0.19	Vb and $_{FIS}$ = 1.000
Within individuals	287	948.50	3.31 Vc	116.84	F_{IT}: -0.17	Vc and F_{IT} = 1.000
Total	573	1614.17	2.83			

AR = administrative regions (Oromia, Amhara, SNNPs, and Beneshangul Gumuz); GR = geographic regions (Northwestern, Western, Southern, and Southwestern Ethiopia); Df = Degrees of freedom; SS = Sum of squares

Table 22 : Overall Nei's heterozygosities, Weir & Cockerham (1984) differentiation measures, Gene flow

Locus	Hs	D_{ST}	Fis	Gis	Fst	Fst*	Gst	Fit	Nm*	HW^c
PE_01	0.23	0.01	0.06	0.07	0.06	0.07	0.06	0.12	3.72	0.008^{**}
PE_02	0.55	0.00	-0.76	-0.76	0.00	0.00	0.00	-0.75	83.08	0.999^{ns}
PE_05	0.24	0.01	0.22	0.22	0.02	0.03	0.02	0.23	10.62	0.000^{***}
PE_06	0.16	0.01	-0.13	-0.12	0.05	0.05	0.04	-0.07	4.75	0.894^{ns}
PE_09	0.36	0.01	0.26	0.25	0.02	0.03	0.02	0.27	12.25	0.000^{***}
PE_12	0.69	0.01	-0.24	-0.25	0.02	0.02	0.01	-0.22	15.38	0.999^{ns}
PE_13	0.05	0.00	0.75	0.72	0.01	0.09	0.01	0.76	22.48	0.000^{***}
PE_15	0.08	0.00	0.136	0.16	0.03	0.05	0.03	0.16	7.33	0.003^{**}
PE_16	0.50	0.00	0.24	0.24	0.01	0.02	0.01	0.25	27.53	0.000^{***}
PE_17	0.40	0.01	-0.33	-0.33	0.03	0.03	0.03	-0.29	7.10	0.999^{ns}
PE_22	0.38	0.01	-0.03	-0.03	0.02	0.01	0.01	-0.01	16.42	0.551^{ns}
PE_23	0.39	0.01	0.25	0.25	0.02	0.02	0.02	0.26	10.17	0.000^{***}
PE_24	0.08	0.00	0.54	-0.03	0.01	0.03	-0.01	0.53	22.48	0.000^{***}
PE_25	0.29	0.02	-0.05	-0.04	0.07	0.07	0.07	0.03	3.13	0.241^{ns}
PE_30	0.18	0.01	0.29	0.27	0.03	0.07	0.03	0.31	7.57	0.000^{***}
PE_31	0.44	0.02	-0.12	-0.10	0.05	0.04	0.05	-0.06	4.85	0.89^{ns}
PE_33	0.49	0.02	-0.15	-0.17	0.05	0.06	0.05	-0.09	4.56	0.979^{ns}
PE_36	0.49	0.00	0.10	0.11	0.01	0.01	0.01	0.11	35.46	0.003^{**}
PE_37	0.53	0.00	-0.66	-0.65	0.00	0.00	0.00	-0.65	62.25	0.999^{ns}
PE_38	0.39	0.02	-0.40	-0.40	0.05	0.05	0.05	-0.33	4.65	0.999^{ns}
Overall	0.35	0.01	-0.12	-0.13	0.03	0.03	0.02	-0.09	18.29	0.999^{ns}

Hs=gene diversity within populations; D_{ST}=Nei's (1978) unbiased average gene diversity between subpopulations; Fis=Inbreeding coefficient within individuals; Fst=Inbreeding coefficient within subpopulations, relative to total or genetic differentiation among populations; Fst*=Weir (1996) Fst estimation using ENA correction method as described in Chapuis and Estoup (2007); Fit=Total genetic differentiation; Gis=Inbreeding coefficient within individuals, adjusted for bias. Gst=Analog of Fst, adjusted for bias or Wright (1951) Fst; Nm=Gene flow estimated from FST=0.25(1- FST)/FST (Nei, 1987); HW^c=Overall Hardy-Weinberg test using Fit; * $p<0.05$, ** $p<0.01$, ***$p<0.001$

Table 23 : **Population pairwise genetic differentiation tests** (F_{ST}) (below diagonal) **and Gene Flow (Nm)** (above diagonal)

Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*
SwSh	0	***12.908***	***9.365***	***10.620***	***18.981***	***16.417***	***11.655***	***8.083***	***9.365***	***7.326***	***11.655***	***11.114***
EWo	0.019	0	***9.365***	***20.583***	***15.375***	***17.607***	***14.456***	***10.167***	***12.250***	***6.893***	***17.607***	***12.250***
Awn	0.026	0.026	0	***9.750***	***7.815***	***9.750***	***11.655***	***9.365***	***6.329***	***15.375***	***14.456***	***8.679***
Gur	0.023	0.012	0.025	0	***12.908***	***12.250***	***15.375***	***15.375***	***16.417***	***8.371***	***18.981***	***17.607***
HKT	0.013	0.016	0.031	0.019	0	***20.583***	***12.908***	***7.563***	***14.456***	***6.160***	***12.908***	***11.114***
IAB	0.015	0.014	0.025	0.020	0.012	0	***20.583***	***8.371***	***9.009***	***6.507***	***11.655***	***12.250***
Jim	0.021	0.017	0.021	0.016	0.019	0.012	0	***9.750***	***9.750***	***9.365***	***18.981***	***16.417***
GGo	0.030	0.024	0.026	0.016	0.032	0.029	0.025	0	***9.365***	***8.679***	***17.607***	***7.815***
WSo	0.026	0.020	0.038	0.015	0.017	0.027	0.025	0.026	0	***6.160***	***12.250***	***8.679***
Wen	0.033	0.035	0.016	0.029	0.039	0.037	0.026	0.028	0.039	0	***9.750***	***6.160***
WSh	0.021	0.014	0.017	0.013	0.019	0.021	0.013	0.014	0.02	0.025	0	***13.639***
YeL	0.022	0.020	0.028	0.014	0.022	0.02	0.015	0.031	0.028	0.039	0.018	0

All Fst values are significant at $p < 0.05$

4.2.4. Genetic distance between the populations

Nei's (Nei, 1972) standard genetic distance between populations ranged from 0.009 to 0.05. The highest pairwise genetic distance (0.05) was observed between four pairs of populations (*Awn* vs *WSo*, *Wen* vs *HKT*, *WSo* and *YeL*). The extent was about six-fold of the minimum distance (0.009) observed between *Gur* and *EWo* populations. The mean genetic distance of each population from the other populations ranged from 0.02 to 0.04 and, in terms of this parameter, *Wen* population is the most distantly related with a mean genetic distance of 0.04 (***Table 24, below diagonal***).

Similarly, *Wen* population showed a relatively highest and significant ($p<0.05$) average number of corrected pairwise differences (PiXY) with all populations, except, *Awn*. On the other hand, *EWo* and *WSh* populations showed the least differentiation with a mean genetic distance of 0.01 and 0.02, respectively, from all other populations and a relatively minimum and non-significant average number of corrected pairwise differences from all other populations except *Wen* (***Appendix 12, Table 1***). Similarly, these populations showed a minimum within population average number of pairwise differences (PiX) whereas, *IAB* showed the largest estimate of such parameter (6.06).

Likewise, Cavalli-Sforza and Edwards (1967) genetic distance (Dc) between the populations, computed without using the INA (**I**nclusion of **N**ull **A**llele) correction method, as suggested by Chapuis and Estoup (2007), provided a larger estimate but the populations remained unchanged in terms of being highly distant or closely distant, except few fluctuations (***Table 24, above diagonal***).

Table 24 : Nei's standard (Nei, 1972) (**below diagonal**) and Cavalli-Sforza and Edwards (1967) (**above diagonal**) populations pairwise and mean genetic distance

pop ID	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*	**Mean**
SwSh	****	***0.147***	***0.175***	***0.192***	***0.146***	***0.136***	***0.167***	***0.184***	***0.192***	***0.188***	***0.176***	***0.169***	**0.022**
EWo	0.014	****	***0.167***	***0.146***	***0.147***	***0.132***	***0.136***	***0.154***	***0.166***	***0.167***	***0.133***	***0.154***	**0.018**
Awn	0.026	0.025	****	***0.191***	***0.180***	***0.166***	***0.180***	***0.196***	***0.199***	***0.145***	***0.169***	***0.189***	**0.028**
Gur	0.022	0.009	0.029	****	***0.169***	***0.158***	***0.171***	***0.150***	***0.148***	***0.197***	***0.152***	***0.155***	**0.020**
HKT	0.012	0.013	0.033	0.020	****	***0.124***	***0.150***	***0.176***	***0.154***	***0.203***	***0.150***	***0.157***	**0.023**
IAB	0.012	0.012	0.023	0.020	0.012	****	***0.131***	***0.165***	***0.177***	***0.194***	***0.164***	***0.155***	**0.021**
Jim	0.021	0.017	0.024	0.018	0.022	0.011	****	***0.178***	***0.189***	***0.190***	***0.154***	***0.160***	**0.021**
GGo	0.027	0.020	0.028	0.017	0.033	0.027	0.028	****	***0.176***	***0.202***	***0.135***	***0.189***	**0.026**
WSo	0.029	0.020	0.046	0.018	0.017	0.030	0.032	0.030	****	***0.208***	***0.145***	***0.192***	**0.030**
Wen	0.036	0.038	0.023	0.042	0.049	0.043	0.035	0.038	0.054	****	***0.191***	***0.201***	**0.040**
WSh	0.018	0.011	0.020	0.011	0.018	0.018	0.014	0.013	0.020	0.033	****	***0.172***	**0.017**
YeL	0.023	0.018	0.032	0.012	0.025	0.02	0.016	0.030	0.031	0.048	0.017	****	**0.025**

Mean genetic distance was calculated from Nei's standard (Nei, 1972) genetic distance (Ds); Cavalli-Sforza and Edwards (1967) genetic distance (Dc) was computed without using the INA (**I**nclusion of **N**ull **A**lleles) correction as described in Chapuis and Estoup (2007)

4.2.5. Cluster analysis and PCoA on the bases of EST-SSR data

The neighbor-joining based cluster analysis of 60 individual samples, randomly selected across the 12 populations (five samples per population), resulted in four major clusters (C1, C2, C3, and C4) with the first three clusters further divided into two sub-clusters. All the four clusters comprised individual plants from different collection zones (geographic regions). However, the samples were more or less grouped according to their geographic region of origin at sub cluster levels (designated as i and ii on each major cluster) although there is considerable intermixes (***Figure 7***). Similarly, unweighted pair-group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) cluster analysis with bootstrap tests (Felsenstein, 1985) has also been conducted at population level, using Nei's standard genetic distance (D_{ST} corrected) (Nei, 1972). Consequently, the 12 populations formed four major clusters (I, II, III and IV) with cluster IV comprising eight populations that formed two sub-clusters (i and ii). (***Figure 8***).

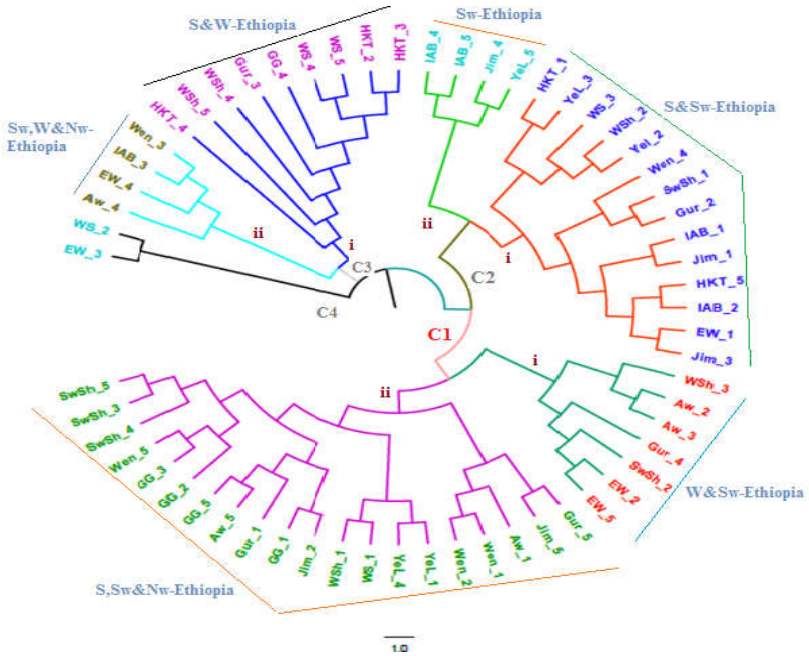

Figure 7: Neighbor-joining tree generated based on simple matching dissimilarity coefficients for 60 individual samples randomly selected from the 12 populations studied. *Aw=Awn, GG=GGo; WS=WSo; EW=EWo*

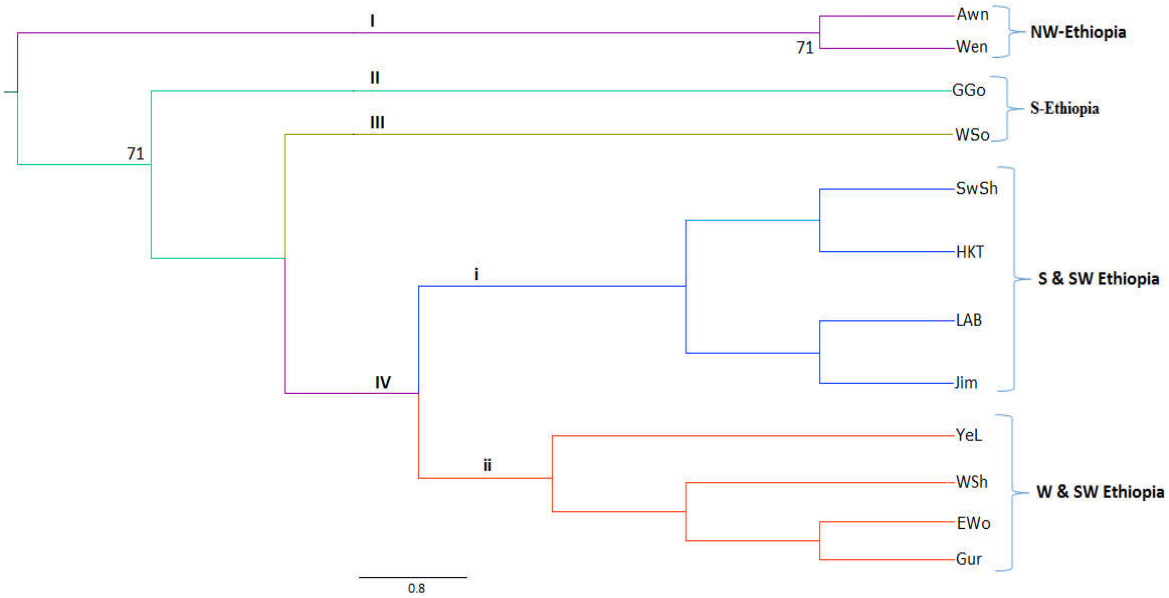

Figure 8: Unweighted pair-group method with arithmetic mean (UPGMA) dendrogram showing genetic relationships among the 12 populations considered based on Nei's unbiased genetic distance. Numbers at the roots of the branches are bootstrap values, and bootstrap values of less than 59% were not shown.

Neighbor-Joining (NJ) tree analysis, based on Nei's standard shared allele distance, grouped the 12 populations into three major clusters (C1, C2, C3, where C2 had two mini clusters, i and ii) with a very minimum bootstrap support (less than 59%) except C1 (99%) (***Figure 9***). Majority of the populations (66.7%) were grouped together under C2 with several hierarchical sub-clusters. The patterns of grouping were similar to UPGMA where, several populations (*Awn* and *Wen*, *HKT* and *WSo*, *Gur* and *YeL*, *IAB* and *Jim*) were tended to group together according to their geographic region of origin but with a very minimum bootstrap support except *Awn* and *Wen* populations. In addition, *Wen*, *WSo*, *YeL*, *Jim*, and *GGo* populations represent older populations in their respective major and mini clusters while the remaining populations represent a relatively recent divergence (recent populations).

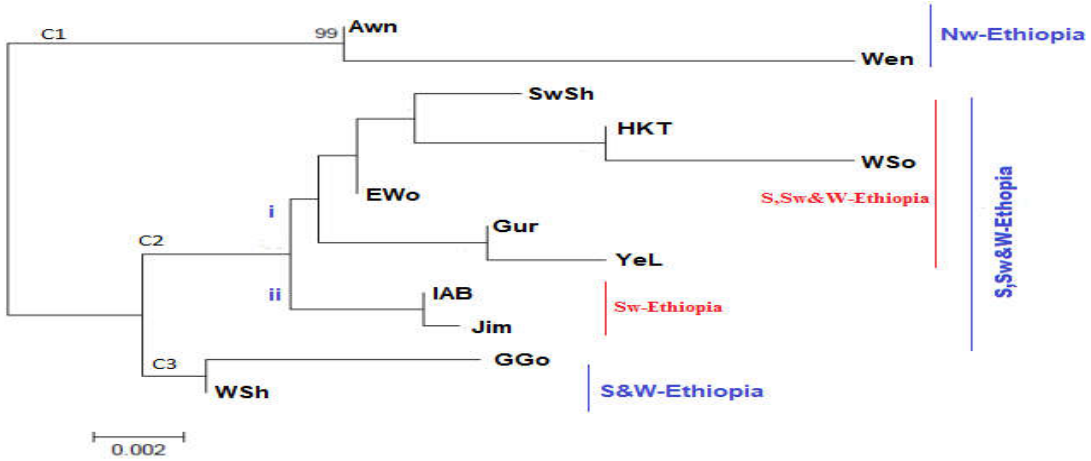

Figure 9: Neighbour- joining (NJ) dendrogram drawn on the bases of Nei's unbiased genetic distances and showing genetic relationships among the 12 Ethiopian potato populations. Number on the branches show bootstrap value and branches without number scored less than 50% of such value

PCoA analysis, conducted using the same samples as NJ-clustering, revealed that the majority of samples were placed at the center of a two-dimensional coordinate plane (***Figure 10***) forming roughly three groups (C1, C2 and C3), showing a tendency for some samples from the same geographic regions to appear together but, poor overall population structure. The first three axes explained 32% (14.1%, 9.6%, and 8.9% variation, in that order) of the total variation, which seems lower.

