ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

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LOCAL USE AND MOLECULAR GENETIC DIVERSITY STUDIES OF ANCHOTE (*Coccinia abyssinica* (LAM.) COGN.) IN WEST WOLLEGA, OROMIA REGION OF ETHIOPIA

BY MESERET ABOSE WEDAJO

Thesis submitted to the school of graduate studies, Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Biology

Addis Ababa, Ethiopia

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GRADUATE PROGRAMMES

This is to certify that the thesis prepared by Meseret Abose entitled:" **local use and molecular genetic diversity study of anchote (*Coccinia abyssinica* (Lam.) Cogn.) in west Wollega, Oromia Region of Ethiopia"** and submitted in partial fulfillment of the requirements for the degree of Masters of Science in Biology complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Anchote (*Coccinia abyssinica*) is a multi-purpose, endemic and valuable food crop of Ethiopia. Currently great emphasis is placed on consumption of food that will provides nutrients and helps in prevention of disease. In spite of this facts, sufficient studies have not been conducted on local use and molecular genetic diversity of anchote. The objective of this study was to assess information on the local use and conservation, and to analyze molecular genetic diversity of anchote in west Wollega Zone of Oromia Regional State, Ethiopia. Local use of anchote data were collected through questionnaires, face to face interview and direct observation of cultivated anchote on farm land. Anchote seeds were collected in the form of gift or purchase from farmers for molecular genetic diversity study. The genetic diversity within and among accessions of anchote collected from six districts of west Wollega was assessed by use of ISSR marker. The result of the study showed that most of the farmers commonly produced, used and had sufficient experience on cultivating, usage, conservation, and management of anchote. Cultivation and management processes were found to be an important factor affecting yield quality and quantity of anchote crop. Six categories of use (food, cash income, medicine, cultural, fodder, and ornament) were recorded. Anchote crop has strong link with socio-economic and cultural, and medicinal purposes than any other crop. Different parts of the crop used for various purpose and the root was most utilized for food, medicinal and cultural purposes. The mother root or "gubo" (in Afan Oromo) is utilized part for medicinal purpose. The amplification of genomic DNA with six ISSR marker yielded 39 scorable loci, of which 79.49% were found polymorphic. The number of observed alleles ranged from 1.3846 - 1.4615 with mean of 1.7949. The effective number of alleles (ne), Nei's gene (h), and genetic diversity estimated by Shannon's information index (I) were 1.4314, 0.2544 and 0.3865 respectively. The total genetic diversity, Ht (0.2544 ± 0.0354) and the average intrapopulation genetic diversity (Hs, 0.1701± 0.0196,) with high level of gene flow (Nm=1.0096) between populations, reflecting high gene differentiation (Gst=0.3312). The AMOVA showed that high value of genetic variation was found within populations (90%), whereas a low value of genetic variance (10%) was found among populations. Cluster analysis of anchote using the NJ, PCoA and UPGMA method showed in traceable patterns of geographic origin and occasional intermixing. This is because of high gene flow among the groups. The obtained information on genetic variation between the genotype and population can be used for future conservation and breeding program of the anchote crops.

Keywords: Anchote, local use, genetic diversity**,** ISSR**,** west Wollega

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List of Acronyms and Abbreviations

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of Molecular Variance
CTAB	Cetyl trimethyl ammonium bromide
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter-simple sequence repeat
NJ	Neighbor joining
PCoA	Principal coordinates analysis
RFLP	Restriction fragment length polymorphism
RAPD	Random amplified polymorphic DNA
SCAR	Sequence characterized amplified region
SSR	Simple sequence repeat
UPGMA	Unweighted Pair Group Method with Arithmetic averages

1 INTRODUCTION

1.1 Background of the study

Plants are used for food, shelter, medicine, clothing, hunting, religious ceremonies and other products (Cotton, 1996). Different plant species have been universally utilized in the preparation of folklore medicines for several diseases, for special ceremonies and for other plant bio-products. The knowledge of such uses had always been transferred orally from generation to generation (Den Eynden *et al.*, 1992). Recently, great emphasis placed on the consumption of food that will provides nutrients to the body and helps in the prevention of diseases (Dike *et al*., 2012). Plants are being explored and engineered to produce recombinant pharmaceuticals, genetically modified food, industrial proteins and other secondary metabolites (Obembe *et al*., 2011).

Among such multipurpose plant species, anchote is an important multipurpose and valuable tuber food crop, endemic and indigenous to Ethiopia (Abera Hora *et al.*, 1995). It is a perennial climbing, monoecious, plant with 2n=20 chromosome number (Bekele Serbessa, 2017). Self and cross-pollinated root crop that belongs to family Cucurbitaceae and genus Coccinia (Edwards et al., 1995). The male and female flowers occur on separate nodes of the same plant and can be propagated both vegetatively and by seeds.

Anchote is adapted to various Agro-ecological zones and distributed in the western and southwestern parts of Ethiopia (Yosef Yambo and Tileye Feyissa, 2013). Particularly, it is widely cultivated for long history and used as traditional consumption in Wollega area of the Oromia Regional State (Amare Getahun, 1973; Abera Hora *et al*., 1995). Furthermore, traditionally produced in western Wollega Zone where the annual rainfall ranged between 762–1016 mm (Daba Mengesha *et al*., 2012). It is always cultivated in rainy season, which depending on agro-ecological zones and takes four to five months to mature to consumable root size. For these reasons, it is considered as a short season crop and, also safely store under the ground, which thus gives additional food security to the population in the times of main crop failures (Habtamu Fufa and Kelbessa Urga, 1997).

Anchote is economically useful as source of food, medicine, varieties of food items for traditional ceremonies and special food for guests (Abera Hora *et al*., 1995; Endashaw Bekele, 2007). Anchote is considered as rich with several medicinal properties and recommended to treat various health problems (Dawit Abebe and Estifanos Hagos,1991; Dandena Gelmesa, 2010). Moreover, anchote has good nutrient composition with a good supplement of vitamins and minerals compared to other tuber crops and regarded as the leading proteinious root crop with high calcium contents (Habtamu Fufa and Kelbessa Urga, 1997; Desta Fekadu, 2011). In spite of enormous uses of anchote nutritionally, its consumption not well known in different parts of the country.

Ethiopia is home to many languages, cultures and religion that have in turn contributed to the high diversity of traditional knowledge and practices of people including the use of plants. In such country, information on particular culture plant use and conservation is significant to conserve existing genetic resource, sustainable use, and food security. Each culture has different perspectives to plant use and application, thus ethnobotany and genetic diversity study of anchote plant are very important with regard to its origin, domestication, widening its genetic diversity and expanding its economic uses.

Understanding genetic resource of indigenous crop like anchote is very important for its further improvement. Particularly it plays a great role in predictable area to improve agricultural production and productivity to solve food insecurity. Various approaches can be employed for genetic characterization of crop plants. Most morphological characterization of anchote crop shows wide range of variation in morphological features (Tilahun Wondimu *et al*., 2014) but very limited work is done on anchote crop using molecular markers. Thus, it may not always allow most accurate information due to the environmental interaction; on the contrary it is well reported that molecular marker methods overcome these problems.

In spite of all the above facts, sufficient studies have not been conducted on ethnobotany and genetic diversity of anchote. Therefore, the aim of this study is to investigate local use and molecular genetic diversity of anchote populations cultivated by the farmers of west Wollega Zone of Oromia Regional state, Ethiopia.