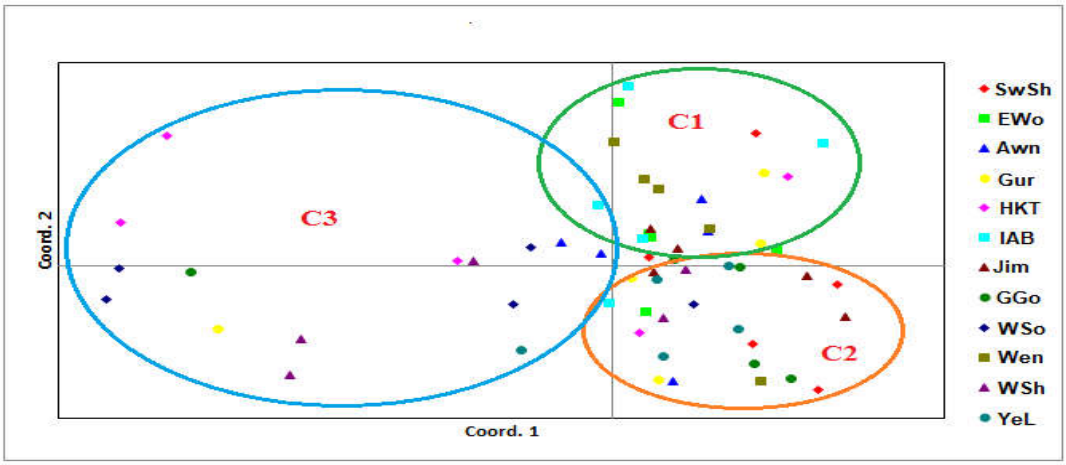

Figure 10: Principal coordinates analysis (PCoA) bi-plot showing the clustering pattern of 60 samples randomly selected from the 12 populations. Samples coded with the same symbol and color belong to the same population. Note: The percentages of variation explained by the first 3 axes (1, 2 and 3) are 14.1%, 9.6%, and 8.9%, respectively.

4.2.6. Structure analysis

Assignment of the 287 individual samples and determination of their population structure was conducted using a model based Bayseian approach with the estimated membership fraction (pre-determined population) ranging from K = 1 to K = 12 and five iterations for each K. On the bases of Gilbert *et al*. (2012) recommendation, Evanno *et al*. (2005) method on STRUCTURE outputs were used for Structure Harvester and predicted K = 3 to be the most likely number of clusters (***Figure 11, A***). Based on this value, Clumpak result (bar plot) showed a wide admixture and hence, no clear geographic origin (collection) based structuring of Ethiopian potato samples or populations. But, few samples from *Awn* and *Wen* (purple colour), *Awn*, *Wen*, *GGo* and *WSo* (Orange colour) seemed to group together (***Figure 11, B***).

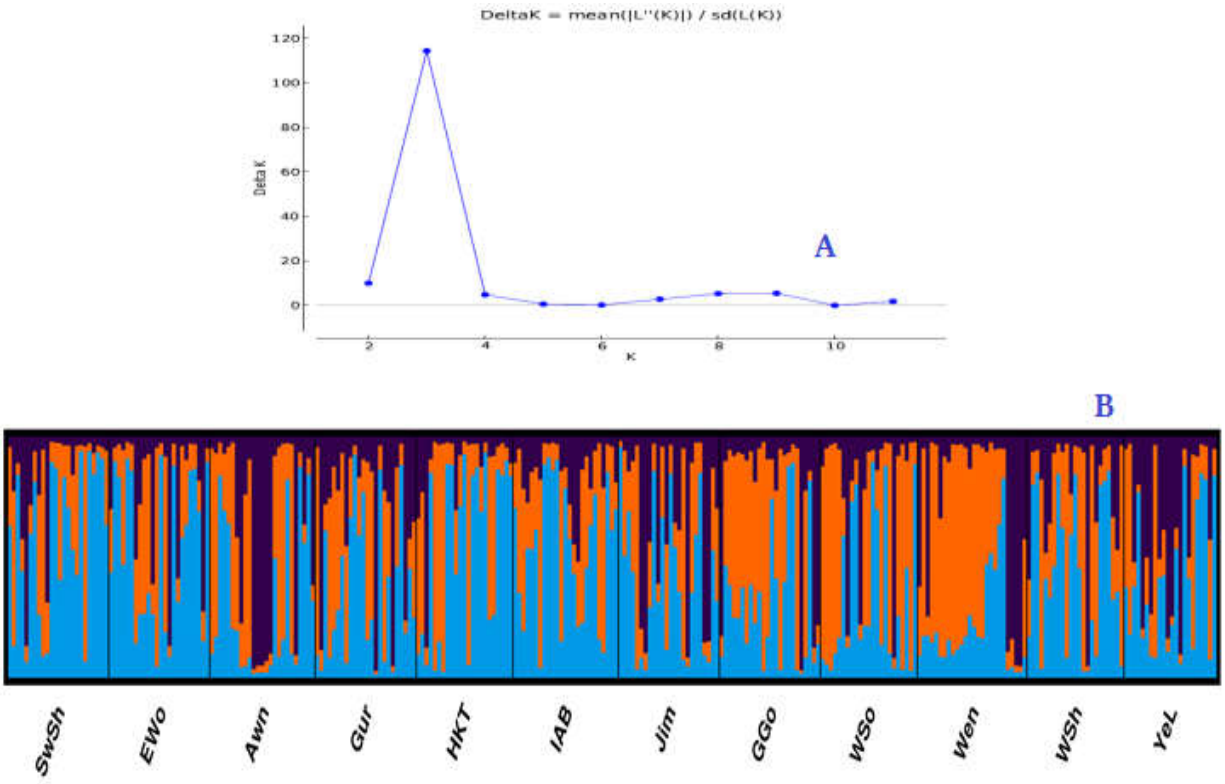

Figure 11: Delta K value estimated using Evano *et al* (2005) method (A) and Bayesian model-based estimation of population structure (K = 3) (B) for the 287 Ethiopian potato individual plants in twelve pre-determined populations

4.2.7. Population genetic bottleneck

Based on the assumption of mutation-drift equilibrium, detection of recent population bottleneck and estimation of effective population size using heterozygosity excess tests, Sign, standardized differences and Wilcoxon, and mode shift method under the three possible mutational models, infinite allele (I.A), two phase (T.P), and step wise (S.M), revealed a non-significant recent bottleneck effects except *Wen* population, collected from Beneshangul Gumuz region, Wenbera woreda, that showed a recent bottleneck under I.A.M and S.M.M (the most conservative model for microsatellite evolution). Likewise, populations from Hadiya-Kembata Tambaro (under I.M.M), Awi nationality zone (under I.A.M and T.P.M) and Wolaita Sodo (under I.A.M and T.P.M) showed a sign of bottleneck, but less likely significant. However, all populations showed no deviation from normal L-shape under mode shift indicator test suggesting large proportion of alleles with low frequencies (***Table 25***).

Table 25 : Statistical significance of the three bottleneck tests under three possible alternative mutational models in 12 Ethiopian potato populations using 20 loci

Population	Mutation model	Significance Test			
		Sign Test*	Standardized Diff. Test (T2)**	Wilcoxon Test***	Mod Shift
SwSh	I.A.M	10:9 (0.478)	0.011 (0.496)	0.5235	Normal L-shaped distribution
	T.P.M	11:8 (0.259)	-0.538 (0.295)	0.7161	
	S.M.M	13:6 (0.025)	-3.420 (0.000)	0.9898	
EWo	I.A.M	9:9 (0.591)	0.150 (0.440)	0.4661	Normal L-shaped distribution
	T.P.M	9:9 (0.551)	-0.362 (0.359)	0.6802	
	S.M.M	10:8 (0.236)	-2.760 (0.003)	0.9700	
Awn	I.A.M	7:12 (0.197)	0.419 (0.338)	0.3254	Normal L-shaped distribution
	T.P.M	9:10 (0.554)	-0.069 (0.473)	0.5856	
	S.M.M	14:5 (0.011)	-2.483 (0.007)	0.9920	
Gur	I.A.M	11:8 (0.200)	-1.954 (0.025)	0.9563	Normal L-shaped distribution
	T.P.M	12:7 (0.080)	-2.921 (0.002)	0.9838	
	S.M.M	17:2 (0.000)	-7.954 (0.000)	1.0000	
HKT	I.A.M	9:10 (0.502)	0.200 (0.421)	0.3840	Normal L-shaped distribution
	T.P.M	10:9 (0.455)	-0.409 (0.341)	0.5078	
	S.M.M	13:6 (0.033)	-3.487 (0.000)	0.9479	
IAB	I.A.M	9:9 (0.525)	0.120 (0.452)	0.4831	Normal L-shaped distribution
	T.P.M	9:9 (0.484)	-0.377 (0.353)	0.6802	
	S.M.M	14:4 (0.003)	-3.489 (0.000)	0.9896	
Jim	I.A.M	12:7 (0.112)	-1.362 (0.087)	0.9091	Normal L-shaped distribution
	T.P.M	13:6 (0.035)	-2.256 (0.012)	0.9601	
	S.M.M	13:6 (0.019)	-6.701 (0.000)	0.9973	
GGo	I.A.M	10:9 (0.428)	-0.592 (0.277)	0.6887	Normal L-shaped distribution
	T.P.M	11:8 (0.217)	-1.364 (0.086)	0.8533	
	S.M.M	13:6 (0.022)	-4.970 (0.000)	0.9953	
WSo	I.A.M	9:10 (0.58629)	-0.470 (0.319)	0.6746	Normal L-shaped distribution
	T.P.M	9:10 (0.57035)	-1.239 (0.108)	0.7910	
	S.M.M	14:5 (0.00724)	-5.187 (0.000)	0.9820	
Wen	I.A.M	6:13 (***0.041***)	1.687 (***0.046***)	***0.0421***	Normal L-shaped distribution
	T.P.M	7:12 (0.217)	1.087 (0.139)	0.1206	
	S.M.M	8:11 (0.450)	0.947 (0.172)	0.6887	
WSh	I.A.M	11:7 (0.181)	-1.342 (0.090)	0.9163	Normal L-shaped distribution
	T.P.M	11:7 (0.156)	-2.077 (0.019)	0.9593	
	S.M.M	15:3 (0.000)	-5.960 (0.000)	0.9996	
YeL	I.A.M	12:8 (0.258)	-1.204 (0.114)	0.8350	Normal L-shaped distribution
	T.P.M	12:8 (0.202)	-2.026 (0.021)	0.9053	
	S.M.M	14:6 (0.023)	-5.675 (0.000)	0.9904	

** observed values of the number of loci with heterozygosity deficiency vs. heterozygosity excess. ** the value of the T2 statistic which represents an average across loci of the deviation of the observed heterozygosity from the expected one (based on the number of alleles in the population), *** the probability for one tail heterozygosity excess. The numbers in parenthesis indicates probability*

4.3. Chromosome of Ethiopian potato

4.3.1. Analysis of mitotic metaphase chromosome spread

Slides with relatively good quality of mitotic chromosome spread were selected and photomicrographs were taken. Cells with under-condensed pro-metaphase and well condensed metaphase stages are readily distinguished. However, in both pro-metaphase and metaphase stages, the centromeric and other morphological features are not discriminable making difficult to handle karyotyping except determination of the chromosome numbers.

Late prophase/prometaphase chromosomes are characterized by differential degree of staining along the arms with regions around the middle of the chromosomes staining darker than distal regions. This may be due to differential degree of condensation of chromatin regions. The darkly staining region may represent constitutive heterochromatin and the less condensed lightly staining regions represent euchromatin (***Figure 12 A***). As chromosome condensation is progressing towards metaphase, the differential condensation and staining disappears and uniformly stained dot-like chromosomes are evident (***Figure 12, B*** and ***C***).

4.3.2. Somatic chromosome number and ploidy level

The 2n chromosome number was counted through visual inspection (counting) in mitotic cells with good chromosome spread under a microscope and from photomicrographs taken from such cells. We found the diploid chromosome number of Ethiopian potato as 2n = c.56 (***Figure 12***). Based on the basic cromosome number report (x=7) for some species of the genus *Plectranthus* so far, we speculated the ploidy level of the crop to be eight (2n = 8x = 56).

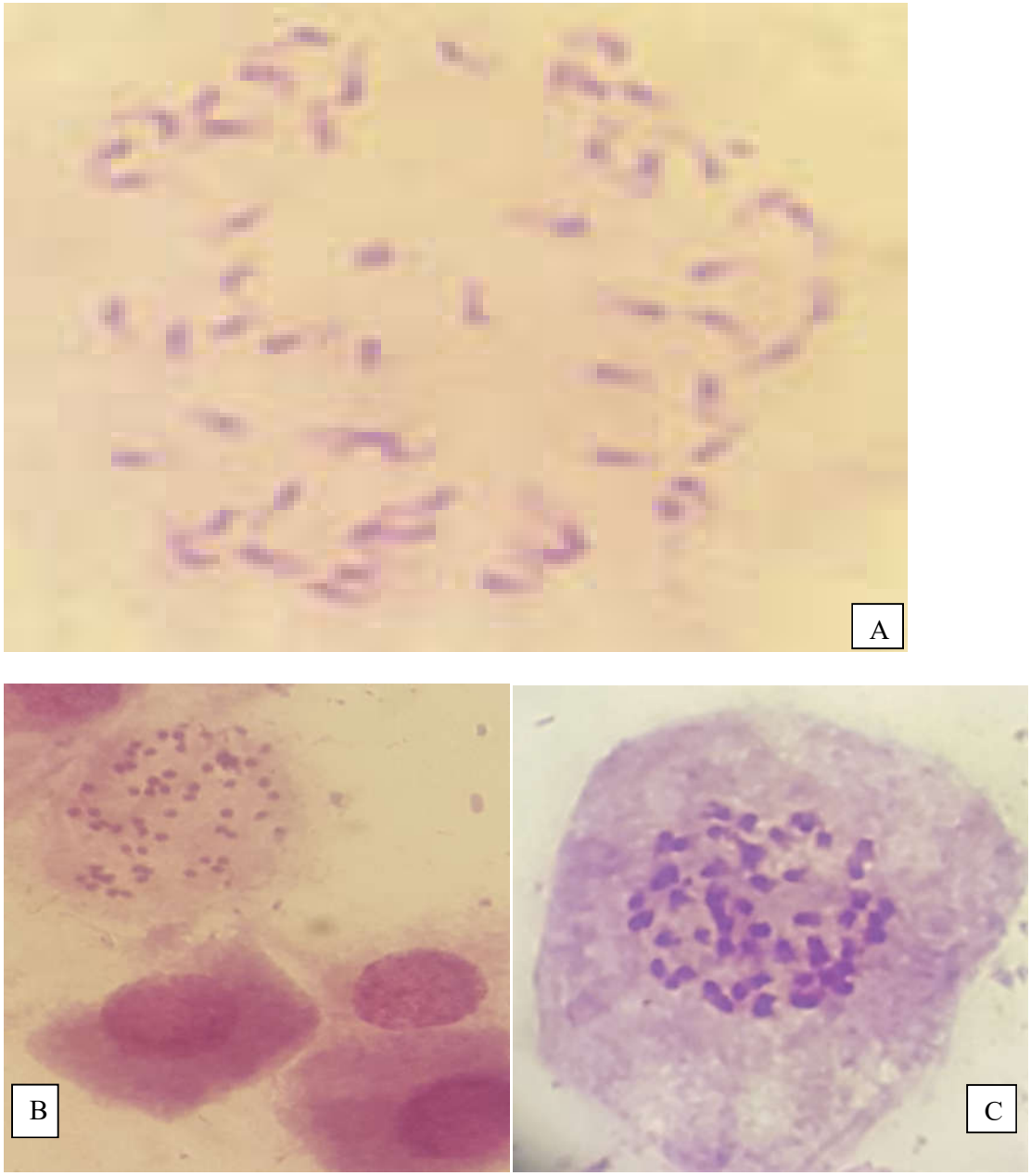

Figure 12: Mitotic chromosome spread of 'Ethiopian potato' at late prophase or pro-metaphase (A) and metaphase stages (B and C)

5. DISCUSSION

Ethiopian potato (*Plectranthus edulis*) is an indigenous tuber crop of Ethiopia that plays an indispensable role in food security of subsistence farmers in areas where it is cultivated. The present study considered the patterns of variations in both qualitative and quantitative agro-morphological traits, tested at multiple locations. In addition, EST-SSRs based marker system as well as cytogenetic analysis have been done to help evaluate the extents of genetic diversity. A total of 174 accessions from diverse agro-ecologies in the country were studied in this regard. The findings of the present study will promote the conservation and breeding efforts of the crop. Results obtained are discussed in the following sections.

5.1. Patterns of Qualitative Agro-morphological Traits Variation

Distributions of the agro-morphological qualitative traits considered in this study revealed a wide range of variations in most of the character states in each groups of characters. For example, pale purple and green were the most frequent while deep purple and deep green were the least frequent leaf color related character states. Similarly, ovate phenotype was the most frequent and round phenotype was the least frequent leaf shape related character states. There are similar reports regarding the existence of wide variability in leaf color in *P. edulis (*Yeshitila Mekbib, 2007), and Taro (*Colocasia esculenta)* (Yared Dagne, 2007; Tewodros Mulualem, 2013) accessions from Ethiopia. A similar trend has been reported on stem and tuber related character states in various root and tuber crops such as *P. edulis* (Woyessa Garedew *et al*., 2006, 2009; Yeshitila Mekbib, (2007), Taro (*C. esculenta)* (Muluneh Tamiru, 2006; Yared Dagne, 2007), and cassava (*Manihot esculenta* Cranz) (Amsalu Nebiyu, 2003) from Ethiopia.

As these qualitative traits were evaluated under common environment and similar growing season, the observed variations among the character states could be attributed to genetic variation within and among the accessions. Hence, they could lay the bases for further improvement programs and conservation measures of the crop. However, some of the phenetic characters, for example, whorl leaf arrangement (2.08%), oval tuber shape (1.75%), deep purple leaf (3.4%) and stem (2.9%) colors, were rare polymorphic phenotypes, that might be resulted from either recurrent (rare) mutation events or natural selection pressure against the traits. Bekele Serbessa (2017) has reported such rare phenetic character states in anchote (*Coccinia abyssinica*) accessions from Ethiopia.

5.2. Variations in Quantitative Traits and Effects of Environmental Factors

All the sixteen quantitative traits revealed a highly significant variation ($p<0.001$) among the accessions in each of the experimental locations as well as when locations are combined. Wayessa Garedew *et al*. (2009), Amsalu Nebiyu (2003) and Baye Birhanu *et al*. (2005) had reported a similar result in *P. edulis*, Irish potato and cassava, respectively. Likewise, the mean performances, phenotypic variance estimate (δ^2_p) and genotypic variance estimate (δ^2_g) revealed wide range of variations. For example, ten traits, days to 50% flower initiation, plant height, number of tubers per hill, days to 50% flowering, flower length, leaf length, number of primary branches per plant, tuber length, tuber dry matter contents, and yield of tubers per hectare of land scored more than five-folds of the minimum mean performance range. Wayessa Garedew *et al*., (2009) and Baye Birhanu *et al*., (2005) reported a similar result in *P. edulis* and Irish potato, respectively except slight variations in the magnitude that could be attributed to differences in the number of samples studied and experimental sites used. The average dry matter contents of Ethiopian potato (21.80% ± 0.94) is comparable with that reported by Wayessa Garedew *et al*., (2009) (20.75%), and Baye Birhanu *et al* (2005) in Irish potato (20%)

but, less than sweet potato (30%), cassava (40%), taro (30%) and yam (27%) (Admasu Tsegaye, 2002).

Such significant and wide range of genetic variation signposts the existence of large genetic variability among the tested accessions and extrapolution to the crop as a whole could be used as a baseline information for further work in conservation and breeding of the crop in any direction (early or late, tall or dwarf, high yielding or low yielding, etc). Similarly, the non-significant variation in location-treatment interaction, and the negligible (nearly zero) variability in genotype-environment interaction (δ^2_{ge}), and the slightly greater (1.78%) or nearly equal estimates of PCV and GCV values in all, except flower length (13.86), suggest the presence of stronger genetic base of the variations than the environment effect. Similarly, effectiveness of testing the accessions at multiple sites, and efficiency in selection for those traits on the bases of phenotypic values except, flower length, could be the likely reasons. The high coefficient of genetic determination (R^2) detected, particularly, in most of the yield contributing traits, also suggest a favourable condition to identify superior genotypes with respect to the traits.

Deshmukh *et al.* (1986) suggested that estimates of phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) could be grouped as high (>20%), medium (10 – 20 %), and low (<10%). Accordingly, five traits such as number of primary branches per plant, number of plants per hill, flower length, number of tubers per plant, and tuber weight per hill revealed high PCV and GCV estimates, suggesting that they are largely governed by genetic factors and had substantial genetic variability as a consequence and hence could be easily improved through selection. Wayessa Garedew *et al*. (2009) had reported a similar result in *P. edulis*. In addition, Baye Birhanu *et al*. (2005), and Ruth and Ramaswamy, (2002) had

reported similar trends for number of tubers per hill and tuber yield per plant in Irish potato and cassava, respectively.

On the other hand, stem girth, internode length, and plant height, scoring moderate PCV and GCV estimates, show the presence of moderate variability among the studied accessions and hence selection on the bases of these traits could be important. However, there is still a need to rely on diverse accessions in order to ensure effective selection on the bases of the remaining traits except flower length that is largely governed by environmental factors as evidenced from its wide range of differences (14.93%) between PCV and GCV estimates.

5.3. Traits Heritability and Tuber Yield Improvement

Heritability can be defined as the percentage of phenotypic variance that is attributed to genetic variance and could be helpful in predicting the expected progress to be achieved through selection process. The heritable portion of the total variation is a point of interest in agro-morphological traits-based genetic diversity analysis. Therefore, to determine the amount inherited from GCV and to get best picture of the amount of advance to be expected from selection, it is necessary to couple both GCV and heritability estimates together, otherwise GCV only provide information on estimates of genetic variability in base population (Burton and Devane, 1953).

According to Singh (2001), heritability can be categorized into very high (≥80%), moderately high (60–79%), medium (40–59%), or low (<40%). In this view, five of the nine traits having very high broad sense heritability (Hb) estimates, such as tuber weight per hill, number of primary branches per plant, number of tubers per hill, plant height, number of plants per hill, days to 50% flower initiation in addition, had medium to high GCV. This suggests the relative small contribution of environment factors to the phenotypes and hence, selection for such

characters could be fairly easy due to high genetic variation. Moreover, some of these traits are supposed to have a direct link with yield of tubers per hectare of land though environmental factors and other quantitative traits play a significant role. Wayessa Garedew *et al*., (2009) reported a similar result for tuber weight per hill (96.5%).

According to Singh (2001), genetic advance (GA) can be defined as the improvement of traits genotypic values for the new population that result from selection relative to the base population, under one cycle of selection at a given selection intensity. To this end, estimates of GA for tuber yield per hectare of land was 1.76t/ha. This indicates that selection of the best 5% high yielding genotypes as parents would improve the mean tuber yield of progenies by 1.76t/ha. Thus, mean genotypic value of the new population for the trait will be improved from 2.01t/ha to 3.77t/ha and the same trend holds true for the remaining traits.

On the other hand, the expected genetic advance values expressed as a percentage of the genotypes mean (GAM) indicates the progress that could be expected from selection of the top 5% of the populations. Jonhson *et al.* (1955) categorized GAM as low (< 10%), moderate (10–20%), and high (>20%). In this regard, tuber weight per hill, number of primary branches per plant, number of tubers per hill, stem girth, plant height, and number of plants per hill, had high GAM. These traits, in addition, had high Hb% suggestting the presence of gene action (may be additive genetic variance) for their expression which is fixable for next generations and thus, guarantee selection in next population since, high heritability estimates coupled with high GAM is usually more helpful in predicting gain under selection than heritability alone (Jonhson *et al*., 1955). Likewise, tuber length, days to 50% flower initiation, leaf length and internode length showed moderate GAM along with high to moderately high (leaf length) Hb% suggesting their relative rewarding nature for selection and greater role of inheritance for non-additive gene action.

Furthermore, Saha *et al*. (1990) indicated that, high GCV coupled with high estimate of Hb% and high GAM provides even better information than single or two of them. In this regard, tuber weight per hill, number of primary branches per plant, number of tubers per hill, and number of plants per hill could be crucial traits in Ethiopian potato improvement programs. Wayessa Garedew *et al*. (2009) reported a similar result for tuber weight per hill and number of tubers per hill.

5.4. Association in the Agronomic Traits and Implication to Improve the Productivity of Ethiopian potato

Both phenotypic and genotypic correlation analysis are highly important in measuring the intensity of association between traits such that selection for a character, particularly in economic and complex traits like yield, results in progress (positively correlated) and/or retrogress (negatively correlated) (Akinwale *et al*., 2011). In this view, the observed phenotypic and genotypic correlation coefficient values were more or less similar among most of the traits studied in terms of both direction (being positive or negative) and significance level. However, the significance level and magnitudes of correlation coefficient values observed in tuber weight per hill and number of tubers per hill, one of the yield contributing traits in root and tuber crops (IITA, 1990), with each other and several other traits indicates the objective and direction of selection and the genetic inheritance of the phenotypic associations. Murat and Vahdettin (2005) and Wayessa Garedew *et al*. (2009) reported a positive phenotypic correlation between tuber yield per plant and plant height, tuber number per hill, tuber diameter in Irish potato and *P. edulis*, respectively. Hence, accessions with large number of tubers, higher number of primary branches, large tuber length, wider stem girth, large tuber diameter, relatively shorter days to flowering and higher tuber dry matter content should be given special attention in

efforts towards tuber yield improvement. Wayessa Garedew *et al*. (2009) and Amsalu Nebiyu (2003) reported similar findings in *P. edulis* and cassava, respectively.

However, the significantly negative genotypic and phenotypic associations in tuber dry matter contents vs tuber length per hill and tuber diameter demands special selection caution to compromise the positive correlation between tuber weight per hill and tuber diameter, and the negative correlation between tuber dry matter contents and tuber diameter since bigger tubers may bear low dry matter contents. Wayessa Garedew *et al*., (2009) reported a similar result.

5.5. Quantitative Traits-based Patterns of Grouping

PCA is a technique used to identify traits that contributed most to the observed variation within a group of genotypes. It offers a practical application in the selection of parental lines during breeding. In this view, variables with coefficients, or elements of eigen vector of large absolute magnitude (close to unity) reflect a strong influence for a given trait and *vice versa* (DeLacy and Cooper,1990). Hence, traits with higher coefficients, usually 0.6 and above, on the PC axes should be considered as more important (Jeffrey, 1980 as cited in Balkaya *et al.* (2010). Similarly, traits with more factor loadings contribute more to the divergence of accessions (genotypes) and are given greater emphasis on selecting the clusters for the purpose of further selection and the choice of parents for hybridization (Jagadev *et al*., 1991). To this end, the first six PC axes (eigen values >0.98) represented a cumulative variance of 76.0% and suggests that traits having high factor loadings, for example; number of plants per hill, number of primary branches per plant, number of tubers per hill, tuber weight per hill, leaf length, and leaf diameter within these axes exhibit great influence on the phenotype of the accessions and could be targets of selection breeding. Furthermore, some of these traits are yield contributing in one way or the other and hence, a good hybridization-breeding program could be initiated by selecting genotypes from these PCs. This result was further supported by factor analysis.

Ahmadizadeh and Felenji (2011) had reported a cummulative variance of 80.1% for the first three PC axes in the evaluation of diversity among Irish potato cultivars in Iran and found yield, harvest index, number of stems and stolon length as having high factor loadings on the first PC axes. Afuape *et al.* (2011) as well reported a cummulative variance of 70.09% for the first three axes in the evaluation of nine sweet potato genotypes and had found total root number, weight of total roots, weight of biomass and biomass dry matter as the important traits. Similarly, Cardi *et al*. (2002) reported ten PC axes with 98.9% cummulative variance for tetraploid potato cultivars with major loading factors from number, size and shape of the leaflet, flowering, the shape of the flower and the number of initial stems.

PCA-biplot- a diagrammatic representation of the genetic diversity pattern of germplasms based on their agro-morphological characteristics, is one of the methods used to separate genotypes into different groups (Okii *et al*., 2014). Similarly, PCA scatter matrix could be used to identify the contribution of each morphological characteristic towards forming groups or clusters. Accessions falling in the same quadrant are assumed to be the same or closely related while those located at an angle of 90º are assumed to have no close relationship (Malik *et al*., 2014). This clustering technique has widely been used in various crops such as anchote (Tilahun Wondimu *et al*, 2014); sweet potato (Afuape *et al.,* 2011); wheat varieties (Malik, *et al.*, 2014); and common bean (Okii *et al.*, 2014). With this concept, the score of individual accessions revealed groupings of most of the accessions in the central quadrant of the plot and high loadings on the first and second principal components. In general, the accessions were found clustered into four but overlapping groups indicating the probability of having exploitable variability among the accessions for planting material selection.

Similarly, cluster analysis, one of the methods used to reveal association between or among genotypes, resulted in eight clusters of woreda-based accessions, with a similar weak trend

observed in individual accessions and populations clustering. Falconer (1981) reported that variation in origin (geographical separation), ancestral relationship, gene frequency and morphology are the probable sources of genetic diversity. Thus, plants differing in either one or more of these factors would differ by significant number of genes and hence, categorizing genotypes (accessions) on the bases of such variations into morphologically similar, more particularly genetically similar groups, is useful in selecting parents for crossing (Souza and Sorrels, 1991). However, it is evidenced that, though genetic diversity is associated with geographical diversity, they are not necessarily directly related. To this end, the weak tendency of association between geographical proximity and genetic diversity for accessions, woreda and population clusters analysis revealed a moderate divergence among the tested accessions. Such geographical and genetic distribution discordance and moderately low divergency is partly due to inter- and intra-ethnic movements leading to continues exchanges of genetic materials or accessions from the same origin might developed different genetic backgrounds and *vice versa*. Similar trends have been reported in *P. edulis* (Wayessa Garedew *et al*., 2013), anchote (Tilahun Wondimu *et al*., 2014), maize (Alom *et al*., 2003), ground-nut (Gemechu Keneni *et al*., 1997), sponge gourd genotypes (Quamruzzaman *et al.*, 2011), and sweet gourd (Masud *et al.*, 1995).

Moreover, traits with maximum contribution to divergence are very important for deciding the purpose of further selection and the choice of parents for hybridization. Kumar and Debey (2003) further suggested that, while selecting genotypes from a particular cluster, inter-cluster distance, cluster mean and percent performance needs to be taken into consideration. Hence, accessions falling under clusters 3, 6, 7 and 8, which had maximum deviation from the total mean, showed higher performance in seven traits, number of primary branches per plant, stem girth, number of tubers per hill, tuber weight per hill, number of plants per hill, and stem girth. These traits are believed to be directly influencing yield and therefore helpful for selection of

parents especially for heterotic breeding. Furthermore, woredas of accessions under these clusters are recommended as superior groups and can be used for further improvement programs since superior lines in terms of genetic diversity and agronomical properties are important for selection for improvement studies (Sandhu and Gopal, 2006; Pandey *et al*., 2005).

Similarly, Singh and Chaudhary (1985) pointed out that divergence analysis helps to identify the diverse genotypes for hybridization purpose and hence, genotypes (clusters) separated by the largest statistical distance show the maximum divergence and are important sources for hybridization. In this view, all the clusters revealed a significant pairwise generalized square distance suggesting the existence of wide range of diversity among the clusters and thus, offers good opportunity for obtaining transgressive segregants. Moreover, crossing of parents for selected traits of interest from those having larger contribution for the largest statistical divergence in clusters 5 vs 7, 6 vs 7, and 8 vs 7 could produce highly desirable recombinants. However, the special advantages of each cluster or genotype within a cluster in selecting parents depends on the specific targets of hybridization (Gemechu Keneni *et al*., 1997). There have been similar reports in *P. edulis* accessions (Wayessa Garedew *et al.,* 2013), sweet potato populations (Tairo *et al.*, 2008; Karuri *et al*, 2010), Irish potato accessions (Ahmadizadeh and Felenji, 2011; Arslanoglu *et al*., 2011; Mondal, *et al*., 2007), and durum wheat landraces (Ahmadizadeh *et al*, 2011).

5.6. Cytogenetic Analysis

Although molecular methods are nowadays the most popular and fast-track tools to study systematics and evolution, it is often essential to note the chromosome number and karyotype of species (Stedje, 1996). Cytological studies, including chromosome number and karyotype analysis have been considered as reliable guides in studying taxonomic and evolutionary relationships of plant species (Soliman, 2000). Hence, well organized information on

chromosome numbers and comparative karyology are fundamental to overall understanding of genome in different species or in morphologically diverse populations within a species (Kumar and Raol, 2002) and, knowledge of chromosome number and behavior might further suggest something of potential interspecific crossability among related species (Chambers, 1961). In view of such fundamental significances, the somatic chromosome number of Ethiopian potato, a morphologically highly diverse crop, was counted to be fifty-six (2n = c.56). However, size (length) of the chromosomes was too small to make detailed cytogenetic analysis, such as karyotyping and determining number of satellite chromosomes, and this hindered making further cytological analysis. At metaphase stage, where fully condensed, the chromosomes have very small size and so morphological features such as centromeres, can not be detected. Thus, it is difficult to determine the arm ratios and describe other morphological features of the chromosomes. The present result is in agreement with several previous reports that states the impracticality of drawing meaningful karyotypic analysis in most taxa of the Lamiaceae in general and the species of *Plectranthus* in particular because of the very smaller size and larger numbers of the chromosomes (De Wet, 1958; Endalkachew Mintesnot, 2007).

Similarly, chromosome number and ploidy level determination has generally been used for taxonomic purposes and as possible clues to the evolution of related groups of species (Garber, 1972; Stace, 2000). In this regard, polyploidy series tips the hierarchical evolutionary levels of descent where, lower levels are primitive and the higher ones are derived, but occasional reductions from higher to lower levels are not impossible (Stebbins, 1971). In addition, information on change in chromosome number could be used to trace possible evolutionary patterns at generic and family levels (De Wet, 1971). In this view, the ploidy level of Etiopian dinich is speculated to be heptaploid (2n = 7x = 56) or octaploid (2n =8x = 56). This speculation was made on the bases of basic chromosome number reports (x=7 or 8) for a number of species of the Lamiaceae at large and several species of the genus *Plectranthus* from Ethiopia, South

Africa, and different parts of Asia, and for other related genera such as *Ascocarydion, Iboza*, *Hemizygia* and *Orthosiphon* (Scheel, 1931; Golubinski, 1938; Furosato, 1940; Vaarama, 1947; Darlington and Wylie, 1955; De Wet, 1958, and Endalkachew Mintesnot, 2007).

A number of species of the genus *Plectranthus*, including *P. coesta, P. striatus,* and *P. wightii* (Krishnappa and Basavaraj, 1982) and *P. rogosus* (Mehra and Gill, 1968) have been reported as being polyploids with a series of basic numbers (x=6, 8, 12 or 16). Similarly, four species of the genus in Ethiopia, *P. caninus* and, *P. ornatus (*x=6 or 10), *P. pseudomarrubioides* (x = 8 or 16), and *P. punctatus* (x = 7 or 8), among which the last had the same chromosome number and morphology as Ethiopian potato, have been reported as being polyploids with a series of basic numbers including x=7 or 8 (Endalkachew Mintesnot, 2007). Furthermore, few genera, such as *Coleus*, a closely related genus to *Plectranthus*, and *Ocimum, O. tenuiflorum* (Datta and Moumita, 2005), *O. lamiifoium* (x= 6 or 7) (Endalkachew Mintesnot, 2007), and *O. grassimum, O. americanum* (Harley and Heywood, 1992), *O. citriodorum, O. minimum, O. tenuiflorum* (Paton and Putievsky, 1996) were reported as having different and a series of basic numbers (x= 6, 7, 8, 9, 10, 12 13, 16 and 19), with a possibility of aneuploid series. A large number of species in the genus *Salvia* (Yildiz and Gucel, 2006; Xun *et al*. 2005), *Satureja* (BC Flora, 2007), and *Thymus* (Morales, 1996), all belonging to the Lamiaceae family, have also been reported as having polyploidy cases and a series of basic chromosome numbers.

In general, the result is, to our knowledge the first report on Ethiopian potato and such a polyploidy case may suggest longer term evolutionary successes that at the beginning, have generated the necessary genetic diversity that has been under positive selection for long periods, and then associated with phenotypic novelty, adaptability and higher fitness. It might also signpost the early selection and domestication events carried out on the crop. In addition,

a comparatively high ploidy level could suggest its recent divergence in the course of evolution (Stebbins, 1974; Sutar *et al*., 2013).

The equal number, and seemingly similar morphology of chromosomes in Ethiopian potato and *P. punctatus*, that is believed to be the wild relative of Ethiopian potato, could indicate the probability of artificial or natural cross hybridization between the two species for selected traits of importance.

Likewise, there are also several reports suggesting diploidy cases as being common in the family Lamiaceae (Esra, *et al*., 2011; Ranjbar and Mahmoudi, 2013; Ranjbar, Mahmoudi and Jahaniyan, 2016). A recent work on *Plectranthus barbatus* (Reis, 2015), a closely related species to Ethiopian potato, has reported a di-ploidy case which in general suggests the possibility of various ploidy levels (wide differentiation) among members of the genus *Plectranthu*s.

Further detailed cytogenetic work, including meiotic behavior analysis, is very important to understand the correct polyploidy type to find out if there might also chromosome variants or cytotypes existing among Ethiopian potato morphotypes from varied agro-ecologies.

5.7. Extents and Patterns of EST-SSRs based Genetic Diversity in Ethiopian potato Populations

Microsatellite (SSRs) marker is one of the widely applicable genomic tools in genetic diversity analysis, marker-assisted selection (MAS), genetic conservation etc. in a number of plant species because of its wide genome coverage, relative abundance, amenability to automation and high throughput genotyping (Kalia *et al*., 2011). The high cost of library screening and clone sequencing in its development is recently compromised by EST-based approach which

is highly cost effective (King *et al*., 2008) and in addition, it is highly transferable across genera and species which is central to species with no or minimal sequence information. One example of such species is Ethiopian potato, an indigenous tuber crop of Ethiopia that plays an indispensable role in food security of subsistence farmers in areas where it is cultivated.