1.2 Objectives of the study

1.2.1 General Objective

- To study the local use and analyze molecular genetic diversity of anchote in west Wollega, Oromia Regional state of Ethiopia

1.2.2 Specific Objectives

- To assess the indigenous knowledge on utilization and conservation of anchote
- To identify use categories, purpose, method of preparation, and usable parts of anchote
- To document farmers knowledge on use, naming, classification, conservation and management of anchote
- To assess molecular genetic diversity among and within anchote populations using ISSR marker

2 LITERATURE REVIEW

2.1 Taxonomy of anchote

Anchote is a perennial climbing, monoecious, plant with 2n=20 chromosome number (Bekele Serbessa, 2017). It is an important self- and cross-pollinated root crop that belongs to the family Cucurbitaceae and genus *Coccinia* (Edwards *et al*., 1995). Cucurbitaceae is one of the most diversified plant families consisting of 120–130 genera (Purseglove *et al*., 1966; Edwards *et al.*, 1995) and 940 to 980 species. The family of Cuccurbitaceae has two large subfamilies: Zanonioideae and Cucurbitoideae, which consists of more than 20 and 100 genera (or 75 spp. and 750 spp.), respectively. Ethiopia and Eritrea, as one flora region, are represented by more than 24 genera of Cucurbitaceae (2 from Zanonioideae and 22 from Cucurbitoideae) and 71 species (2 from Zanonioideae and 69 from Cucurbitoideae) (Edwards *et al.*, 1995).

The genus *Coccinia* belongs to subfamily Cucurbitoideae and possesses about 30 extant species in the palaeotropics, all restricted, in their distribution, to Africa except *Coccinia grandis* [(L.) Voigt.], which is disseminated to other continents such as America, Asia, and Australia (Schaefer and Renner, 2011).

The species status in the genus *Coccinia* [Wight & Am. (1834)] needs further investigations (Edwards *et al*., 1995). A total of 10 *Coccinia* species have been recorded in Ethiopia and Eritrea: eight in the Flora of Ethiopia and Eritrea (Edwards *et al*., 1995), and the remaining two species C. *ogadensis* Thulin, described by Thulin (2009) and C. *microphylla* Gilg by Holisten and Renner (2011). Among these species *Coccinia abyssinica* is the only species cultivated for its edible tuberous root (Habtamu Fufa and Kelbessa Urga, 1997).

A total of eight species of *Coccinia* found in Ethiopia (Jeffrey et al., 1995), only five are fully named according to the rules for giving scientific names to plants in the Flora of Ethiopia and Eritrea (Bekele Sarbessa, 2017). Those correctly named include *C. schliebenii* Harms (1932), *C. adoensis* (Hochst. Ex. A. Rich.) Cogn., *C. abyssinica* (Lam.) Cogn., *C. megarrhiz*a C. Jeffrey, and C. grandis (L) Voigt (syn. C. indica Wight & Arn.); and those not fully named are: *Coccinia*. sp = Bally 12989, *Coccinia* sp. = Burger 2947A, and *Coccinia* sp. = Gilbert & Jones 129 (Edwards et al., 1995).

2.2 Geographic origin and distribution

Understanding crop plant's geographic origin and distribution is very important for breeding, genetic improvement, and conservation managements activities of the crop. This is because of the nearby availability of the wild type and related species, which can provide adaptive value as well as broaden the genetic base of a crop species through outcrossing. In fact, the center of origin is, usually considered, as center of diversity (Sebastian, 2011).Therefore, determination of center of origin of a crop plant is important to conserve its genetic diversity, especially for those species, which are vulnerable to ecosystem fragmentation (degradation) and other anthropogenic pressures.

Cucurbitaceae species are distributed in the tropics and subtropics of both the Old and New Worlds, with hotspots of diversity in Southeast Asia, West Africa, Madagascar and Mexico (Schaefer and Renner, 2011). However, Schaefer *et al*. (2009) observed the great disjunction between related genera in their geographical distribution. These authors reasoned out that there have been many successful long-distance dispersals (by different mechanisms, usually by birds) between Asia and Africa, back to Asia, between Africa and South America, and from Asia to Australia (Schaefer *et al*., 2009; Holstein and Renner , 2011)

Holstein and Renner (2011) used molecular technology to estimate the center of origin and diversification dates of wild *Coccinia* species in eastern and southern Africa, and estimated to be around 6–7 million years ago. The genus has a broad range of agro–ecology from semi–arid habitat to moist forest (Holstein and Renner , 2011).

The genus *Coccinia* occurs in wide range of areas including the western, central, southeastern, and northern parts of the Ethiopia (Edwards, 1991). However, the well-known species for its food, medicine, and other socio-cultural values, in western and southwest Ethiopia, is only *Coccinia abyssinica* (Lam.) Cogn. (Amare Getahun, 1973).

Anchote is distributed in the western and southwestern parts of Ethiopia. Particularly, it is widely cultivated and used in Jimma, Illu-Abba-Bora and Wollega areas of the Oromia Regional State (Habtamu Fufa and Kelbessa Urga, 1997; Amare Getahun, 1973). It was also indicated that it occurs in Gonder, Gojam and Bale areas although its extent of cultivation and utilization was not pointed out (Zemede Asfaw, 1997). The plant seems to have its center of origin and diversity in the western and southwestern parts of Ethiopia (Edwards *et al*., 1995).

2.3 Importance of anchote

Anchote is an important and valuable tuber food crop, endemic and indigenous to Ethiopia that is commonly produced and used in Ethiopia specifically western Wollega (Amare Getahun, 1973; Abera Hora *et al*., 1995). It is utilized for human food, animal feed, medicine, and other socio-cultural values (Amare Getahun, 1973). The root is the most important sources of food, but sometimes people use its leaves as a vegetable (Abera Hora *et al*., 1995). Although information on nutritional and anti-nutritional contents of anchote's leaf and seed is limited, the root has been better studied and reported as possessing a higher nutritional content than other common and widespread root and tuber crops (Habtamu Fufa and Kelbessa Urga, 1997). The study on use and value of anchote is very limited. Therefore, the importance and ethnobotanical use knowledge of local people should be studied.

Anchote is the rich in protein, vitamins, and minerals like iron, calcium, and phosphate compared to other tuber crops (Habtamu Fufa and Kelbessa Urga, 1997). From a total of 100 g fresh weight, anchote tuber consists of 74.93 g moisture, 3.25 g protein and 327 mg calcium (Desta Fekadu, 2011). Anchote is considered as food security crop (Abreham Bekele *et al*., 2014). There is available information on biochemical aspects of anchote, but very limited studies have been reported on its molecular aspects. Therefore, there is a need to conduct further studies of genetic diversity using molecular markers specifically in major growing area, which is western Wollega of Oromia Regional State, Ethiopia.

Different *Coccinia* species have been reported as a folklore medicine to treat various diseases such as skin diseases, diabetes, and gonorrhea in many countries (Kuriyan et al., 2008; Blanca *et al*., 2011; Munasinghe *et al*., 2011; Sivaraj *et al*., 2011). As an example, the crude leaf extract of *Coccinia grandis* exhibited a broad range of anti-bacterial activities (Sivaraj *et al.*, 2011). Similarly, in Ethiopia ***C.*** *abyssinica* has been recommended to treat individuals suffering from bone fracturing, displaced joints and other diseases such as gonorrhea, tuberculosis, and cancer (Dawit Abebe and Estifanos Hagos,, 1991; Dandena Gelmesa , 2010). Anchote is not only a valuable tuber food crop but also used for cultural values (Reinhard and Admasu, 1994).