5.7.1. EST-SSR markers development and validation for Ethiopian potato

So far, there is no report on the extents of molecular diversity in general and EST-SSRs based marker system in particular for Ethiopian potato. Hence, as the first step in the development of genomic tools and resources that can promote the conservation and breeding of this crop, we developed and validated 20 polymorphic EST-SSRs. This work reports the transferability of SSR markers from *P. barbatus* to *P. edulis* and hence it enriches the limited reports on cross-genera (Karaca *et al*., 2013) and cross-species (Mussa Adal *et al*., 2015) transferability of molecular markers, and molecular marker based genetic diversity analyses of Lamiaceae species. In the present study, the screening of 3263 *P. barbatus* ESTs resulted in 301 sequences (9.2%) containing SSRs. This proportion suggests that EST-SSRs are less abundant in *P. barbatus* than in *Salvia miltiorrhiza* (14.7%) (Xu *et al*., 2013) and *Lavandula* species (18.8%) (Mussa Adal *et al*., 2015), and slightly more abundant in *P. barbatus* than in *Mentha piperita* (8.4%) (Kumar *et al*., 2015), which are all Lamiaceae species. The results also suggest that the abundance of the EST-SSRs in *P. barbatus* is higher than their abundance in cereal crops such as rice (4.7%), sorghum (3.6%), barley (3.4%) and maize (1.4%) (Kantety *et al*., 2002). However, other factors such as the SSR search criteria and size of the dataset might have partly contributed to the differences (Varshney *et al*., 2005).

A maximum of two alleles are expected per individual plant at single copy microsatellite locus in diploid species. In the present study, a maximum of two alleles per plant were detected at each of the 20 loci, indicating that they are all single copy and have disomic inheritance

regardless of the so far reported basic chromosome number and ploidy level for several members of the genus *Plectranthus* (De Wet, 1958; Morton, 1962; Endalkachew Mintsnot, 2007) and the ploidy level, heptaploidy or octaploidy, we speculated for the crop. However, the speculation needs further in-depth cytogenetic analysis that needs to be coupled with the type of polyploidy to come up with correct justification. The expected (source species i.e. *P. barbatus*) and observed (individual plants analyzed i.e. *P. edulis*) allele size (fragment size in bp) showed slight differences. This disparity could be attributed to chromosomal rearrangements during the evolution of the Ethiopian potato genome, strand slippage during DNA replication, or the effectiveness of the gene marker used for fragment scoring.

The EST-SSR markers developed in the present study contained di-, tri- penta- and hexa-nucleotide repeats. Studies have shown that di, tri and tetra-nucleotide repeat SSRs are the most commonly used motifs in molecular genetic studies (Selkoe and Toonen, 2006). Tri-nucleotide repeat motifs were relatively abundant. This is attributed to the fact that they are more frequent in the EST's coding regions, unlike in non-coding regions, in almost all the taxa studied (Toth *et al.,* 2000; Varshney *et al*., 2005; Eujayl *et al*., 2004; Wang *et al*., 2011) because of the positive selection for specific amino-acid stretches (Metzgar *et al.,* 2000) or the prevalent selection against frameshift mutations in these regions for dinucleotides and other non-triplet repeat motifs (Li *et al.,* 2002).

As length and total size of perfect array of microsatellites increases, the frequency of repeats decreases and hence the informativeness increases (Metzgar *et al.,* 2000; Katti *et al.,* 2001) owing to the higher mutation rates in longer microsatellites (McConnell *et al.,* 2007; Lagercrantz *et al.,* 1993). In agreement with this, the average number of repeats in dinucleotide SSRs is higher (8.7) than trinucleotides SSRs (5.7) among the SSRs developed in the present study. Similarly, the informativeness in terms of a number of alleles, GD and PIC was higher

for di-nucleotide repeats than trinucleotides, suggesting higher rate of evolution for SSRs with shorter repeat motifs than SSRs with longer repeat motifs.

The use of molecular markers for efficient selection of genotypes with desirable traits and enhancing the efficiency of breeding by allowing effective simultaneous selection of various desirable traits is a well-established approach (Dudley, 1993; Edwards *et al*., 1992). Hence, the large number of alleles detected in the present study suggests the suitability of microsatellites in general and those developed in this study in particular for genetic linkage and QTL mapping of desirable traits followed by marker assisted selection (MAS) in breeding programmes. However, most of the detected alleles were rare and scarce suggesting minimum selection pressure against the alleles. Otherwise, clonally propagating crops are expected to bear less proportion of such alleles as compared to seed-propagating crops. Moreover, the higher number of private alleles observed at several loci (Example: PE_13, PE_31, PE_36, PE_15) could offer a good opportunity to evaluate Ethiopian potato genetic materials for the association of particular alleles with traits of interest and for conservation. Such alleles are useful in comparing diversity between species or populations (Mahmodi *et al*., 2013) and also for measuring genetic distinctiveness of individuals in a population (Kalinowski, 2004).

The average percent polymorphism per population revealed in the present study across the 20 loci (94%) is by far greater than the level reported by Kumar *et al*., (2015) for 13 accessions of *M. piperita* (61%), which shares the same family with Ethiopian potato. However, it was similar with that reported for 28 alfalfa accessions (97%) (Wang *et al.,* 2013) and 37 *Opium poppy* accessions (96%) (Selale *et al*., 2013). Such high percent polymorphism together with the PIC values obtained, which provides an estimate of the discriminatory power of a locus (Marulanda *et al*., 2014), and the allelic diversity suggest great potential of the markers for use in future genetic studies. However, the informativeness of a considerable number of loci is low

and hence, there is a need to develop more highly informative EST-SSRs or other type of DNA markers that are suitable to characterize Ethiopian potato genetic resources for efficient conservation and breeding.

The loci studied revealed differences between Ho and He in which half of them showed excess heterozygosity that led to a significant departure from HWE across populations. Such excess heterozygosity is expected in historically outcrossing species that maintain their heterozygosity through vegetative propagation, or if other factors such as natural and artificial selection pressure favor heterozygosity, or minor genotyping errors like null alleles we detected might have contributed (Morin *et al*., 2009). Similar results have been reported in sweet potato (Zawedde *et al*, 2014; Gao *et al*., 2011). In addition, Fisher's exact test, assuming HWE and collapsing less frequent alleles revealed a significant ($p<0.05$) and higher (49.47%) pairwise genotypic linkage disequilibrium compared to other cereal crops like maize (*Zea mays* L.) (9.7%), (Remington *et al*. 2001). If the loci are not linked, the observed higher LD could be an effect of currently declining population size, a low recombination rate or natural selection (Gupta *et al.,* 2005) or genetic isolation between populations because of the usually practiced clonal propagation method unlike outcrossing populations that are assumed to have relatively low LD (Jin *et al*., 2010). Hence, SSR markers in general and the developed loci in particular are powerful in detecting the breeding nature of tuber crops which is the key for further breeding programs and conservation measure.

In general, the current markers detected a wide range of genetic variation among the entire populations. Moreover, the polymorphic information contents of the loci across populations look wider (0.05 – 0.66). However, despite the range, considerable number of loci are in the range of slightly informative. Thus, a thorough screening procedure should be applied to

identify highly variable polymorphic loci that are suitable to group the crop genetic resources into certain classes for efficient conservation and breeding.

5.7.2. Levels of genetic diversity among populations of Ethiopian potato and implication for selection and conservation

Genetic diversity of plant species is determined by several factors including reproductive biology, life (evolutionary) history, range of geographic distribution, and different environmental factors that cause mutations to create differences within a species or population (Loveless and Hamrick, 1984; Hamrick and Godt, 1996, Milgroom, 1996). Rampersad *et al*. (2013) suggested that high genetic variation may be a function of population size i.e larger and older populations have higher levels of maintained gene diversity compared to a recently colonized habitat because of the sufficiently enough time in the older population to allow mutational events to introduce new genetic variants and decrease the effects of drift and, thereby, increase the allele frequencies to quantitative levels.

Higher genetic diversity is important in increasing fitness and, therefore, reducing the likelihood of local extinction (Futuyma, 2008). However, the mean observed heterozygosity (0.39), Shannon's information index (0.61) and Nei's gene diversity (0.35) obtained in the present study showed a medium level of genetic variation within populations. This could be mainly due to a relatively narrow genetic basis of the populations that resulted from limited germplasm resources accessible to farmers, or due to reduction in population size both due to natural as well as human factors, such as replacing cultivation of Ethiopian potato by other crops. In addition, farmers' preferences for selected traits of economic importance (Wang, *et al.*, 2011) and asexual mode of reproduction of the crop (clonal propagation) (Mignouna and Dixon, 1997; Emperaire *et al.*, 2001; Raji *et al*., 2007) could have contributed to limited genetic variation in Ethiopian potato populations.

Wen, *WSo*, and *Awn* populations are genetically more diverse than the other populations as estimated by parameters such as gene diversity, heterozygosity and Shannon diversity index and hence the areas representing these populations could be considered as genetic diversity hot spots and a potential *in-situ* conservation sites for Ethiopian potato. Among the populations, *EWo* has the least genetic diversity, which might suggest current rapid genetic erosion from the area (population bottleneck) (Hawks, *et al*., 2000) or intensive artificial selection pressure to maximize tuber yield. However, there is no clue on recent population bottleneck effect for this population which might be due to the fact that clonally propagating crops are less affected by drift irrespective of their reduction in population size.

Evaluation of populations allelic richness (allelic diversity) and private allelic richness (private allelic diversity) is an alternative criterion to detect the extent of genetic diversity particularly in populations with different size and hence, their long-run evolutionary potential that especially targets conservation and management programs (Petit *et al*., 1998; Simianer, 2005; Foulley and Ollivier, 2006) since the effects of selection is limited to the initial allelic composition than allelic frequencies (levels of heterozygosity) (Hill and Rasbash, 2009). In addition, measure of allelic richness is useful for inferring the evolutionary histories of populations (Castric and Bernatchez, 2003) and to test reductions in population size (Cornuet and Luikart, 1996) as it is more sensitive to the presence of rare alleles (Leberg, 1992) which is prominent in this study, and population bottlenecks (the current status of the crop) compared to expected heterozygosity. In this regard, *Gur*, *Jim*, *WSo* and *WSh* populations are the top four in that order, and hence are more interesting in terms of genetic and evolutionary studies on this crop. Moreover, these populations except, *WSo* bear a relatively high proportion of private alleles which may indicate certain level of independent evolution of their gene pools that allowed maintenance of private alleles at a population level (Slatkin, 1985).

Deviation from HW-equilibrium in several populations for many of the developed loci also indicate the excess or deficient heterozygosity that is partly attributed to the usual clonal propagation practice or minor SSR genotyping errors like null alleles (Morin *et al.,* 2009).

5.7.3. Population differentiation and genetic partitioning

AMOVA revealed that Ethiopian potato has very low genetic differentiation among the populations, geographic regions and administrative regions, which accounted only for 3% of the total genetic variation in each of the cases. The result is in line with previous reports on clonally propagating crops, such as enset (*E. ventricosum*) (Temesgen Magule *et al*., 2015; Dagmawit Chombe and Endeshaw Bekele, 2011), as such species tend to be more diverse within populations (but largely lower than sexually reproducing species) than among populations. Likewise, F_{ST} averaged across all loci (F_{ST} = 0.03) and pairwise F_{ST} for all pairs of populations (highest value = 0.07) was generally low to moderate on the bases of Wright (1943) and Hartl and Clark (1997) suggestions, that group the levels of differentiation in to low (0.00-0.05), moderate (0.05-0.15) and high (>0.15). Wright (1943) indicated that genetic differentiation among populations can be considered high if the value of F_{ST} is greater than 0.25. This could be partly attained if gene flow, which is a powerful force to decrease differentiation among populations, is low (Nm<1) (Slatkin, 1987) or if genetic drift removes rare or scarce alleles and hence, increase private alleles within populations (Papetti *et al.,* 2012). Hence, the present study showed that Ethiopian potato has very little population sub-structuring. The low population differentiation is supported by high gene flow (mean Nm=18.29) owing to hsitorical step-wise pollen movement across populations, contemporary germplasm exchange largely in the form of tubers and rarely in the form of seeds through sharing common markets among several of the adjacent areas where different populations were collected. This study also showed the minimal effects of regions or geographic origins of

populations on genetic variation in Ethiopian potato. This could be partly explained by the extensive exchange of tubers as planting materials among farmers (gene flow), common origin of the populations, the clonally propagating nature of the crop in which only a limited number of individuals contribute tubers to the next generation, which gradually leads to recent or old population bottlenecks and hence facilitate genetic drift.

A pair-wise population genetic differentiation analysis resulted in a seven-fold variation in F_{ST} value among pairs of populations (ranging from 0.01 to 0.07). The highest population differentiation was observed between *Wen* and *HKT*, *Wen* and *WSo*, *Wen* and *YeL* as well as between *Awn* and *WSo* populations. *Wen* population showed the highest (0.05) pairwise Nei's standard genetic distance with *WSo* and *HKT* populations and is the most genetically distinct population with a mean Nei's standard genetic distance 0.04. This can be partly explained by the fact that *Wen* and *Awn* populations were collected from a relatively pocket location and are separated from the other populations with a relatively longer geographic distance that probably restricted recent seed and tuber exchange. Hence, these populations may serve as potential sources of new genetic variation of important traits that can be used in breeding programs and as potential parental sources. Accessions from Ilu Aba Bora zone *(IAB* population) showed the highest average number of pairwise differences (**Pix**) within population that might be attributed the larger number of woredas (collection sites) covered and the result implies a large amount of genetic diversity of the crop in this zone to be preserved.

In terms of the loci, half (ten) of them were represented by excess heterozygosity (negative Fis), indicating that the crop is not suitable for inbreeding because of its clonal propagation nature that in root and tuber crops preserves heterozygosity and thus keeps the original allele diversity in the population intact or nearly as high as it was. The other possible reason is selection favoring heterozygosity as it provides the best adaptive fitness. Whereas, the positive

indices in the remaining loci refers to the existence of selection against heterozygosity for certain traits (Kof *et al.,* 2008). Such disparity in fixation indices within a genome provides important insights into the reproductive biology, genome regions under evolutionary processes (or selection) and demographic history of a population (Holsinger and Weir, 2009). The value for Fst and Gst showed a slight variation for several of the loci which might be attributed to the difference in algorithms while considering mutation effect and hence, more markers could be applied to minimize the biasness introduced by any one locus (Frankham *et al.,* 2002).

5.7.4. Genetic relationship and structure among populations

Neighbour joining cluster analysis in which each population is represented by five individual plants revealed a weak clustering pattern confirming low genetic differentiation among the populations and suggesting that the genetic background of Ethiopian potato populations does not always correlate with their geographical origin. Although UPGMA and PCoA analyses also showed a certain level of populations clustering according to their geographical regions, the clustering pattern is weak to support the concept of "isolation by distance" (Wright, 1943). Similarly, structure analysis revealed a close relationship (weak sub-division) among the samples from the 12 collection zones, and in general, three inferred groups (K=3) with potential admixtures have been observed. It is interesting to indicate that all individual plants analyzed have alleles originated from the three clusters, which supports the presence of a strong gene flow that led to poor population differentiation.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Ethiopian potato accessions, evaluated at multiple sites, revealed various phenetic character states and sufficient genetic-based variability in all the quantitative traits considered except flower length. In addition, several traits, particularly the yield related ones, had high heritability, genetic advance as a percent of mean and genotypic coefficients of variation. Such a rich phenetic and genetic-based variability could be used as broad-based raw materials that offer breeders a good opportunity for successful selection in a number of directions and for appropriate conservation measures.

Cytogenetic analysis, the first report on the crop, revealed a very small sized somatic chromosome whose number is 2n=7x or 8x=c.56, where polyploidy is the most likely case in view of the basic chromosome number reports (x=7or 8) thus far in *Plectranthus* species and other taxa of Lamiaceae. Such chromosome data and polyploidy situation could be used as a baseline information for further study targeting breeding and crossing the crop (hybridization) with other closely related species and/or for mapping its genes on their respective chromosomes.

Molecular genetic diversity analysis, as the first report, again resulted in the development and evaluation of 20 new EST-SSRs from cDNA sequences of *P. barbatus* deposited in the GenBank. All those markers were polymorphic in the populations studied and are valuable genetic tools to help evaluate the extent of genetic diversity and population structure of not only Ethiopian potato but also of various species in the family Lamiaceae. The markers detected a larger number of alleles, some of which were private alleles that may be linked to important agronomic traits. The study also showed the potential of EST-SSR markers in

defining how Ethiopian potato genetic diversity is structured, and hence contribute to the development of better *in-situ* and *ex-situ* management strategies as well as selection criteria for the germplasm to be used in breeding programs for the improvement of various desirable traits in this crop. Among the 12 administrative zones and woredas considered, Wenbera, Awi and Wolaita have populations with a relatively higher genetic diversity, and hence can be considered as hot spots for *in-situ* conservation of Ethiopian potato as well as sources of desirable alleles for breeding purposes.

Analysis of genetic relationship in Ethiopian potato samples, in all the cases, revealed a weak geographic region of origin-based pattern suggesting common origin of the germplasms (common genetic base) resulted from historical or contemporary gene flow because of several factors such as cultural, ethnic, social, and/or economic linkage among the society.

Overall, this study could offer baseline information that promote further studies to exploit the high economic and endogenous values and to stop and reverse the current rapid genetic erosion of Ethiopian potato.

6.2. Recommendations

Regardless of the wide range of variations and reasonable diversity among the entire accessions for the qualitative and most quantitative agro-morphological traits tested across multiple locations, it may be of importance to further broaden the genetic base of the study through intensive germplasm accessions from all Ethiopian potato growing areas of the country to support genetic improvement of tuber yield and to reveal additional potential sites for conservation and development of best-performing varieties.

As the first report, the present study is only limited to 20 EST-SSRs which are not large enough to unveil the actual extents of genetic diversity in Ethiopian potato populations. Hence, more

of such markers as well as other appropriate and up-to-date molecular marker systems are important to generate a more reliable and exploitable data in this regard for use in efficient breeding and conservation.

Similarly, cytogenetic analysis, as the first report only presented the mitotic chromosome count and speculative ploidy level based on only few accessions. Thus, further detailed works including meiotic behavior analysis, chromosome counts for more accessions covering wide areas where the crop is grown is needed in order to confirm the chromosomes count, the correct ploidy level and type because there might exist chromosome variants or cytotypes among Ethiopian potato morphotypes and/or accessions varying in geographic origin.

From the observation during sample collection, the crop is highly marginalized and only limited to marginal plots of land that are not meant for production of other crops or grazing, not because of its less importance. Hence, immediate and collaborative efforts of all stakeholders are needed to take appropriate actions, including *ex-situ* and *in-situ* conservation measures, and creating awareness among younger farmers in order to stop or decrease the rapid local gene pool loss of the crop and to bring to production and the market chain.

Promotion of the crop should be given special emphases

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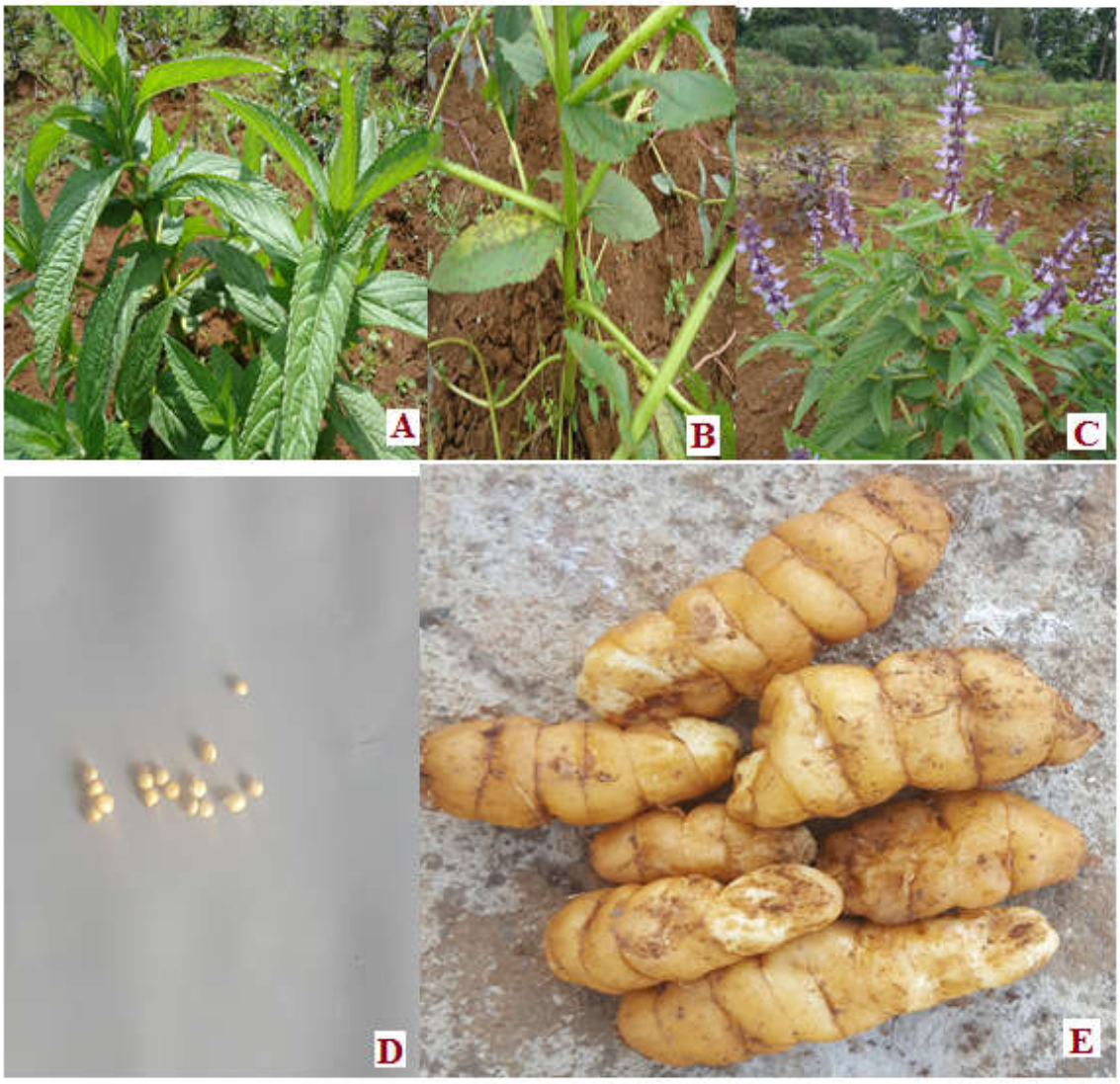
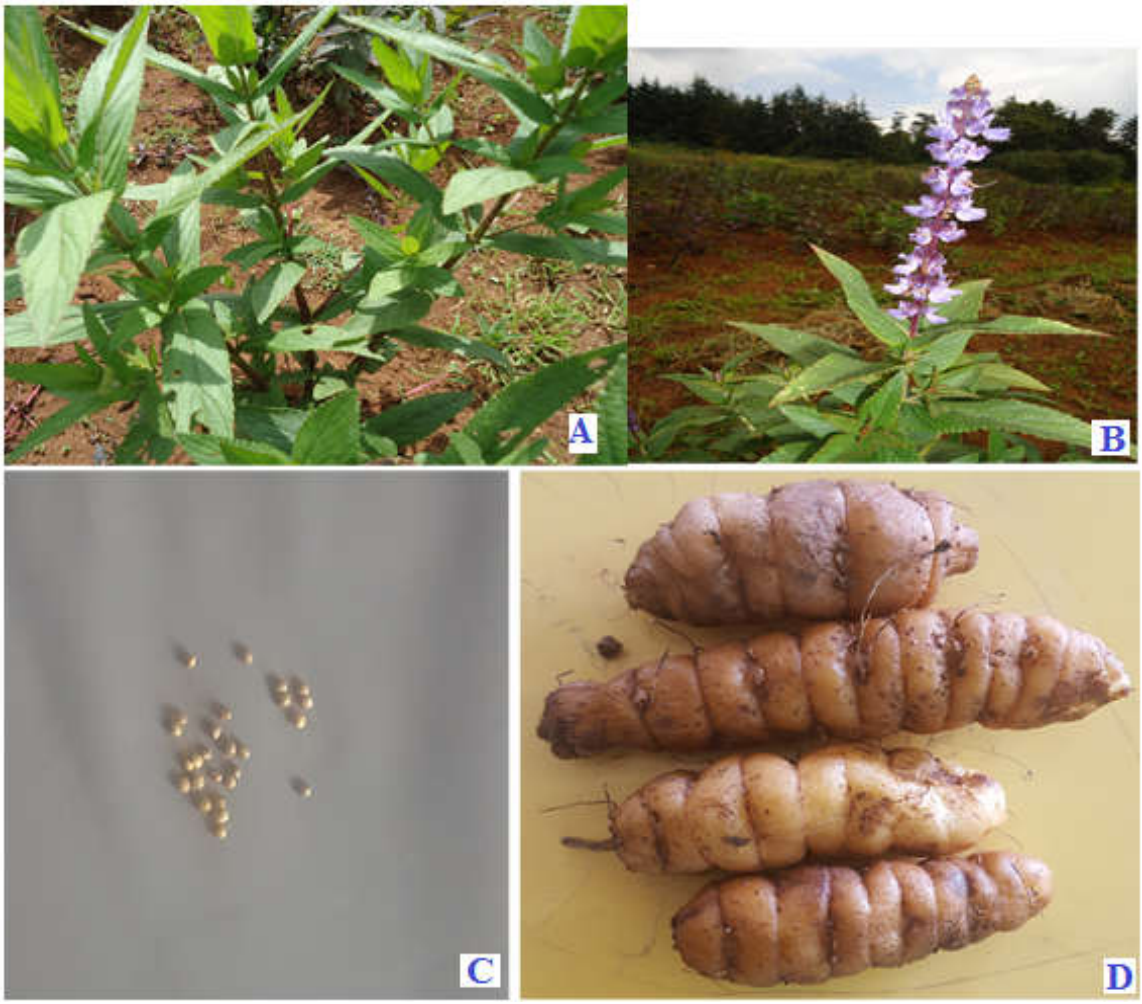
8. APPENDICES

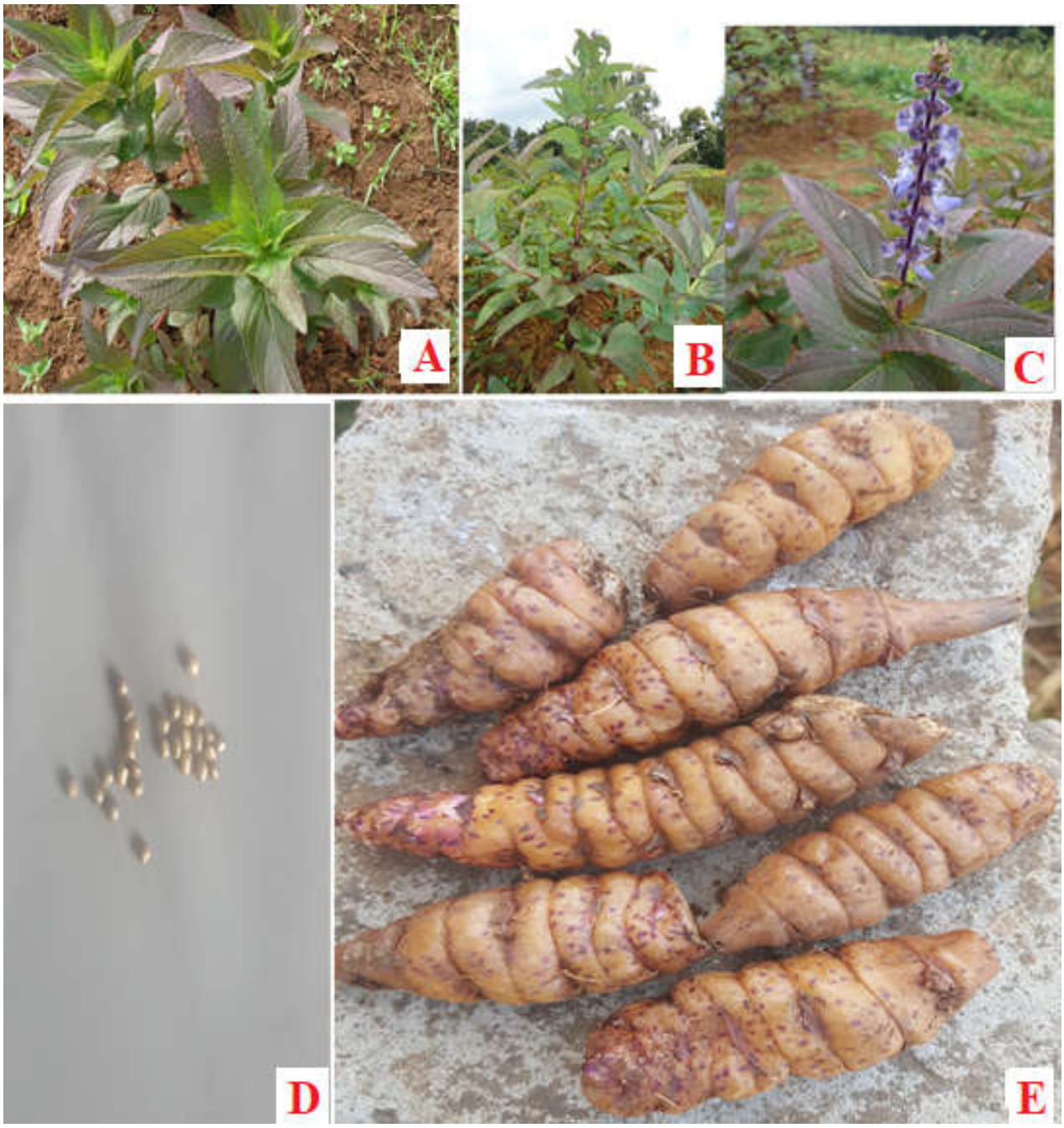
Appendix 1: Ethiopian potato major morphotypes



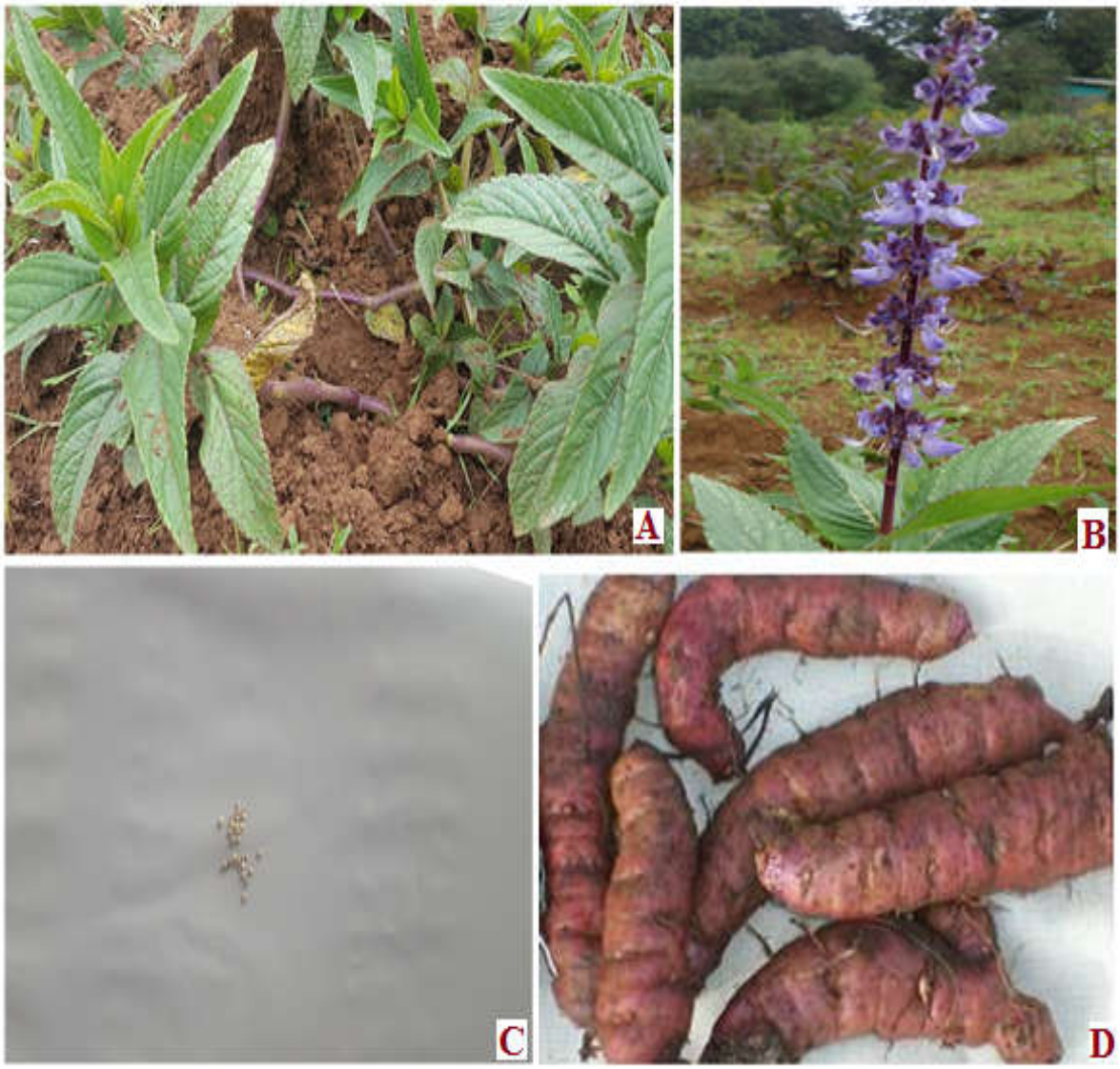
Appendix 1, Figure 1: Morphotype 1: Deep green leaf color (A); fully green stem color (B); purplish flower color (C); less ringed to smooth tuber texture (E); less to no tuber hair (E); creamy white tuber color (E); very whitish seed color (D)



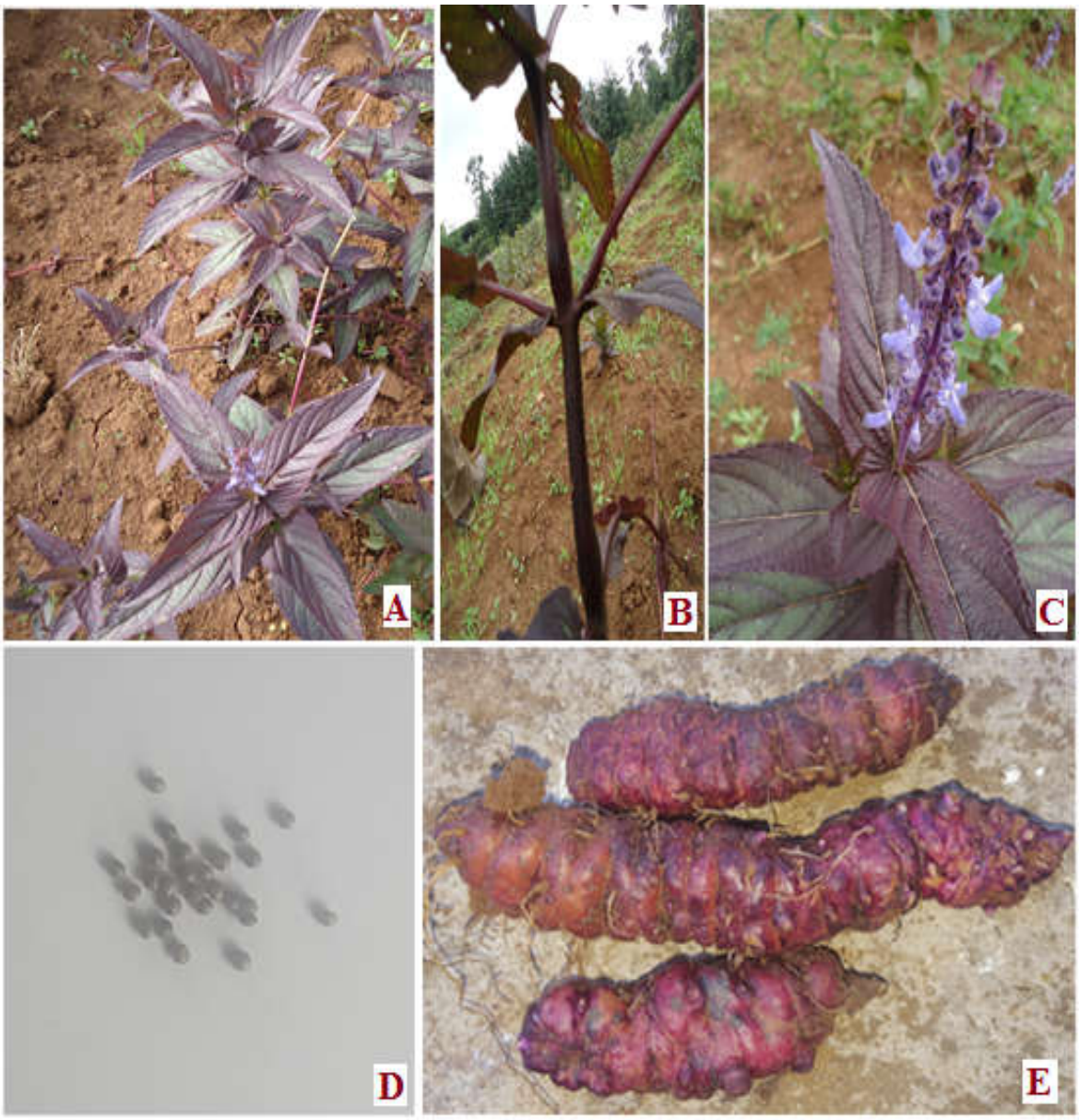
Appendix 1, Figure 2*:* Morphotype 2: Green and soft leaf color (A); purple stem color (A); purplish white flower color (B); creamy tuber color (D); moderately ringed tuber texture (D); less tuber hair (D); whitish seed color (C)



Appendix 1, Figure 3*:* Morphotype 3: Pale purple leaf color (A); pale purple stem color (B); purple flower color (C); creamy purple tuber color (E); ringed tuber texture; less hairy tubers (E); purplish white seed color (D).