2.4 Propagation of anchote

Anchote is a tuberous perennial with trailing annual shoot, of several meters long, that climbs a support by means of its simple tendrils (Edwards *et al*., 1995). It undergoes an annual cycle of death and regeneration of herbaceous shoots, the condition known as hemicryptophytic life form (Schaefer and Renner, 2011).

The male and female flowers occur on separate nodes of the same plant. The two flowers bloom at different time (a dichogamy situation called protandry) (Edwards *et al*., 1995). This nature, obviously, invites outcrosses and prevents inbreeding. As protandrous species tend to be pollinated by bees or flies (Sargent and Otto, 2004), anchote is mainly pollinated by bees (Edwards *et al*., 1995).

Anchote can be propagated both vegetatively and by seeds. In the vegetative propagation, tubers are planted and used as seed sources during the next growing season. Some tubers may also be left in soil for regrowth (as 'guboo') for the coming season. The commonest way of anchote propagation is via seeds, and both cross and self-pollination may occur in anchote (Amare Getahun, 1969; Abera Hora *et al*., 1995; Jeffrey *et al*., 1995).

2.5 Challenges of anchote production and productivity

Anchote is traditionally produced in western Wollega Zone where the annual rainfall ranged between 762-1016 mm (Daba Mengesha *et al*., 2012). It is always cultivated in rainy season, which depending on agro-ecological zones takes four to five months to mature to consumable root size. For these reasons it is considered as a short season crop. It can also safely be stored under the ground, which thus gives added food security to the population in the times of main crop failures (Habtamu Fufa and Kelbessa Urga, 1997).

Anchote production and productivity is affected by Agro-ecological conditions and other factors like pests (Abera Hora *et al*., 1995). The pests include both domestic and wild animals. Porcupine, warthog, and wild pig are among a few wild animal pests of the crop. The former two pests eat anchote root by digging into the soil whereas the others consume the foliage and damage the crop by trampling on it. For commercial and higher quality anchote production, the planted area should be fenced or properly protected from damage by animal pests. Fruit boring insects can also damage the fruit and hence, reduces the production (Abera Hora *et al.*, 1995).

2.6 Genetic resources and its diversity

Genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation, desirable quality, and pest and disease resistance. Genetic divergence analysis estimates the extent of diversity existed among selected genotypes (Mondal, 2003). The grouping of genotypes indicated that geographical distribution need not necessarily be the indicator of genetic divergence (Verma and Sachan, 2000; Jeena and Sheikh, 2003). Genetic diversity measures individual variation and reflects the frequency of different types in a population (Falconer *et al*, 1996).

Genetic diversity study involves analysis of variations at individual, group, or at population levels (Mohammedi and Prasanna, 2003). The genetic diversity issue of a crop is directly related to its pollination biology. For cross-pollinating plants, genetic diversity may maintain easily unlike that of strictly self-pollinating plants. In the latter case, breeders use different methods for crop improvement such as artificial selection, hybridization, mutation breeding, and other molecular techniques (Ngampongsai *et al*., 2009).

Diversity analysis of germplasm collection can facilitate reliable classification of accessions and identification of subsets of important accessions with possible utility of specific breeding purpose, i.e., basic step for further improvement, breeding programs, cultivar release, and conservation management (Haussmann *et al*., 2004; Akhtar *et al*., 2007). These genetic characterization processes (involving both marker and analytical method choices) have different approaches with objective of the study, level of resolution required, resource and time constraints (Mohammedi and Prasanna, 2003). One may wish to characterize a crop plant by comparative anatomy, morphology, embryology, and physiology separately or complemented by molecular markers, which actually vary based on the taxonomic level of the study materials (Weising *et al*., 2005). For accessions' morphological characterization, the International Plant Genetic Resources Institute (IPGRI) descriptors have been available since its first release of descriptor list in 1977 (Bioversity International, 2007).Even though most agricultural studies focused on morphological description, nowadays there is a tendency to use more stable and reliable molecular markers. (Mohammedi and Prasanna, 2003).

For accurate and unbiased estimate of genetic diversity, by any of these markers, there are some basic considerations during sampling, experimentation, and bio-geographical distribution record, as well as choice of data analysis tools (Meirmans, 2015). Perennial root crops, similar to anchote, that can be stored for many years in the soil and sprouts on the setting of rainy season, constitute a reference genetic material on which as many molecular markers can be accumulated as desired, without limitation on the quantity of DNA (De Vienne *et al*., 2003).

2.6.1 Morphological diversity of anchote crop

Farmers, taxonomists, and breeders use the most commonly and easily observable markers called morphological descriptors for classification and evaluation of yield or other trait of interest (Singh and Parab, 2015)

As different scholars reported that anchote exhibits a great range of diversity in foliage, fruit, and root morphologies (Dandena Gelmesa , 2010; Tilahun Wondimu *et al*., 2014),which could add values as ornamental plant beside its importance as food. It is also occurring in its “wild” form that provides gene flow and ensures broad genetic diversity with little differentiation (Edwards *et al*., 1995).

Morphological studies of anchote crop revealed that wide ranges of variation in phenotypic traits. Based on pheno-morphic and agronomic traits of anchote, major quantitative traits such as fruit weight, fruit length, seed weight, petiole length, root yield and vine length are contributing to the overall diversity of anchote population (Tilahun Wondimu *et al*., 2014). Phenotypically anchote characterized with large fruit diameter and lowest seed number per locule have high protein contents in its leaves and fruit (Desta Fekadu, 2011). The root, fruit, leaves, flower, and the stem of anchote vary in size, shape, and color in agro-morphological traits of anchote accessions (Bekele Serbessa, 2017). However, phenotypic trait such as deep green and purple stem colors are rarely observed traits in different agro-morphological traits (Bekele Serbessa, 2017). In such crop having high phenotypic variation, the detailed genotypic study is needed.

Its unique physical features can characterize *Coccinia abyssinica*. The shoots have simple tendrils by which it climbs up support. The tubers vary in shape depending on environmental condition. It is highly affected by the age, soil physical conditions, anchote type (genetic) and cultivation managements. The common shapes of anchote roots are spherical and conical, whereas other shapes are due to soil structure that affects its normal growth. The outer part (skin) of anchote root is grayish, without exception, while the edible internal part has mainly two colors white, and "reddish" (Abera Hora *et al*., 1995).The root can be stayed in the soil without being damaged and enters into dormancy until the next rainy season. It increases in size and accumulate nutrients every growing season.

Anchote has ovate or broadly ovate, scabrid punctuate above, crispate-setulose on veins beneath, 7.5–15 x [7.5–17] cm, margin sinuate-denticulate, shallowly to deeply palmately 5–lobed, lobes triangular to ovate, sometimes laciniate leaves. Petiole length ranges between 1.5–16 cm, spreading-setulose. Coiling tendrils are simple, usually attach with support. Flowers of separate sexes on the same plant is observable (Edwards *et al*., 1995). Male and female flowers separate; raceme-like clustered male flowers of variable number; solitary female flowers; fruits ellipsoid, dark green with longitudinal lines of white spots, red with lines of paler spots when ripe, with variable dimensions; seeds broadly asymmetrically ovate in outline, compressed, covered in fibers (Edwards *et al.*, 1995) ; stem and leaf are solid and not hallow like most other cultivated cucurbitaceous crop (Abera Hora *et al*., 1995).

The farmers select the good "variety" based on some phenotypic and nutritional based characters such as root color, root yield, and ease of cooking as well as palatable taste. Such preference on limited traits, by farmers, of the crop may result in ending up with narrow genetic diversity, and finally leads to extinction. Therefore, such artificial selection can affect the genetic diversity of anchote, which is also common for other crop (Lasalita-Zapico *et al*., 2010). The other threat for such orphan crop is an introduction and advertisement of other, alternative, high yielder crops like Irish potato, cassava, and others (Abera Hora *et al*., 1995).

2.6.2 Molecular genetic diversity and population structure analyses

The methods based on morphological features are commonly used but they not always allow the most accurate information. This is because of morphological markers are characterized by their insufficient polymorphism, dominant, and affected by environmental conditions at different developmental stages (De Vienne *et al*., 2003).Therefore; molecular markers have been complementing it for better characterization of genetic diversity, population structure, and evolutionary studies (Sessions, 1996).

Molecular genetic diversity and population structure studies of a crop plant have great importance for germplasm collection, breeding programs and genetic resource conservation and management of the crop (Wen *et al*., 2010; Lu *et al*., 2011). Particularly it plays a great role in predictable area to improve agricultural production and productivity to solve food uncertainty in developing world. For instance, efficient utilization of anchote genetic resources requires comprehensive and systematic collection, evaluation and description.

One of the breakthroughs in biological sciences is the invention of Polymerase Chain Reaction (PCR) that amplifies DNA in vitro (Mullis and Faloona, 1987). Since then, molecular marker techniques separated into non-PCR based and PCR-based depending on the way in which they produce different DNA fragments.

The non-PCR markers include RFLP and its modifications and PCR-based markers include RAPD, AFLP, ISSR, SSR, etc. (Botstein *et al*., 1980; Williams *et al.*, 1990; Vos *et al*., 1995). The PCR-based molecular markers used for plant genetic diversity analyses with different power of genome sampling and generating polymorphisms (Weising *et al*., 2005).

Assessment of genetic diversity carried out by several types of markers in plants among which molecular markers have shown advantages over others because of their neutrality and feasibility. Another reason is that they do not depend on age and tissue type and are not influenced by the environmental conditions (Da Mata *et al*., 2009).

Many breeders select various molecular markers for specific plant crop to realize its genetic diversity for breeding program and conserve and store in the form of superior plant genetic resource such as gene bank and DNA library to preserve genetic material for long period. The detail information on genetic variability and divergence of valuable food crop is an important tool for many breeding programs (Wen *et al*., 2010; Lu *et al*., 2011). Therefore, the assessment of molecular genetic diversity of anchote crop with more suitable molecular marker helps to provide correct picture of crop extent of diversity.

In spite of availability of many molecular markers for crop genetic diversity study, very limited work has been done on anchote crop using molecular markers. Abreham Bekele *et al*. (2014)

were the first to make appreciable efforts to describe anchote's genetic diversity using ISSR molecular marker approaches and their findings revealed that the existence of significant genetic variations within and among the populations. In addition, the SSR marker-based studies of *Coccinia abyssinica* showed that the germplasm samples collected from different agroecological zones and treated under different experimental conditions showed variation among and within accessions (Bekele Serbessa, 2017). So far, more evidences were accumulated for nutritional or chemical composition and morphological description of anchote crop but detailed studies needed aspect of its molecular genetic diversity.

2.6.3 Inter-simple sequence repeat

ISSR markers are a class of molecular markers based on inter-tandem repeats of short DNA sequences. PCR amplification of these regions using a single primer yields multiple amplification products that can be used as a dominant multilocus marker system for the study of genetic variation in various organisms (Da Mata *et al.*, 2009). These regions lie within the microsatellite repeats and offer great potential to determine intra-genomic and inter-genomic diversity compared to other arbitrary primers, since they reveal variation within unique regions of the genome at several loci simultaneously. They exhibit specificity of sequence-tagged-site markers, but need no sequence information for primer synthesis enjoying the advantage of random markers (Vijayan, 2005). The primers used in ISSR analysis can be based on any of the SSR motifs (di-, tri-, tetra- or penta-nucleotides) found at microsatellite loci, giving a wide array of possible application products, and can be anchored to genomic sequences making either side of the targeted simple sequence repeats (Da Mata *et al*., 2009).

The ISSR marker is mostly dominant genetic markers just like amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) markers, and their derivatives (Vijayan, 2005). Dominant markers do not allow clear distinction between homozygote and heterozygote. These markers, however, usually produce multiple DNA fragments (each of which is considered a locus) in a single reaction, allowing the generation of a large number of loci across the genome of any species without the need to first know the DNA sequences of the target regions. Apart from its usage as genetic markers, these dominant markers can be used as initial steps for the development of co-dominant markers: RAPD for the development of single-locus co-dominant 'sequence characterized amplified region (SCAR)' markers, and ISSR for the development of single-locus co-dominant microsatellite markers (Adibah *et al*., 2012).

The generation of ISSR markers makes use of microsatellite sequences that are highly variable and ubiquitously distributed across the genome, at the same time achieving higher reproducibility compared to using RAPDs and less costs in terms of time and money compared to using AFLPs. All these make ISSR an ideal genetic marker for various studies, most notably on genetic variation/diversity, DNA fingerprinting, and phylogenetics (Adibah *et al*., 2012).

In the assessment of genetic diversity, molecular markers based on DNA have many advantages. Among the polymerase chain reaction (PCR) based marker techniques, inter-simple sequence repeats (ISSR) are one of the simplest and widely used markers. This group of molecular markers is widely applicable because it is rapid, inexpensive, require small amount of template DNA, and unlike SSR marker, do not require prior designing of primer sequence (Vijayan, 2005). ISSR marker has been efficiently used for the study of molecular genetic diversity in different seed crops like anchote (Abreham Bekele *et al*., 2014).

Since the nature of anchote crop is characterized with self and open cross pollinated (Edwards *et al*., 1995), the crop has been expected to have abundant molecular variability and tremendous scope for development of improved varieties and characterization of germplasm. The studies conducted on pheno-morphic and agronomic traits of anchote crop through multivariate analysis of quantitative traits exhibits broad range of quantitative morphological traits diversity (Tilahun Wondimu *et al*., 2014). Similarly, few studies on molecular marker-based characterization of anchote showed the abundant molecular diversity in anchote crops (Abreham Bekele *et al*., 2014; Bekele Serbessa, 2017). For more characterization, molecular marker methods have been used with success to identify and determine relationships at the individual and population level of anchote crop especially focusing on the suggested area for its center of origin and diversity, west Wollega Zone, Oromia Region, Ethiopia.

3 MATERIALS AND METHODS

3.1 Description of the study area

The present study was conducted in western Wollega zone, Oromia Region state of Ethiopia. The Zone was selected for the long history of anchote cultivation status and usage by native Oromo peoples (Amare Getahun, 1973; Abera Hora *et al*., 1995).

The study was conducted in six woredas (districts). A total of 12 kebeles, two kebeles representing each woreda were selected. The kebeles are selected because they are major anchote growing areas. Information on anchote distribution was obtained from the Departments of Agriculture and Natural Resource of the Zone (Table 1**)**.