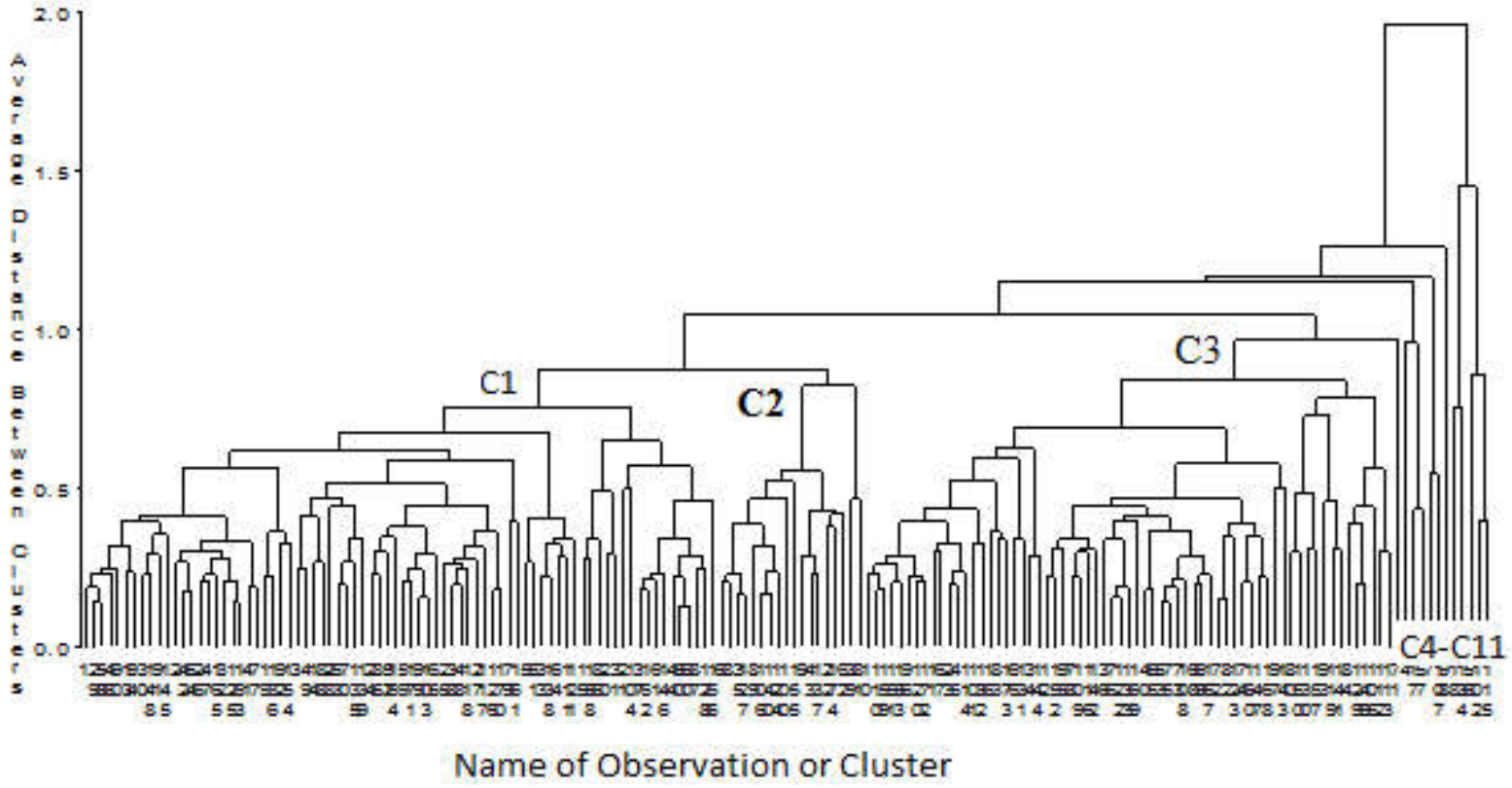
Apendix 1, Figure 4*:* Morphotype 4: Green leaf color (A); pinkish stem color with running type of growth (A); purple flower color (B); purple tuber color (D); less ringed tuber texture (D); lsee hairy tubers (D); purplish white seed color (C)



Appendix 1, Figure 5*:* Morphotype 5:. Deep purple leaf color (A); deep purple stem color (B); deep purple flower colour (C); highly ringed tuber texture (E); highly hairy tuber (E); blakish seeds color (D)

Appendix 2, Figure 1*:* Unweighted Neighbor-joining tree using the simple matching dissimilarity matrix based on 20 microsatellite markers for the 287 individual plants of Ethiopian potato collected from Ethiopia

The populations are color coded and individual plants are represented by numbers as follows: *SwSh* (5-28), *EWo* (30-53), *Awn* (55-79), *Gur* (81-104), *HKT* (106-128), *IAB* (130-154), *Jim* (156-179), *GGo* (181-204), *WSo* (206-228), *Wen* (230-255), *WSh* (257-279), and *YeL* (281-302).



Appendix 3, Figure 1: Grouping of the 174 individual accessions based on average linkage method

Appendix 3, Table 1*:* List of accessions included under each cluster in Appendix 3, figure 1

Cluster	Accessions
1(77)	ED001, ED002, ED003, ED005, ED013, ED014, ED015, ED019, ED020, ED021, ED024, ED025, ED026, ED027, ED028, ED029, ED030, ED031, ED032, ED033, ED037, ED038, ED041, ED042, ED044, ED046, ED047, ED048, ED049, ED050, ED051, ED052, ED054, ED058, ED059, ED060, ED061, ED063, ED064, ED065, ED070, ED077, ED079, ED085, ED86, ED087, ED088, ED090, ED091, ED092, ED094, ED099, ED103, ED104, ED111, ED117, ED118, ED121, ED125, ED126, ED128, ED135, ED136, ED138, ED139, ED145, ED146, ED148, ED154, ED161, ED162, ED163, ED164, ED165, ED166, ED170, ED171
2(18)	ED006, ED008, ED009, ED022, ED035, ED039, ED043, ED062, ED081, ED089, ED100, ED106, ED124, ED127, ED137, ED140, ED155, ED174
3(67)	ED010, ED011, ED012, ED016, ED023, ED034, ED036, ED040, ED045, ED053, ED055, ED066, ED067, ED068, ED069, ED072, ED073, ED074, ED075, ED076, ED078, ED080, ED082, ED083, ED084, ED093, ED095, ED096, ED097, ED101, ED105, ED108, ED109, ED110, ED112, ED113, ED114, ED116, ED119, ED120, ED122, ED123, ED129, ED130, ED131, ED130, ED131, ED132, ED133, ED141, ED142, ED143, ED144, ED147, ED149, ED150, ED151, ED152, ED153, ED157, ED159, ED160, ED167, ED168, ED169, ED172, ED173
4(2)	ED102, ED115
5(2)	ED017, ED057
6(2)	ED007, ED107
7(2)	ED018, ED134
8(1)	ED056
9(1)	ED004
10(1)	ED071
11(1)	ED098

Numbers in parenthesis indicate number of accessions grouped in to the cluster; ED stands for 'Ethiopian potato'

Appendix 4, Table 1: Combined mean performance values for 16 characters of 174 Ethiopian potato accessions evaluated at Holeta and Ambo research centers

Trt	NPB	SG	NPPH	IL	PH	LL	LD	DFI	DF	FL	TL	TD	TN	TW	TDM	YTPH
ED001	5.20	2.89	1.76	5.46	42.03	13.24	4.24	143.42	153.29	9.44	16.06	1.50	40.25	1.46	22.64	2.91
ED002	3.38	3.20	2.14	5.24	41.30	12.37	4.48	138.10	154.07	10.20	13.95	1.70	26.91	1.24	20.67	2.48
ED003	3.33	3.33	2.10	5.77	41.70	11.16	3.64	112.90	150.83	10.88	13.26	1.76	19.63	0.24	21.60	0.47
ED004	3.25	2.65	3.40	6.50	44.17	11.42	3.27	145.13	155.65	9.50	16.95	1.96	26.62	1.14	21.44	2.27
ED005	2.04	3.83	2.04	5.67	43.00	13.62	4.39	152.10	167.57	10.70	13.60	1.60	9.96	0.09	21.65	0.17
ED006	3.82	3.37	1.63	5.63	41.38	11.53	3.88	151.54	168.64	10.50	15.19	1.73	21.87	1.10	21.94	2.19
ED007	3.04	1.78	2.16	5.30	41.30	12.35	3.96	114.64	151.89	10.00	16.50	1.81	25.70	0.86	20.10	1.71
ED008	2.89	2.66	2.09	5.55	42.35	12.87	4.07	152.52	161.99	10.47	13.67	1.61	21.95	0.72	21.86	1.43
ED009	5.29	3.40	2.07	5.45	49.83	12.67	4.18	143.75	158.47	12.04	17.03	1.88	34.69	1.58	22.35	3.15
ED010	6.64	3.44	2.13	5.95	40.20	13.79	4.23	145.40	155.55	11.80	13.38	1.49	37.53	1.48	23.26	2.96
ED011	6.14	3.21	2.02	4.56	43.00	12.53	4.35	126.50	149.52	9.70	14.39	1.60	36.69	1.48	22.77	2.95
ED012	5.04	3.57	1.72	5.61	42.60	13.11	4.28	144.00	157.32	12.70	17.74	1.87	31.85	1.57	23.02	3.14
ED013	3.32	3.00	2.32	5.54	37.46	11.21	3.79	143.92	155.57	10.25	16.87	1.97	19.16	0.76	20.22	1.52
ED014	2.00	3.68	2.33	4.62	41.92	11.93	4.47	136.50	161.00	10.80	16.55	1.84	14.67	0.10	20.04	0.19
ED015	4.64	2.50	3.67	6.28	48.61	11.68	4.02	144.62	164.02	14.50	16.98	1.98	28.78	1.58	21.00	3.16
ED016	3.57	2.92	2.29	5.63	48.75	11.58	3.75	162.09	173.44	11.88	16.59	2.01	18.96	0.71	20.76	1.42
ED017	5.50	4.38	2.93	5.88	39.67	15.50	5.13	147.00	161.00	14.20	14.32	1.55	33.70	0.98	22.67	1.96
ED018	6.05	3.95	2.36	5.30	38.00	11.55	3.57	152.88	171.15	11.22	14.37	1.78	33.54	1.03	23.00	2.05
ED019	5.57	3.49	2.52	5.68	40.50	12.45	3.72	135.66	164.06	8.06	16.86	1.97	29.84	1.24	21.22	2.48
ED020	3.57	2.97	1.98	4.45	41.00	11.37	4.22	152.83	170.19	10.25	16.24	1.99	21.37	1.01	21.07	2.02
ED021	5.64	2.87	2.18	5.65	44.41	11.63	3.90	156.10	171.31	28.45	16.16	1.96	30.42	1.43	21.76	2.85
ED022	4.28	3.71	1.31	6.02	51.77	13.46	3.87	144.40	155.68	12.65	13.73	1.88	24.77	1.05	20.73	2.09
ED023	4.55	3.62	1.86	6.65	49.34	12.47	4.33	146.13	157.65	8.90	14.09	1.54	24.15	0.72	23.65	1.43
ED024	4.64	3.65	3.39	5.56	42.20	11.63	3.87	144.60	155.28	9.77	16.35	1.84	26.25	1.30	21.50	2.59
ED025	4.73	2.57	2.50	5.65	33.33	11.45	3.49	143.67	154.44	10.13	14.16	1.76	23.96	0.55	22.83	1.10
ED026	7.40	3.60	2.00	5.64	33.00	10.87	3.67	141.67	151.69	10.75	14.72	1.63	38.74	1.27	22.95	2.54
ED027	4.55	3.11	2.25	4.20	37.84	11.92	4.02	161.66	171.04	10.55	15.04	2.13	25.48	0.67	19.06	1.34
ED028	4.55	3.33	3.14	4.95	41.33	12.42	4.15	154.63	161.37	10.50	14.56	1.80	27.01	0.62	22.25	1.24
ED029	2.68	2.41	2.26	4.51	34.27	11.11	3.36	152.10	170.58	10.31	15.39	1.74	16.21	0.35	21.98	0.69
ED030	6.38	4.04	2.20	6.00	45.50	13.52	4.00	152.88	167.93	15.40	13.19	1.61	38.23	1.63	23.27	3.26
ED031	5.82	3.46	1.35	5.32	36.58	12.26	4.04	137.42	163.82	14.67	13.42	1.62	31.62	1.05	22.98	2.09
ED033	3.07	3.39	2.14	4.94	34.33	10.61	3.98	153.92	163.94	13.77	15.69	1.84	21.77	0.77	22.42	1.54
ED034	5.07	3.72	2.25	5.29	38.42	11.77	3.72	148.40	152.19	12.13	14.42	1.86	28.36	1.00	21.34	2.00
ED035	6.15	3.73	1.84	5.44	41.71	13.28	4.49	143.50	156.02	12.60	15.48	1.92	26.95	1.17	21.93	2.34
ED036	8.04	2.41	2.50	6.37	41.73	11.36	4.10	144.60	153.22	10.20	16.93	1.64	42.36	1.61	22.75	3.22
ED037	6.24	3.87	2.74	5.49	45.23	13.90	4.37	144.80	155.42	11.70	16.68	1.65	31.94	0.97	21.91	1.93
ED038	5.24	3.22	2.05	5.35	42.89	12.60	4.03	136.80	149.12	11.65	14.04	1.88	28.94	1.32	23.05	2.64

ED039	5.04	4.00	1.85	6.40	46.30	11.99	4.23	144.60	157.02	8.70	13.86	1.97	28.34	1.18	21.28	2.36
ED040	5.32	3.54	1.88	3.85	35.29	12.02	3.96	153.42	163.69	9.42	17.85	1.77	29.71	1.70	21.09	3.39
ED041	7.44	3.52	1.90	5.98	43.25	14.37	4.47	151.97	166.57	11.05	18.22	1.71	32.41	1.50	20.79	2.99
ED042	2.57	3.52	1.47	4.91	37.75	13.03	4.02	151.92	166.19	12.00	16.34	1.80	21.66	0.64	20.84	1.28
ED043	2.82	3.27	2.15	5.26	37.25	11.48	4.14	153.42	163.44	12.29	15.45	1.78	21.08	0.58	21.79	1.16
ED044	7.34	2.81	2.20	4.35	33.09	10.24	3.78	155.00	167.72	8.71	16.24	1.62	40.40	1.62	22.07	3.24
ED045	5.07	3.46	1.95	5.65	42.58	12.96	3.97	148.11	160.94	11.71	16.08	1.95	26.10	1.22	21.10	2.43
ED046	5.32	3.54	2.83	6.55	42.33	13.78	4.14	144.42	155.94	10.00	16.71	2.07	28.53	1.05	19.90	2.10
ED047	3.57	3.68	2.73	6.27	42.50	11.86	4.04	145.41	158.44	10.73	15.02	1.73	18.17	0.84	20.71	1.67
ED048	4.00	2.25	2.25	5.50	41.67	14.23	4.62	145.50	157.89	14.17	15.12	1.95	19.27	0.84	22.18	1.67
ED049	6.07	3.09	1.53	5.90	46.67	11.49	3.68	146.08	156.57	11.38	16.95	1.90	33.76	1.47	21.47	2.93
ED050	4.57	2.97	1.68	5.43	34.88	11.17	3.49	163.79	172.44	13.08	17.17	1.84	27.08	1.52	20.98	3.04
ED051	5.32	3.52	3.47	5.48	45.65	12.18	4.00	152.79	169.82	10.96	14.52	1.70	26.52	1.22	22.57	2.43
ED052	4.68	3.41	2.03	5.51	39.03	12.33	4.33	137.10	148.53	14.00	15.34	1.85	25.25	0.77	22.32	1.53
ED053	3.38	2.84	2.42	3.68	41.34	10.04	3.63	151.25	162.53	11.60	15.26	1.74	18.48	0.74	21.33	1.47
ED054	4.32	2.73	1.59	4.93	39.50	8.50	2.99	156.42	167.19	11.68	13.96	1.81	23.49	0.68	21.86	1.35
ED055	7.40	2.67	2.64	4.38	39.05	11.40	3.58	148.17	156.44	10.88	15.63	1.70	36.67	1.60	23.45	3.20
ED056	4.84	2.72	3.34	5.39	39.00	13.41	4.06	144.80	157.42	9.50	15.68	1.78	26.56	1.07	22.43	2.14
ED057	4.64	2.82	1.87	5.90	44.92	12.89	4.19	145.80	157.62	15.63	16.86	1.87	24.92	1.73	20.36	3.45
ED058	7.11	3.35	2.43	5.71	39.33	12.99	3.88	143.92	155.94	11.00	15.09	2.00	34.08	1.50	22.40	2.99
ED059	2.57	3.56	2.05	5.08	43.17	11.08	3.94	145.92	157.32	11.63	15.60	1.76	13.74	0.09	21.55	0.18
ED060	6.57	3.90	2.20	5.33	42.63	12.22	4.05	163.54	172.69	11.75	15.33	2.00	31.04	1.23	21.64	2.45
ED061	3.38	3.62	2.51	5.83	38.12	11.04	3.36	161.25	174.13	11.05	14.78	1.82	19.79	0.92	21.90	1.83
ED062	5.15	3.18	2.28	4.11	33.96	12.12	4.34	154.16	168.32	8.46	14.18	1.92	27.50	1.23	22.02	2.46
ED063	2.44	3.41	1.67	5.50	39.77	13.11	4.16	154.95	164.02	13.30	16.91	2.01	20.52	0.64	20.66	1.27
ED064	3.57	3.72	1.83	5.73	43.42	13.02	3.77	157.66	168.44	12.75	13.23	1.66	22.19	0.68	22.36	1.36
ED065	2.93	3.38	2.59	4.91	38.92	13.37	4.26	148.44	167.78	14.63	14.76	1.68	20.10	0.55	21.29	1.10
ED066	4.57	3.47	2.46	5.37	45.68	13.61	4.57	145.92	157.69	13.92	15.17	2.04	26.59	1.17	21.24	2.34
ED067	6.15	3.44	2.04	5.53	44.92	13.06	4.14	145.42	154.07	10.83	17.46	1.99	28.96	1.55	20.82	3.10
ED068	3.08	3.84	1.28	5.70	38.22	11.28	3.50	139.30	153.42	13.20	16.04	1.73	15.80	0.10	21.97	0.19
ED069	9.14	4.25	1.48	5.56	47.37	12.43	3.99	145.00	155.02	11.38	16.20	1.76	44.54	2.04	22.41	4.08
ED070	6.07	3.32	2.45	5.23	41.50	12.64	4.07	153.67	164.32	11.46	15.98	1.77	31.45	1.45	22.60	2.90
ED071	5.90	3.44	2.51	5.42	37.58	12.43	4.06	152.48	165.19	10.39	15.80	1.85	31.97	1.26	22.43	2.52
ED072	4.57	3.00	2.39	5.81	43.17	13.16	3.96	152.67	169.07	11.18	16.25	1.80	27.81	1.05	21.16	2.09
ED073	5.44	3.34	2.46	6.00	48.30	13.48	4.09	150.40	168.52	13.95	16.48	1.66	28.38	0.81	23.21	1.62
ED074	7.90	3.64	2.34	5.63	42.33	12.40	4.18	143.17	157.07	14.78	14.60	1.77	42.20	2.07	23.12	4.14
ED075	4.98	2.81	2.01	5.60	41.67	10.55	4.34	148.30	161.93	11.43	13.07	1.64	25.70	0.67	22.44	1.33
ED076	5.90	3.58	1.18	4.05	36.27	10.09	3.49	145.92	161.44	10.05	14.35	1.80	26.72	0.99	22.19	1.97
ED077	3.57	3.54	3.08	4.54	30.71	11.09	3.74	146.17	156.69	10.13	15.74	1.87	20.53	0.73	20.68	1.46
ED078	4.57	3.34	2.93	4.41	30.33	11.29	3.79	161.67	170.19	12.83	13.41	1.71	29.71	1.07	23.20	2.14
ED079	4.74	3.29	1.96	4.99	38.60	10.97	3.27	147.20	154.82	10.50	14.39	1.83	27.39	1.02	21.95	2.03