Table 1 Description of the district and kebele administrations included in the study

No	District(woreda)	Kebele	Altitude	Mean T^o	Annual RF
1	Lalo Asabi	Hatusi Siban	1823m	19	800mm-2000mm
		Jarso Damota	1790m	18	800mm-2000mm
2	Guliso	Guji Warabu	1575m	17	800mm-2000mm
		Seda Birbir	1643m	18	800mm-2000mm
3	Ganji	Sage Guji	1760m	17	800mm-2000mm
		Kapi Gurracha	1622m	17	800mm-2000mm
4	Boji Chokorsa	Charaki Kobara	1740m	18	800mm-2000mm
		Gombo Kobara	1722m	18	800mm-2000mm
5	Ayira	Hojin Gudina	1626m	17	800mm-2000mm
		Lalisa Birbir	1602m	16	800mm-2000mm
6	Yubdo	Alaku Gabriel	1723m	18	800mm-2000mm
		Alaku Dacha	1690m	17	800mm-2000mm

(Source; Agriculture and Natural Resource Woreda Office of west Wollega Zone).

3.2 Local use study

The local use study was carried out from November to December 2017. Permission to conduct the study was obtained from Agriculture and Natural Resource Office of the Zone, Agriculture and Natural Resource Office of each woreda and from each representative kebeles. The volunteer research teams were selected and formed from both kebeles of each woreda for execution of the study.

In this study, all available and relevant information about the study area was gathered. The primary and secondary data sources were used for determination of number of kebeles, location of farmland of the farmer and on the availability of cultivated anchote. This facilitated the collection of data on local use of anchote and seed collection for genetic diversity study.

3.3 Sampling

In sampling technique, purposive sampling method was employed for selection of samples, zones, woredas, and kebeles. Six woreda/districts and two kebeles (Kebeles are the lowest administrative units) from each woreda (12 kebeless), were selected purposefully based on availability and cultivation status of anchote. Seventy-two (57 males and 15 females) selected purposely from 12 kebeles (6 farmers per kebeles). Out of the total farmers, 24 farmers or households were selected purposely based on recommendations from the local people, local authorities, Agriculture, and Natural Resource Woreda Office for the seed sample collection. The survey focused on investigation of local use and molecular genetic diversity of anchote population cultivated by west Wollega Zone of Oromia Region, Ethiopia.

3.3.1 Data collection

Diverse methods of data collection were used in order to assess local knowledge of anchote in the study area. The data collection of the study conducted mainly through questionnaires, face-to-face interview, and direct observation of cultivated anchote on farmland.

Both general information and the indigenous knowledge on anchote were gathered by using semi structured and close ended questionnaires and interviews. From the selected representative kebeles (12 kebeles), 72 respondents were selected for interview and questionnaires data collection. The semi structured open and close-ended questionnaires were first prepared in English language then translated for respondents into their local language (Afan Oromo).

3.3.2 Data analysis

Both qualitative and quantitative data were analyzed by using simple descriptive statistical methods like frequency and percentage.

3.4 Genetic Diversity Analyses Using ISSR markers

3.4.1 Plant material

Anchote seed samples were collected from six woredas/districts of west Wollega zone of Oromia Region from households in the form of gift or purchase. Then the seed samples collected from six representative populations (two individuals from each kebeles, of a total of 12 kebeles) were planted in pots at College of Natural Science, Addis Ababa University.

The seed samples of twenty-four accessions of anchote successfully germinated at different germination time. The young leaves of each individual samples were randomly selected and used for DNA extraction.

3.4.2 DNA extraction

The DNA was extracted from 300 mg fresh leaves using the modified CTAB (cetyl trimethylammonium bromide) method with some modification (Doyle and Doyle , 1987). Young leaves of each sample were ground to fine powder, using liquid nitrogen with pestle and mortar. The fine powder of each sample was transferred to 2 ml Eppendorf tube and then 700 µl of pre-warmed (65^{o}C) CTAB solution with 0.2 β- mercapto-ethanol was added immediately and shaken gently to obtain slurry. The tubes were then incubated in water bath at 65^{o}C for 30 minutes. The mixture was removed from water bath and centrifuged at 13000 rpm for 7 minutes. The supernatant was carefully poured to new tube, and incubated in pre-warmed CTAB solution for 30 minutes with tissue pellet and centrifugation at 13000 rpm was repeated. The supernatant was collected, transferred to 1.5 ml new Eppendorf tube and 600 µl chloroform was added and shaken carefully for 5 minutes. Then centrifuged at 13000 rpm for 7 minutes and chloroform extraction was repeated. The supernatant was transferred to new Eppendorf tubes and the, DNA was precipitated with 2/3 volume of cooled isopropanol and placed at -20^{o}C for 2 h. This was followed by centrifugation at 13000 rpm for 15 minutes. The DNA pellet was collected and 200 µl of 70% ethanol was added and centrifuged at 13000 rpm and ethanol was discarded. The DNA pellet was air-dried and re-suspended in 100 µl TE buffer and kept at 4^{o}C overnight and continued with DNA precipitation followed by addition of 0.5 volume 7.5M ammonium acetate (4 $^{\circ}$C), 2 volume of cooled absolute ethanol and gently mixed by inverting the Eppendorf tubes and kept on ice for 2 h at -20°C and centrifuged at 13000 rpm for 35 minutes. The supernatant was discarded and DNA pellet was rinsed using 200 µl of 70% ethanol followed by the centrifugation at 13000 rpm for 15 minutes. This was repeated with 3M sodium acetate. The DNA pellet was left to dry at room temperature and 100µl TE buffer was added to each tube. The isolated DNA was kept in -20°C freezer and its quality was checked by gel electrophoresis using 2.5% agarose gel (Fig. 1). The concentration was determined by nano drop spectrophotometer at 260 nm and the quality was checked at 260 /280 nm. The DNA concentration ranged from 276.6-2187.9 ng/µl, which were later diluted to optimized constant concentration of 100 ng/µl.

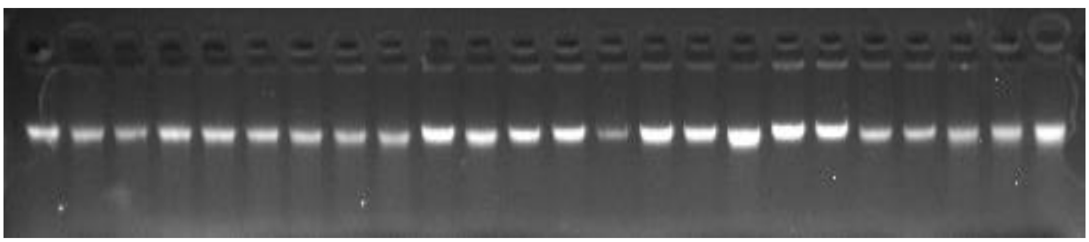


Figure 1 Genomic DNA after electrophoresis on 2.5% agarose gel

3.4.3 PCR Amplification

Ten ISSR primers were screened and six of them produced reproducible and polymorphic bands and used for further amplifications (Table 6). The BIORAD T100™Thermal cycler was used for the amplification of DNA. Each reaction was performed in 25 µl reaction volume containing 17.5 µl of deionized sterile water, 2.5 µl of buffer, 0.5 µl of dNTPs, 2.5 µl $MgCl_2$, 0.5 µl Taq polymerase, 0.5 µl ISSR primer and 10 ng DNA template. After mixing the PCR components, the reaction was carried out in BIORAD T100™Thermal cycler (UK) using the following conditions; Initial denaturation at 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 49-53°C for 1 min based on specific annealing temperature of the primer, extension at 72°C for 1 min and final extension at 72°C for 10 minutes.

The amplified products were electrophoresed in 2.5% of agarose in 1xTBE buffer at constant voltage of 100V for 3.5 h. The gel was then stained with EZ-Vision® Bluelight DNA Dye solution. The DNA was visualized on UV and documented by using gel documentation system. The size of amplified ISSR fragments was estimated by running 100 bp ladder (Solis BioDyne, Estonia) in the gel as standard size marker (Figure 6).

3.4.4 Data analysis

The data from the ISSR markers analysis was scored for presence (1) and absence (0) of bands. Faint and unclear bands were not included or counted. Various software's were used for analysis of the binary data matrix. The POPGENE 32 software was used to measure the following parameters under assumption of Hardy-Weinberg Equilibrium (Yeh *et al*, 1999): observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity(h) (Nei, 1978), number of polymorphic loci (PL), Percentage of polymorphic loci (PPL) and Shannon's information index of diversity (*I*). Both Nei's gene diversity (h) and Shannon's information indices (*I*) were used to calculate genetic diversity for each population. The amount of gene flow between the populations was calculated using population differentiation [(Nm = 0.5 (1-Gst)/ Gst], according to McDermott and McDonald (1993).

Jaccard's similarity coefficient was used to estimate similarity between pairs of populations from NTSYS-pc version 2.2 (Rohlf, 2000). Analysis of molecular variation (AMOVA) was used to estimate genetic variance within and among each population using Arlequin version 3.01 (Excoffier *et al*., 2006)

Cluster analysis was performed to construct dendrograms with both Unweighted Pair Group Method with Arithmetic averages (UPGMA) tree using NTSYS- pc version 2.02 and neighbor joining (NJ) tree using Free Tree 0.9.1.50 (Pavlicek *et al*, 1999) using Jaccard's coefficient of similarity. Excel add-ins (GenAIx) ver.6.502 Software version was applied for genetic distance matrix between each pair of individuals and populations using binary (dominant) distance method that were used for principal components analysis (Peakall and Smouse, 2012).

4 RESULTS

4.1 Local use of anchote study

4.1.1 Socio-demographic characteristics

The data collected from each respondent or farmers on their sex, age, family size, residence, and religion and education status are indicated in table 2. In this study, out of 72 households, males constituted 79.17% while females were 20.83 %.

In this investigation, all possible age groups of households or respondents were included. Thus, age groups categorized into three. Farmers in this all age groups have good understanding of value, use, and conservation of anchote. Out of three age groups 41.67% were found in the range of 38-48 whereas the range from 21-37 and above 49 were 20.83% and 37.5% respectively.

The majority of respondents (66.67%) were protestant whereas 20.83% orthodox, 4.17% Muslims and 8.33% were others.

Concerning the family size, out of the total number of respondents, 54.17% had five and above members. In addition, respondents who had 4 and 2 family members were 33.33% and 9.72% respectively, whereas the 2 (2.78%) of respondents were single. This result indicated that most respondents had large family size with an average of 5.8.

According to table 2, the residence status of respondents was categorized into four groups (born there, lived for 20 years, 10 years and <10 years). Most of the respondents (94.44%) are the group category who lived there since birth. Few respondents were lived for 20 years (2.78%) whereas others lived for 10 years and <10 years (each 1.39% only). The respondent’s residence status shows the link between Oromo ethnic class and anchote crop.

Table 2 Respondents' background information

Variable	*Alternatives*	Frequency(n=72)	Percentage (%)
Sex	Male	57	79.17
	Female	15	20.83
Age	21-37	15	20.83
	38-48	30	41.67
	>49	27	37.5
Religion	Orthodox	15	20.83
	Protestant	48	66.67
	Muslim	3	4.17
	Other	6	8.33
Family size	>5	39	54.17
	4	24	33.33
	2	7	9.72
	Single	2	2.78
Residence	Since birth	68	94.44
	For 20 years	2	2.78
	For 10 years	1	1.39
	For <10 years	1	1.39
Educational status	Cannot read and write	24	33.33
	Can read and write	22	30.56
	Primary school	21	29.17
	High school & above	5	6.94

Educational backgrounds of sampled respondents or farmers are grouped into four educational categories: cannot read and write, can read and write, primary school and high school or above.

Educational backgrounds of sampled farmers constitute, 33.33% of respondents cannot read and write. About 30.56% of the respondents who have no formal education but they can read and write, 29.17% of them attended elementary school and 6.94% of respondents attended high school and above.

The level of education is one of the demographic features of households, which has crucial role to preserve valuable traditional knowledge for both future generation and other communities (Den Eynden *et al*., 1992). Educational level of sampled households can play significant role to increase information about conservation, and promoting uses of anchote crop.

4.1.2 Indigenous classification method and cultivation status of anchote

Anchote is Afan Oromo name for *Coccinia abyssinica*, which all respondents used as vernacular name in the study area. Local people or farmers classify anchote as Red (Diimaa; in Oromo) and White (Adii; in Oromo). Their classification is based on morphological character such as root color and root shape. According to their traditional knowledge, ‘‘Ancootee adii’’or white anchote is preferred to ‘‘Ancootee Diimaa’’ or Red Anchote. This is because white Anchote can be easily cooked and easily processed for whatever use purpose as the farmers described. This information on cultural interest is important to conserve both phenotypic features.

Most of the farmers in the study area reported that the crop has been under cultivation since long time and utilized for different purposes. As shown in table 3, most of respondents or farmers (83.33%) have been cultivating anchote and 16.67% of respondents were not cultivating anchote.

The land size of most farmers (41.66%) for anchote production were ≤ 0.03 hectares while 27.78% and 13.89% of respondents had 0.06 and 0.13 hectares for anchote production respectively (Table 3). In general, the average land size for anchote production of respondents was 0.024 hectare that seems to be insufficient for family size.

Table 3 Anchote cultivation land size in hectares

Variable	Alternatives	frequency (n=72)	Percentage (%)
Land size (ha)	0.13	10	13.89
	0.06	20	27.78
	≤ 0.03	30	41.66
	0	12	16.67

Anchote is cultivated in home garden and field (Fig. 2). Most farmers cultivate anchote in home gardens mainly for seed generation and conservation. In addition, farmers do this to overcome the problem of anchote crop pest like porcupine (root pest), birds (seed or flowers pest) and seed-bearing failures of young anchote crop. These knowledges are appreciable for conservation, preservation and sustainable use of anchote crop.

Farmers described that anchote crop is usually cultivated in rainy season from April to December in agroecological zone of study area. It produces consumable root within five months. According to the farmers, cultivation of anchote requires fertile soil particularly for its rapid growth and to produce large size root shape with good quality. In fact, anchote root size, cultivated in more fertile area such home garden is larger compared to cultivated anchote in poor fertile soil based on observation made on farmland.

According to the information obtained from local farmers, anchote crop is cultivated via seed and root. In seed cultivation, farm land for anchote is prepared by burning grass and different waste materials of other plant material on the soil for anchote production. Then after ploughing the seeds are sown and well-watered with rainy water. On the other hand, the whole root of the crop is planted to establish “mother” plant, called “**Gubo**” around the home garden, which serves as the seed source for further planting and seed conservation (Fig. 2). Sometimes the root cutting is employed for anchote propagation.