ED080	4.82	3.29	2.87	5.62	37.50	12.61	3.88	153.04	162.44	10.02	16.09	1.84	23.13	0.53	21.36	1.05
ED081	6.84	3.17	1.48	5.98	42.73	13.85	4.62	150.40	158.97	11.95	17.40	1.99	29.92	1.69	20.81	3.38
ED082	5.00	3.43	1.83	4.83	42.67	15.18	4.87	154.50	164.50	14.50	14.26	1.74	25.90	0.78	22.69	1.56
ED083	4.57	3.25	2.32	3.87	44.17	10.97	3.73	155.29	169.57	10.38	15.67	1.82	26.82	1.70	21.63	3.40
ED084	3.00	2.83	1.50	6.00	41.00	16.40	5.07	151.50	172.00	10.00	16.90	2.12	18.42	0.48	19.48	0.96
ED085	5.57	3.27	1.72	5.28	40.04	12.58	4.28	157.39	168.32	9.75	13.86	1.64	31.81	1.18	23.33	2.35
ED086	3.48	3.42	2.20	4.82	40.58	10.69	3.73	148.30	161.63	11.00	17.09	1.76	19.43	0.76	21.82	1.52
ED087	7.11	3.54	1.68	5.30	38.58	12.05	3.41	144.42	165.19	11.25	14.36	1.82	27.14	0.77	20.74	1.54
ED088	3.34	4.46	1.72	5.35	39.70	15.36	5.55	145.80	156.12	12.60	15.10	1.81	18.37	0.57	21.65	1.13
ED089	4.07	2.70	2.62	5.47	40.96	13.51	4.17	160.14	169.94	11.88	13.41	1.53	23.01	0.39	21.82	0.77
ED090	5.73	3.60	2.76	5.81	36.42	13.97	4.38	147.39	159.87	11.79	16.18	1.81	25.72	1.20	21.54	2.40
ED091	6.91	3.64	1.41	4.56	44.03	13.36	3.91	163.30	171.13	13.50	14.73	1.91	29.12	0.85	21.92	1.68
ED092	4.57	3.51	1.96	5.25	47.17	13.42	4.30	161.04	170.44	11.13	18.05	1.83	22.58	0.87	20.99	1.74
ED093	6.40	3.72	2.04	5.72	44.08	13.69	4.33	155.50	167.07	12.38	17.61	1.90	26.97	1.42	21.91	2.83
ED094	4.90	3.45	2.12	5.14	41.58	13.09	3.98	152.04	166.94	12.25	15.32	1.88	24.96	0.81	21.18	1.62
ED095	6.94	3.42	2.85	5.35	46.42	14.53	4.23	155.91	167.07	10.76	15.92	1.95	28.25	1.09	21.05	2.18
ED096	4.68	3.49	2.58	5.38	45.60	12.97	3.79	145.50	151.33	12.80	15.55	1.79	24.82	1.00	21.00	1.99
ED097	4.64	3.42	3.11	4.30	46.40	13.12	4.69	145.70	159.42	10.20	15.61	1.78	24.52	1.15	21.98	2.30
ED098	4.64	3.49	2.08	5.20	39.85	13.85	4.47	145.80	156.22	12.63	14.68	1.79	23.42	0.69	22.19	1.38
ED099	5.65	4.22	2.17	5.25	50.50	12.33	3.58	148.17	159.69	11.50	13.90	1.67	25.40	1.09	22.81	2.18
ED100	6.98	3.84	1.61	5.49	48.00	13.01	3.99	147.30	151.93	14.60	14.01	1.68	35.45	1.50	23.16	2.99
ED101	2.57	3.64	2.62	4.63	42.42	13.21	3.98	163.92	172.94	12.30	14.72	1.68	19.23	0.56	21.32	1.12
ED102	3.82	4.15	1.29	4.73	46.33	13.84	4.50	152.35	161.32	13.00	13.46	1.48	19.07	0.12	21.80	0.24
ED103	5.53	3.73	1.64	3.78	43.10	13.04	4.23	155.10	165.13	11.87	14.88	1.80	27.81	0.87	20.95	1.73
ED104	3.32	3.55	2.74	5.23	44.21	13.38	4.09	156.60	166.57	13.50	16.30	1.96	19.38	0.62	19.65	1.24
ED105	6.57	3.91	2.29	5.88	43.34	14.42	4.40	141.17	162.84	14.31	16.24	1.79	30.06	1.36	21.46	2.72
ED106	4.40	3.95	2.66	5.21	41.25	12.78	3.72	147.89	158.07	12.46	14.13	1.82	20.43	0.90	21.15	1.79
ED107	5.50	1.98	2.50	4.67	39.50	14.25	4.50	144.50	156.00	10.00	14.73	1.83	25.86	1.05	23.01	2.10
ED108	4.55	2.50	1.70	6.00	44.17	14.50	4.53	140.63	150.65	10.00	17.42	1.92	25.79	1.45	19.72	2.90
ED109	5.48	3.64	2.02	5.46	36.22	12.39	3.90	145.17	157.19	12.29	12.87	1.66	24.18	0.64	21.99	1.27
ED110	1.57	3.13	1.72	5.38	45.08	14.17	4.26	144.92	154.69	12.65	15.76	1.73	11.77	0.10	21.45	0.20
ED111	3.32	3.61	1.12	4.39	47.17	13.53	4.34	152.92	164.64	10.46	16.11	1.83	17.61	0.34	21.42	0.67
ED112	4.57	4.10	2.68	5.58	51.25	13.00	4.26	152.67	163.94	12.14	15.64	1.84	26.01	1.04	22.31	2.07
ED113	8.50	3.38	2.00	4.88	35.92	13.72	4.90	150.25	166.00	11.72	12.63	1.72	39.80	2.03	22.10	4.05
ED114	9.57	3.84	3.02	5.66	51.58	13.29	4.35	127.92	140.44	11.79	16.67	1.46	42.68	1.57	23.75	3.13
ED115	4.90	3.54	2.52	4.60	36.52	12.99	3.81	143.17	153.82	11.17	15.40	1.69	20.81	0.70	21.19	1.39
ED116	5.57	4.11	2.02	5.28	40.33	12.50	4.02	149.57	160.94	13.13	15.13	1.72	27.56	1.09	22.13	2.18
ED117	6.40	3.13	2.08	5.24	39.08	11.83	3.83	145.67	156.94	12.75	13.45	1.71	29.32	0.94	22.69	1.87
ED118	5.38	3.00	1.36	5.55	43.17	11.17	3.90	140.63	152.15	14.25	18.37	1.77	28.86	1.80	21.34	3.59
ED119	5.00	4.16	1.83	5.50	51.56	13.62	4.32	145.50	164.25	14.77	13.00	1.58	21.15	0.43	23.29	0.86
ED120	6.32	3.22	2.66	4.91	42.98	13.10	3.91	154.62	166.17	9.72	15.70	2.04	29.02	0.96	21.65	1.91

ED121	6.19	3.68	1.87	6.83	41.00	13.18	3.93	140.67	151.19	12.33	15.22	2.00	29.78	1.06	21.73	2.11
ED122	8.64	3.92	1.94	4.75	48.10	12.29	3.80	165.10	172.12	11.37	13.43	1.85	40.16	2.03	23.01	4.06
ED123	7.65	4.03	2.44	5.56	45.20	11.60	3.68	163.88	173.28	11.83	15.35	1.67	36.24	1.08	22.39	2.14
ED124	6.55	3.73	2.70	5.05	49.78	14.47	4.28	154.63	167.65	11.67	17.99	1.89	30.07	1.44	20.19	2.87
ED125	6.07	4.01	2.26	5.92	45.00	12.56	3.84	157.09	174.69	12.13	14.88	1.91	28.61	1.01	21.44	2.01
ED126	4.82	3.51	2.16	5.94	47.75	13.03	3.66	142.17	152.39	11.50	16.10	1.85	24.65	0.73	20.67	1.46
ED127	3.91	3.27	1.63	5.24	48.90	13.38	4.25	149.00	159.02	10.95	15.16	1.79	18.70	0.80	21.60	1.59
ED128	3.34	3.21	1.67	5.38	46.60	12.30	4.56	146.00	157.17	12.06	13.90	1.77	16.04	0.09	22.36	0.17
ED129	6.64	3.61	2.09	6.08	49.30	13.62	4.53	141.30	153.62	12.48	14.96	1.89	28.85	0.96	21.78	1.91
ED130	8.65	3.70	1.95	5.17	35.67	11.69	3.74	146.92	159.19	13.08	15.29	1.52	37.42	1.55	23.53	3.10
ED131	7.05	2.70	2.06	4.50	40.00	14.00	4.75	142.63	152.90	10.00	15.35	1.39	32.42	1.41	23.53	2.81
ED132	8.98	3.25	1.76	5.00	39.83	13.28	3.94	144.58	151.78	11.00	14.58	1.87	38.88	1.41	21.87	2.81
ED133	6.07	3.77	2.46	4.89	43.08	11.26	3.51	155.67	167.69	12.31	14.11	1.74	29.82	1.14	22.73	2.28
ED135	7.84	3.66	2.20	5.47	43.92	12.83	4.40	152.20	163.02	10.10	14.90	1.48	32.64	1.20	23.19	2.39
ED136	5.57	3.16	1.80	4.68	42.59	11.67	4.07	152.67	163.89	10.17	16.02	1.90	24.44	0.93	21.18	1.85
ED137	5.32	3.37	1.71	5.47	45.02	12.55	4.03	150.29	160.44	12.43	15.42	1.82	27.59	1.12	21.20	2.23
ED138	7.07	3.21	3.10	6.41	51.71	13.30	4.05	143.79	154.69	14.70	17.04	1.86	30.40	1.29	21.58	2.58
ED139	5.74	3.47	2.56	4.95	40.56	13.23	4.60	147.35	161.47	12.00	15.38	1.73	25.87	1.07	20.95	2.13
ED140	6.32	3.11	1.91	5.08	37.05	12.41	4.42	145.92	159.32	11.96	17.10	1.81	29.08	1.31	21.47	2.61
ED141	3.50	3.72	2.17	5.83	53.00	15.18	5.02	146.00	161.00	12.25	12.11	1.67	19.61	0.22	21.20	0.43
ED142	5.55	1.98	2.36	4.75	39.50	11.02	3.92	143.63	155.65	10.67	15.98	1.87	26.19	1.22	21.24	2.43
ED143	5.54	3.22	1.46	5.05	39.53	12.79	4.00	139.20	152.12	11.53	13.51	1.72	25.56	0.77	21.62	1.53
ED144	3.64	2.95	1.52	5.82	44.55	11.88	4.08	147.20	158.22	9.95	14.14	1.68	20.23	0.27	21.60	0.53
ED145	5.82	2.91	1.40	5.23	40.38	10.88	3.43	153.67	165.69	12.08	14.40	1.79	29.70	1.35	22.04	2.70
ED146	4.64	2.94	2.11	6.05	39.70	12.70	4.23	147.20	158.22	11.00	15.76	1.71	26.37	0.32	20.98	0.64
ED147	6.10	2.76	3.56	4.70	23.50	5.00	1.89	149.25	160.29	13.67	17.48	1.94	31.36	1.42	20.82	2.82
ED148	5.57	3.31	2.53	5.20	36.88	11.49	3.40	138.67	150.69	11.25	14.34	1.70	29.57	1.01	21.42	2.01
ED149	6.57	3.30	2.10	4.90	38.50	11.63	3.53	142.79	153.69	10.07	14.45	1.71	31.01	0.91	22.15	1.82
ED150	6.05	4.14	2.53	5.83	58.50	12.23	3.88	149.13	163.15	10.23	13.44	2.00	31.34	1.27	22.63	2.54
ED151	5.31	3.19	2.27	5.27	39.70	10.95	3.51	146.15	157.33	12.10	13.33	1.59	24.93	0.57	23.03	1.13
ED152	4.64	3.32	1.12	4.42	47.93	12.52	3.86	145.00	156.02	12.68	14.40	1.72	23.40	0.72	23.22	1.44
ED153	7.55	3.98	2.64	6.27	46.42	12.18	3.62	140.63	150.65	13.53	17.45	2.03	28.97	1.63	20.81	3.26
ED154	2.57	3.67	2.16	5.81	43.08	12.91	4.17	144.92	156.94	10.29	15.31	1.84	17.66	0.79	21.11	1.58
ED155	7.08	2.97	2.29	5.90	37.30	10.23	3.60	138.30	149.33	9.80	15.47	1.68	33.61	1.19	22.01	2.37
ED156	5.55	3.71	2.24	5.50	42.17	12.85	4.13	145.13	152.90	12.50	14.84	1.77	24.14	1.01	22.92	2.01
ED157	3.57	3.25	1.56	5.75	36.67	13.08	4.23	154.67	165.69	11.79	14.86	1.78	17.31	0.85	21.01	1.70
ED158	5.57	3.73	2.62	4.76	43.08	13.67	3.96	145.67	157.44	12.75	15.26	1.84	27.77	1.09	21.60	2.18
ED159	4.57	3.32	1.57	4.61	34.08	11.94	3.99	152.67	163.69	12.39	15.30	1.81	23.63	0.99	22.49	1.98
ED160	2.00	4.13	3.89	6.00	50.33	16.00	5.03	113.00	147.00	12.00	17.14	1.91	11.00	0.07	21.77	0.14
ED161	8.29	3.85	1.48	5.85	46.00	13.24	4.49	154.75	164.02	12.75	13.98	1.62	38.21	1.12	22.71	2.23
ED162	6.94	3.48	2.14	4.33	37.67	12.22	4.16	143.00	154.62	11.08	16.50	1.88	34.00	1.61	22.44	3.22

ED163	5.08	3.46	2.55	5.77	36.73	12.92	3.86	148.30	162.73	11.93	13.12	1.65	20.77	0.37	22.36	0.73
ED164	4.32	3.43	1.95	4.63	39.25	12.62	3.85	146.17	156.82	12.63	14.62	1.72	19.18	0.42	22.10	0.84
ED165	5.73	3.67	3.24	5.22	48.75	13.42	4.07	145.92	157.69	12.09	13.67	1.61	24.41	0.48	22.24	0.95
ED166	6.74	3.87	2.37	6.36	50.50	13.46	3.79	160.00	169.42	12.59	14.50	1.81	30.65	1.08	21.83	2.15
ED167	8.68	3.65	2.40	5.19	38.40	13.40	3.92	146.30	155.03	10.90	14.03	1.84	37.65	1.20	23.09	2.40
ED168	9.50	4.05	1.87	6.00	56.42	15.33	5.20	150.50	161.00	11.20	14.07	1.65	40.00	1.57	21.15	3.13
ED169	4.34	3.57	2.02	5.30	39.80	12.95	4.08	155.20	166.22	12.40	15.28	1.80	17.80	0.68	19.78	1.36
ED170	5.34	3.60	1.88	5.06	38.80	13.82	4.09	152.20	163.02	10.63	14.25	1.52	23.46	0.56	24.24	1.11
ED171	3.19	3.43	2.26	4.52	37.46	12.48	3.98	155.67	167.19	10.60	16.98	1.85	19.42	0.78	20.92	1.56
ED172	3.57	3.53	1.72	4.83	43.38	11.50	3.68	155.79	165.69	11.06	14.83	1.89	19.98	0.58	20.42	1.16
ED173	2.64	3.41	1.82	4.54	37.20	11.85	3.83	155.10	167.02	10.98	15.06	1.83	17.23	0.65	21.60	1.29
ED174	3.82	3.45	1.71	4.85	39.58	11.45	3.62	150.67	161.94	11.24	15.51	1.92	19.37	0.56	21.70	1.11
Mean	**5.19**	**3.40**	**2.18**	**5.32**	**42.03**	**12.56**	**4.05**	**148.18**	**160.75**	**11.75**	**15.28**	**1.79**	**26.71**	**1.01**	**21.80**	**2.01**
LSD(5%)	**0.97**	**0.38**	**0.38**	**0.71**	**3.32**	**1.86**	**0.60**	**1.69**	**1.99**	**3.93**	**1.70**	**0.19**	**3.62**	**0.19**	**1.52**	**0.38**
CV(%)	**13.36**	**10.20**	**16.03**	**12.55**	**7.28**	**13.21**	**13.17**	**1.00**	**1.14**	**11.63**	**9.10**	**9.42**	**12.07**	**17.85**	**6.26**	**17.91**

Appendix 5: Estimates of ANOVA for the three test locations

Appendix 5, Table 1*:* ANOVA result for location 1 (Holeta season 1)

Variables	Rep (**1**)	Block(Rep) (**2**)	Trt (**173**)	MSE (**157**)	CV (%)	R2
NPB	0.95	1.87*	5.01***	0.48	13.39	0.93
SG	2.65**	0.14	0.45***	0.18	12.63	0.77
NPPH	0.39	0.11	0.57***	0.16	18.85	0.81
IL	0.001	5.62***	0.84**	0.56	13.81	0.68
PH	203.63***	5.92	44.32***	11.39	7.79	0.84
LL	24.3**	4.05	2.04	2.28	10.4	0.55
LD	2.47***	0.01	0.21	0.21	9.65	0.58
DFI	1.62	1.11	111.39***	2.56	1.08	0.98
DF	0.02	0.36	75.81***	3.79	1.21	0.96
FL	22.15**	10.01**	5.65***	2.06	12.38	0.79
TL	0.001	0.18	4.34**	2.77	10.98	0.67
TD	0.01	0.03	0.05**	0.03	10.33	0.66
TN	5.64	10.38	87.02***	11.23	12.47	0.91
TW	0.02	0.03	0.37***	0.03	17.66	0.94
TDM	0.18	0.47	2.58	2.23	6.85	0.6
YTPH	0.09	0.14	1.46***	0.12	17.66	0.94

* = significant at p<0.05; **= highly significant at p<0.01; ***= highly significant at p<0.001; numbers in brackets represent degrees of freedom

Appendix 5, Table 2*:* ANOVA result for location 2 (Holeta season 2)

Variables	Rep(1)	Block(Rep)(2)	TRT(173)	MSE(157)	CV%	R2
NPB	0.61	0.05	5.10***	0.44	12.89	0.93
SG	0.46*	0.21	0.37***	0.08	8.60	0.83
NPPH	0.39*	0.25*	0.49***	0.08	13.33	0.87
IL	4.52***	1.26*	0.70***	0.28	9.91	0.75
PH	760.27***	6.50	43.80***	6.46	6.05	0.89
LL	1474.77***	4.94	2.35	2.53	12.78	0.83
LD	213.01***	0.74	0.23	0.25	12.54	0.87
DFI	0.01	0.52	122.43***	2.08	0.97	0.98
DF	6.78	3.46	87.46***	2.21	0.93	0.97
FL	1.19	3.88	3.23***	1.56	10.81	0.70
TL	0.02	1.03	3.16***	1.76	8.75	0.66
TD	0.02	0.001	0.04**	0.03	8.93	0.62
TN	0.24	0.35	85.77***	8.20	10.70	0.92
TW	0.003	0.04	0.36***	0.02	13.73	0.95
TDM	0.30	0.38	1.76	1.37	5.36	0.59
YTPH	0.01	0.14	1.44***	0.07	13.75	0.95