Figure 2 Anchote productions in field

Since anchote is perennial climbing crop, most farmers cultivate anchote with maize which supports the tendril of the plant and the plants are grown on it (Fig. 2). Some farmers plant dry or branched trees for young growing anchote plants. Farmers perceive that unless anchote climb up on maize or trees, it will not produce root.

Almost all farmers in the study area have principal mechanism for anchote seed management and conservation. Anchote mother plant or Gubo (in Oromo) is planted in home garden and maintained through its generation to generation. Accordingly, the result of this study revealed that most of the time females were more responsible and involved in seed management and conservation in the study area.

As it is, shown on figure 3, when anchote seeds become matured or the fruit became red, farmers collect the seeds in plastic bag or pot. The collected seeds are mixed with ash and stored for few days until it loses water. Then the seeds are exposed to sun light to dry for further seed storage.

Figure 3 Farmers knowledge of anchote seed collection, processing, and storing**.**

In general, most farmers have sufficient experience on cultivating, usage, conservation, and management of *Coccinia abyssinica*. However, the use of vegetative or root planting may affect the genetic diversity.

4.1.3 Use categories of anchote

The local people use anchote for many purposes. The purposes of use as individual and group categories are analyzed. Five use categories namely: Food, cash income, medicinal and cultural value and other purposes were recorded. The use percentage of each use categories as the group and individual is presented in Table 4.

All respondents or farmers used anchote for various purposes whether they cultivated it or not. Moreover, each farmer uses cultivated anchote for different purposes. As listed in Table 4, 33.33% of farmers used anchote for food and cash income. In addition, 27.78% of farmers used for food, medicine and cultural value. Among respondents, 16.67% of them used for food only and similarly, 16.67% for food, medicine and cash income. In addition, 5.55% of the farmers used for food, cash income, medicinal, cultural value, and other. This result implies anchote is used for various purposes within this study area.

All farmers use anchote for food among all representative kebeles. As it is presented in table 4, 55.56% of famers used anchote for cash income whereas 50 % use for medicine. The other farmers (33.33%) use for cultural value (during various ceremony) purposes. In addition, others (5.55%) use for other purposes such animal feeding, rare chickens under its shade and as ornamental plant.

Anchote is preferred to other food by local people. This is because of its nutritional benefits and duration of consumption or consumed throughout a year. Another reason is that it can be cultivated in small land sizes or in-home garden. In general, anchote utilized over other food for different socio cultural and economic purposes in the area.

Table 4 Use categories of anchote in west Wollega

Use categories	Frequency(n=72)	Percentage (%)
As group		
Food, cash income,	24	33.33
Food, medicine, cultural value	20	27.78
Food, medicine, cash income	12	16.67
Food	12	16.67
Food, cash income, medicinal, cultural and other	4	5.55
As individual		
Food	72	100
Cash income	40	55.56
Medicinal	36	50
Cultural value	24	33.33
Other	4	5.55

4.1.4 Pattern use of anchote

The mode of preparation, form of uses and using purposes of anchote described by farmers are illustrated in Table 5. Different parts of anchote are used in nutrition, medicine, and fodder for animals. The roots were the most used part by local people for food, medicine, cash income, and cultural purposes. All farmers used anchote root for food. The roots are frequently used as food crop and prepared in different ways. In most cases the roots were boiled, then peeled, ground as "lanqaxa"; in Afan Oromo (finely ground form of root after boiled and peeled) or chopped into pieces after boiling and prepared with butter and eaten with enjera. In other cases, anchote dish is prepared from its roots that are peeled and chopped into pieces with knife before boiling and cooked with butter, onion, and pepper, then eaten with enjera.

According to local people, anchote roots are used in treatments of injured bone, swelling body, malaria, tuberculosis, gonorrhea, and overall health of child born women and lactation (Table 5). Particularly the roots of mother plant or Gubo (in Oromo) that is preserved in soil for more than four years is used for medicinal purpose. Interview with farmers in Ganji woreda indicated that two individuals whose legs and hands injured were treated with anchote root that survived in the soil for seven years. In addition, swelling body was treated with the roots of gubo anchote from Lalo asabi woreda.

The mode of preparation for medicinal purpose is either as Lanqaxa (Oromo) or other special way that root dried and ground to powder and prepared as broth with butter, onion and ginger. For local people, the broth preparation is effective to treat injured bone, swelling body, defend against malaria, tuberculosis, gonorrhea, and mothers for lactation.

Anchote crop contributes to socio-economic value of local peoples. About 36.11% and 19.44% of farmers used anchote roots and seeds for gaining income respectively (Table 5). Anchote roots are more available in local market and anchote dish is also available in different hotels and restaurants in the study area. However, the product of anchote is not available in super markets despite its economic values for local farmers.

As indicated in Table 5, 33.33% of respondents used anchote for cultural value purposes. This implies anchote is popular cultural food in the area and is used during different ceremony such as "Meskel" holyday, Thanks giving, New Year or at any holyday and kinship. Anchote food is commonly used during "Meskel" holyday and invitation of different kinship when various ceremonies occur according to cultural character of community in the study area. These uses may link to both cultural and religion order.

With respect to the leaves and whole plants of *C. abyssinica*, 2.78% of respondents use for animal feeds while 2.78% found the plant to be useful for fencing or rare chicken and as ornamental. However, the leaves and seeds were not found to be used for human food and treatment of infections in the present study.

Table 5 Uses of different parts of anchote, methods of preparation, form of use and purpose of use

Parts used	Use categories	Mode of preparation	Form of use	Purpose of use	Percentage
Roots	Food	Peeled, and chopped into pieces with knife before cooking and cooked with butter, onion and pepper, and eaten	Eat with enjera as cook	For Body building and gaining energy	100
		cooked, peeled and Ground and cooked root or chopped into pieces	Eat as lanqaxa or chopped with enjera		
	Cash income	Preparation of the matured root	Selling in local market	Gating income/ in cash	36.11
	Medicine	Cooking the root in water, peel and grind to powder/as lanqaxa	Eating with enjera as cooked	Swelling body Malaria and TB	50
		Roots are dried and ground to powder and broth is made with butter, onion and ginger	Drunk the liquid as broth or eaten with enjera	Treatment of Injured bone and gonorrhea	
				Child born women/lactation	
	Cultural (historical value)	The root is cooked in water, peeled and ground to lanqaxa	Eaten as lanqaxa with enjera	Meskel holyday	33.33
				Kinship	
Seeds	Cash income	Seed are collected and mixed with ash and dried	Sold in market	Cash income	19.44
Leaves	Fodder	Harvesting of the leaves	Served as forage	Animal feeds	2.78
Whole plants	Ornament	Plant around the house/on fence	Served as ornamental flower	Ornament, Rare chicken	2.78

4.2 Molecular genetic diversity of anchote

4.2.1 ISSR profile, band variation and polymorphism

Six primers produced 39 loci across 24 individuals. The remaining primers were considered unsuitable due to poor amplification. Out of these 39 loci, 31 loci were polymorphic, indicating rich allelic diversity in sampled populations. The size of amplified bands ranged between 200-1500 bp (Fig. 4). The number of polymorphic bands ranged from 2-7 attributing to 31 total polymorphic bands and 5.167 average polymorphic bands per primers. The percentage of polymorphic bands were 66.67% for primer 824 and 881, 71.43% for primer 826, 75% for primer 827, 87.5 % for primer 841 and 100% for primer 825. Out of all primers used in this study primer 881 produced only 3 bands and 2 polymorphic bands. Primer 824, 826, 827 and 841 generated 4, 5, 6 and 7 polymorphic bands respectively (Table 6). All the six primers generated 79.49% polymorphic bands at species level. This indicate that existence of abundant molecular diversity at population level