* = significant at p<0.05; **= highly significant at p<0.01; ***= highly significant at p<0.001; numbers in brackets represent degrees of freedom

Appendix 5, Table 3*:* ANOVA result for location 3 (Ambo season 1)

Variables	Rep(1)	Block(Rep)(2)	Trt(173)	MSE(157)	CV(%)	R2
NPB	1.14	1.64*	4.58***	0.51	13.63	0.93
SG	1.76***	0.13	0.30***	0.09	8.78	0.84
NPPH	0.42	0.67**	0.42***	0.12	15.62	0.85
IL	4.69**	0.11	0.74**	0.50	13.71	0.71
PH	77.94**	17.73	42.08***	11.16	8.32	0.86
LL	103.79***	11.91*	5.43**	3.23	17.55	0.75
LD	5.31**	0.27	0.51	0.42	20.51	0.67
DFI	3.36	38.65***	98.81***	1.96	0.94	0.99
DF	38.98**	1.13	80.97***	4.61	1.33	0.96
FL	0.54	75.2***	52.07***	1.91	11.43	0.98
TL	2.99	1.71	3.79*	2.68	10.51	0.69
TD	0.01	0.02	0.05**	0.03	9.01	0.74
TN	9.40	28.18	73.93***	12.81	13.49	0.90
TW	0.002	0.07	0.28	0.05	22.5	0.89
TDM	0.39	0.32	2.23	2.19	6.81	0.61
YTPH	0.01	0.27	1.14***	0.22	22.72	0.89

* = significant at p<0.05; **= highly significant at p<0.01; ***= highly significant at p<0.001; numbers in brackets represent degrees of freedom

Appendix 6, Table 1*:* Allelic richness per locus per population based on minimum sample size of 18 diploid individuals

Loci/Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*	**Locus Mean**
PE_01	3.00	1.82	4.00	3.61	2.00	1.00	3.49	3.74	3.56	3.00	3.74	2.00	**2.91**
PE_02	2.94	2.75	4.44	2.78	3.00	3.00	3.99	2.96	3.00	2.00	3.78	2.00	**3.05**
PE_05	3.69	1.86	4.91	2.78	3.56	2.70	2.74	2.00	3.70	3.78	3.70	4.45	**3.32**
PE_06	1.78	1.99	1.75	2.00	1.99	2.00	2.00	2.00	2.00	2.00	2.79	1.97	**2.02**
PE_09	2.69	3.95	2.72	4.57	2.98	2.94	4.70	3.74	4.35	2.69	4.56	2.82	**3.56**
PE_12	4.00	4.81	4.70	5.49	4.00	5.65	4.00	5.75	5.57	5.71	4.95	4.97	**4.97**
PE_13	1.00	1.00	1.78	4.48	1.00	1.00	1.00	1.94	1.96	2.60	1.00	1.86	**1.72**
PE_15	2.00	1.00	1.00	1.00	2.00	2.72	1.97	2.50	1.00	1.00	1.96	1.97	**1.68**
PE_16	3.99	3.94	5.88	3.75	4.74	3.94	3.99	3.82	4.95	4.00	4.52	3.86	**4.28**
PE_17	2.94	2.00	2.00	2.96	2.97	3.44	2.82	2.00	2.00	2.00	2.00	2.00	**2.43**
PE_22	2.00	2.96	2.00	4.63	3.00	3.00	2.99	2.99	4.96	2.00	2.99	3.81	**3.11**
PE_23	2.98	2.00	2.93	3.97	3.00	3.69	4.89	3.00	3.00	3.70	3.90	3.76	**3.40**
PE_24	1.99	2.57	1.72	1.96	1.82	1.94	2.57	1.00	2.00	1.72	1.00	3.76	**2.00**
PE_25	2.00	2.00	1.93	2.75	2.00	2.00	2.00	3.49	2.78	1.99	2.00	2.00	**2.24**
PE_30	2.57	2.88	2.00	3.82	1.78	2.44	3.92	2.99	2.78	2.00	2.95	1.82	**2.66**
PE_31	2.75	2.75	3.00	4.56	5.61	2.98	4.25	4.49	4.97	2.86	3.90	6.24	**4.03**
PE_33	3.69	2.94	3.92	2.00	2.78	3.65	2.94	2.00	2.96	3.91	2.00	2.00	**2.90**
PE_36	4.68	3.00	3.00	3.00	3.82	2.97	4.52	3.00	3.00	3.78	3.00	3.00	**3.40**
PE_37	3.00	2.99	2.93	3.50	2.00	3.00	2.78	2.75	2.96	2.00	3.57	2.97	**2.87**
PE_38	2.00	2.00	2.00	2.96	2.00	2.00	2.00	3.69	2.00	2.00	2.00	2.00	**2.22**
Pop Mean	**2.78**	**2.56**	**2.93**	**3.33**	**2.80**	**2.80**	**3.18**	**2.99**	**3.17**	**2.74**	**3.01**	**2.96**	

Appendix 7, Table 1: Private Allelic Richness across populations and loci based on minimum sample size of 18 diploid individuals

Loci/Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*	**Locus Mean**
PE_01	0.000	0.000	0.217	0.818	0.000	0.000	0.000	0.000	0.000	0.000	0.783	0.000	**0.152**
PE_02	0.000	0.001	0.751	0.000	0.000	0.000	0.062	0.000	0.000	0.000	0.783	0.000	**0.133**
PE_05	0.110	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.311	0.001	0.252	0.036	**0.059**
PE_06	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.818	0.000	**0.068**
PE_09	0.163	0.000	0.003	0.953	0.000	0.942	0.060	0.000	0.000	0.027	0.004	0.055	**0.184**
PE_12	0.000	0.008	0.005	0.035	0.000	0.026	0.000	0.031	0.783	0.071	0.000	0.012	**0.081**
PE_13	0.000	0.000	0.783	2.730	0.000	0.000	0.000	0.942	0.208	0.822	0.000	0.078	**0.463**
PE_15	0.000	0.000	0.000	0.000	0.000	0.720	0.000	0.750	0.000	0.000	0.957	0.000	**0.202**
PE_16	0.000	0.000	0.942	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	**0.079**
PE_17	0.942	0.000	0.000	0.049	0.272	0.027	0.010	0.000	0.000	0.000	0.000	0.000	**0.108**
PE_22	0.000	0.000	0.000	0.825	0.000	0.000	0.000	0.000	1.030	0.000	0.000	0.007	**0.155**
PE_23	0.000	0.000	0.000	0.003	0.000	0.075	1.217	0.000	0.000	0.720	0.001	0.002	**0.168**
PE_24	0.000	0.031	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	1.009	**0.089**
PE_25	0.000	0.000	0.000	0.041	0.000	0.000	0.000	0.791	0.049	0.000	0.000	0.000	**0.073**
PE_30	0.000	0.000	0.000	0.000	0.000	0.000	0.942	0.000	0.000	0.000	0.000	0.000	**0.078**
PE_31	0.000	0.188	0.000	0.000	0.283	0.000	0.970	0.113	0.009	0.214	0.002	1.798	**0.298**
PE_33	0.750	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	**0.063**
PE_36	0.341	0.000	0.000	0.000	0.205	0.000	1.785	0.000	0.000	0.783	0.000	0.000	**0.259**
PE_37	0.000	0.000	0.002	0.001	0.000	0.000	0.000	0.000	0.004	0.000	0.783	0.000	**0.066**
PE_38	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.791	0.000	0.000	0.000	0.000	**0.071**
Pop Mean	**0.12**	**0.01**	**0.14**	**0.28**	**0.04**	**0.09**	**0.25**	**0.17**	**0.14**	**0.13**	**0.22**	**0.15**	

Appendix 8, Table 1: Test of Deviation from Hardy-Weinberg Equilibrium across Loci and populations

Loci/Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*
PE_01	0.061^{ns}	1.000^{ns}	0.003**	0.139^{ns}	1.000^{ns}	mono	1.000^{ns}	1.000^{ns}	1.000^{ns}	0.116^{ns}	1.000^{ns}	1.000^{ns}
PE_02	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
PE_05	0.038*	1.000^{ns}	0.093^{ns}	0.007**	0.196^{ns}	0.122^{ns}	1.000^{ns}	0.138^{ns}	0.004**	0.033*	0.050^{ns}	0.111^{ns}
PE_06	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}	1.000^{ns}	0.127^{ns}	1.000^{ns}	1.000^{ns}	0.068^{ns}	1.000^{ns}
PE_09	0.064^{ns}	0.230^{ns}	0.010*	0.005**	0.137^{ns}	0.024*	1.000^{ns}	0.044*	0.525^{ns}	0.000***	0.079^{ns}	0.477^{ns}
PE_12	0.001**	0.126^{ns}	0.002**	0.010*	0.000***	0.141^{ns}	0.589^{ns}	0.000***	0.000***	0.001**	0.053^{ns}	0.074^{ns}
PE_13	mono	mono	1.000^{ns}	0.000***	mono	mono	mono	0.022*	0.022**	0.020*	mono	1.000^{ns}
PE_15	1.000^{ns}	mono	mono	mono	1.000^{ns}	0.141^{ns}	0.024*	1.000^{ns}	mono	mono	0.022*	1.000^{ns}
PE_16	1.000^{ns}	0.000***	0.000***	0.646^{ns}	0.033*	0.017*	0.126^{ns}	0.529^{ns}	0.153^{ns}	0.069^{ns}	0.012*	0.847^{ns}
PE_17	1.000^{ns}	0.273^{ns}	0.052^{ns}	0.001**	0.083^{ns}	0.705^{ns}	0.103^{ns}	1.000^{ns}	0.001**	0.063^{ns}	0.144^{ns}	0.005**
PE_22	0.289^{ns}	0.052^{ns}	1.000^{ns}	0.308^{ns}	0.430^{ns}	0.033*	0.058^{ns}	0.781^{ns}	0.015*	1.000^{ns}	1.000^{ns}	0.369^{ns}
PE_23	0.133^{ns}	1.000^{ns}	0.029*	0.026*	0.005**	0.048*	0.008**	0.003**	0.000***	0.178^{ns}	0.250^{ns}	0.001**
PE_24	0.064^{ns}	0.023*	1.000^{ns}	0.022*	1.000^{ns}	0.021*	0.023*	mono	0.070^{ns}	1.000^{ns}	mono	0.008**
PE_25	0.176^{ns}	0.538^{ns}	1.000^{ns}	0.004**	0.008*	1.000^{ns}	0.021*	1.000^{ns}	0.430^{ns}	0.115^{ns}	0.132^{ns}	0.139^{ns}
PE_30	1.000^{ns}	1.000^{ns}	1.000^{ns}	0.002**	1.000^{ns}	1.000^{ns}	0.007**	0.036*	1.000^{ns}	0.003**	1.000^{ns}	1.000^{ns}
PE_31	0.340^{ns}	1.000^{ns}	0.000***	0.091^{ns}	0.019*	0.016*	0.000***	0.596^{ns}	0.000***	0.000***	0.007**	0.310^{ns}
PE_33	0.001*	0.000***	0.000***	0.289^{ns}	0.000***	0.000***	0.150^{ns}	0.126^{ns}	0.408^{ns}	0.000***	0.020*	1.000^{ns}
PE_36	0.076^{ns}	0.306^{ns}	0.050^{ns}	0.483^{ns}	0.190^{ns}	0.026**	0.002**	0.356^{ns}	0.363^{ns}	0.024*	0.650^{ns}	0.021*
PE_37	0.000***	0.000***	0.000***	0.001**	0.008**	0.000***	0.000***	0.000***	0.000***	0.000***	0.011*	0.000***
PE_38	0.051^{ns}	0.143^{ns}	0.273^{ns}	0.017*	0.051^{ns}	0.000***	0.001**	0.064^{ns}	1.000^{ns}	0.550^{ns}	1.000^{ns}	0.017*

ns=not significant, * P<0.05, ** P<0.01, *** P<0.001, mono=mono nucleotide repeats at the locus

Appendix 9, Table 1: Pairwise Linkage Disequilibrium over the entire population computed for the 20 EST-SSR loci (significance level = 0.0500) (By GDA)

Locus	PE_01	PE_02	PE_05	PE_06	PE_09	PE_12	PE_13	PE_15	PE_16	PE_17	PE_22	PE_23	PE_24	PE_25	PE_30	PE_31	PE_33	PE_36	PE_37	PE_38
PE_01	0.00																			
PE_02		0.00																		
PE_05		*	0.00																	
PE_06		*		0.00																
PE_09		*			0.00															
PE_12						0.00														
PE_13		*				*	0.00													
PE_15		*				*		0.00												
PE_16		*							0.00											
PE_17		*	*	*			*	*		0.00										
PE_22		*									0.00									
PE_23	*	*	*	*	*	*	*	*	*	*	*	0.00								
PE_24	*	*	*	*	*	*	*	*	*	*	*	*	0.00							
PE_25		*	*		*	*	*	*		*		*	*	0.00						
PE_30		*			*					*		*	*		0.00					
PE_31		*										*		*		0.00				
PE_33		*										*	*	*			0.00			
PE_36	*	*		*			*	*		*		*	*	*			*	0.00		
PE_37	*	*	*	*	*		*	*		*	*	*	*	*	*		*	*	0.00	
PE_38		*		*			*			*	*	*	*	*	*			*	*	0.00

* = significant at $p<0.05$

Appendix 10, Table 1*:* Micro-Checker estimation of Null alleles across populations and loci, and large allele dropout detection for each locus over the entire populations

Locus/Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*	Large allele dropout
PE_01	no	no	yes	no	no	no	no	no	no	no	no	no	***NS***
PE_02	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_05	no	no	no	yes*	no	no	no	no	no	no	no	no	***NS***
PE_06	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_09	no	no	yes	yes	no	no	no	no	no	yes	no	no	***NS***
PE_12	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_13	no	no	no	yes	no	no	no	yes	yes	yes	no	no	***NS***
PE_15	no	no	no	no	no	no	yes	no	no	no	yes	no	***NS***
PE_16	no	yes	yes*	no	yes	yes	no	no	no	yes	yes	no	***NS***
PE_17	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_22	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_23	no	no	no	no	no	no	no	yes*	no	no	no	yes*	***NS***
PE_24	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_25	no	no	no	yes	no	no	yes	no	no	no	no	no	***NS***
PE_30	no	no	no	yes	no	no	yes	yes	no	yes	no	no	***NS***
PE_31	no	no	no	no	yes	no	no	no	yes	no	no	no	***NS***
PE_33	no	no	yes	no	no	no	no	no	no	yes*	no	no	***NS***
PE_36	no	no	no	no	no	no	yes	no	no	no	no	yes	***NS***
PE_37	no	no	no	no	no	no	no	no	no	no	no	no	***NS***
PE_38	no	no	no	no	no	no	no	no	no	no	no	no	***NS***

no = population or locus with no detectable null allele and possibly in Hardy Weinberg equilibrium; Yes = Loci and populations showing signs of null allele; * = Loci and populations having stuttering; ***NS*** = Non-significant at 95% confidence interval

Appendix 11, Table 1: Weir (1996) estimation of population pairwise Fst using the ENA correction as described in Chapuis and Estoup (2007) (FreeNA)

Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*
SwSh	0.000											
EWo	0.017	0.000										
Awn	0.037	0.037	0.000									
Gur	0.033	0.015	0.044	0.000								
HKT	0.011	0.013	0.042	0.027	0.000							
IAB	0.009	0.010	0.026	0.025	0.006	0.000						
Jim	0.024	0.022	0.031	0.021	0.020	0.006	0.000					
GGo	0.039	0.031	0.039	0.015	0.045	0.035	0.033	0.000				
WSo	0.034	0.028	0.059	0.014	0.014	0.034	0.034	0.035	0.000			
Wen	0.049	0.054	0.016	0.047	0.060	0.053	0.040	0.044	0.060	0.000		
WSh	0.019	0.009	0.025	0.013	0.014	0.017	0.009	0.012	0.021	0.044	0.000	
YeL	0.027	0.020	0.046	0.011	0.027	0.020	0.016	0.039	0.032	0.066	0.016	0.000

Appendix 12, Table 1: Population average pairwise differences: Average number of pairwise differences between populations (PiXY) (*above diagonal*), Average number of pairwise differences within population (PiX) (*diagonal element*), and Corrected average pairwise difference (PiXY-(PiX+PiY)/2) (*below diagonal*)

Pop	*SwSh*	*EWo*	*Awn*	*Gur*	*HKT*	*IAB*	*Jim*	*GGo*	*WSo*	*Wen*	*WSh*	*YeL*
SwSh	**5.63**	*5.35*	*5.80*	*5.85*	*5.69^{ns}*	*5.83^{ns}*	*5.65*	*5.77*	*5.88*	*5.66*	*5.22*	*5.79*
EWo	0.03	**4.99**	*5.48*	*5.37^{ns}*	*5.41*	*5.53^{ns}*	*5.30*	*5.38*	*5.48*	*5.37*	*4.84^{ns}*	*5.38*
Awn	0.15	0.15	**5.66**	*5.90*	*5.96*	*5.94*	*5.64*	*5.77*	*6.08*	*5.49^{ns}*	*5.26^{ns}*	*5.87*
Gur	0.15	0.00^{ns}	0.19	**5.75**	*5.87*	*6.02*	*5.68*	*5.70*	*5.83^{ns}*	*5.73*	*5.21^{ns}*	*5.70^{ns}*
HKT	0.00^{ns}	0.03	0.25	0.11	**5.76**	*5.93^{ns}*	*5.74*	*5.91*	*5.84^{ns}*	*5.84*	*5.31*	*5.85*
IAB	-0.01^{ns}	0.01^{ns}	0.08	0.11	0.02^{ns}	**6.06**	*5.74^{ns}*	*5.98*	*6.12*	*5.86*	*5.42^{ns}*	*5.96*
Jim	0.10	0.07	0.08	0.07	0.13	-0.02ns	**5.46**	*5.69*	*5.83*	*5.47*	*5.10^{ns}*	*5.59^{ns}*
GGo	0.19	0.12	0.17	0.06	0.26	0.18	0.19	**5.54**	*5.87*	*5.59*	*5.11^{ns}*	*5.81*
WSo	0.12	0.04	0.31	0.01^{ns}	0.02^{ns}	0.15	0.16	0.16	**5.88**	*5.87*	*5.32^{ns}*	*5.91*
Wen	0.23	0.26	0.05^{ns}	0.25	0.35	0.22	0.13	0.22	0.32	**5.22**	*5.14*	*5.71*
WSh	0.05	0.00^{ns}	0.09	-0.02^{ns}	0.08	0.05	0.02^{ns}	-0.01^{ns}	0.03^{ns}	0.18	**4.70**	*5.20*
YeL	0.15	0.07	0.22	0.01^{ns}	0.16	0.11	0.04	0.22	0.14	0.28	0.03^{ns}	**5.64**

ns = non-significant; all the rest except the diagonals are significant at $p<0.05$