Table 6 List of ISSR primers with their scored bands and polymorphism

Primer Code	Primer sequences (5'–3')	Annealing Temperature(°C)	Size (bp)	Total Bands	Polymorphic Bands	Percentage of Polymorphism (%)
824	TCTCTCTCTCTCTCTCG	53	210-1500	6	4	66.67 %
825	ACA CAC ACA CAC ACA CT	49	210-900	7	7	100.00 %
826	ACA CAC ACA CAC ACA CC	53	200-900	7	5	71.43 %
827	ACACACACACACACACG	51	200-1500	8	6	75.00 %
841	GAG AGA GAG AGA GAG A(CT)C	52	200-900	8	7	87.50 %
881	GGGTGGGGTGGGGTG	49	450-900	3	2	66.67 %
Total			200-1500	**39**	**31**	
Mean				**6.5**	**5.167**	**79.49%**

Nei's gene diversity and Shannon information index of the primers used in this study ranged from 0.20 – 0.41 and 0.29 – 0.59 respectively. Among all the primers used for further analysis of molecular genetic diversity, primer 825 or $(AC)_8T$ produced 7 (100%) polymorphic bands across all genotypes. On the other hand, the highest Nei's gene diversity (0.41) and Shannon information index (0.59) were exhibited by primer 825. In contrast, primer 826 showed the least Nei's gene diversity (0.20) and Shannon information index (0.29).

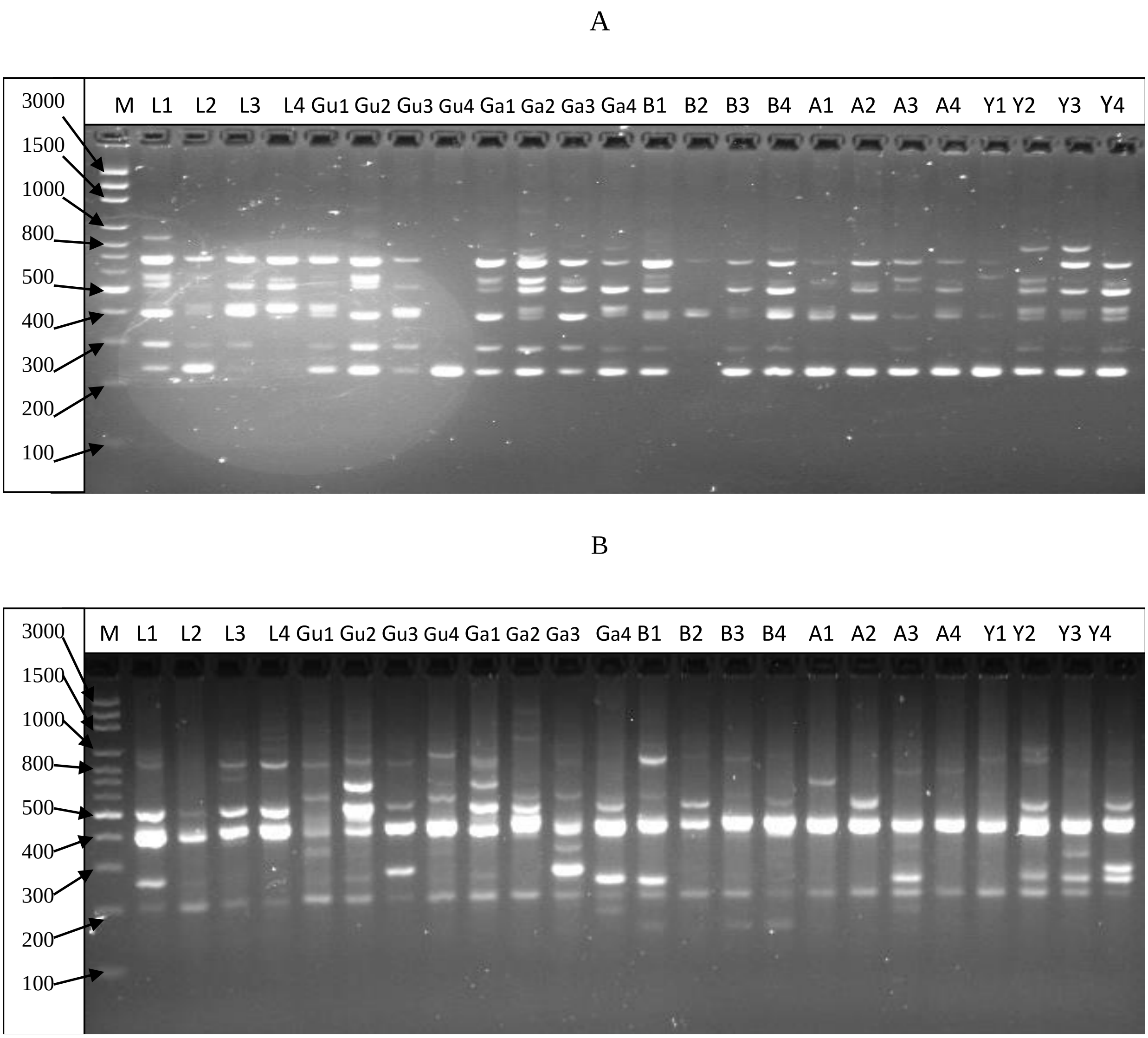


Figure 4 ISSR marker profile generated by primer 825 (A) and 827 (B)

NB. Molecular marker (100bp DNA ladder, Solis Biodyne; Estonia); lanes L1-L4(Lalo asabi); lanes Gu1-Gu4 (Guliso); lanes Ga1-Ga4 (Ganji); Lanes B1-B4 (Boji chokorsa); lanes A1-A4 (Ayira); Y1-Y4 (Yubdo).

4.2.2 Genetic diversity and population structure

The obtained results from genetic polymorphism analysis in different populations of anchote, such as number of observed alleles (na), effective number of alleles (ne), gene diversity (h), Shannon's information index (I), number of polymorphic loci (PL), and percentage of polymorphic loci (PPL), are presented in Table 7. The results showed that within the six studied populations, the number of observed alleles ranged from 1.38 - 1.46. The maximum number of observed alleles (1.46) and number of effective alleles (1.40) was found in Gulliso population and the lowest number of effective alleles (1.25) was detected in Ganji population. Based on the h and I value, Guliso population showed the highest genetic diversity whereas Ganji population showed lowest genetic diversity.

In the present study, the analysis of genetic polymorphism obtained with ISSR markers demonstrated that the highest percentage of polymorphic loci (46.15%), largest number of polymorphic loci (18) and the highest gene diversity (h) and Shannon's information index (0.2104 and 0.2978) were found in Guliso population. The lowest values (0.1423) were shown by Ganji population as compared to the other populations, which may be linked to vegetative propagation prevailed in the area. Populations of Lalo Asabi and Yubdo showed equal gene diversity values (0.1729), whereas Ayira and Boji Chokorsa populations exhibited nearly the same values (0.16) of gene diversity.

Table 7 Analysis of genetic diversity in the 6 populations of anchote.

Pop	NPB	PPB	Na	Ne	h+SD	I + SD
Lalo asabi	17	43.59 %	1.4359	1.3042	0.1729+0.2098	0.2533+0.3011
Gulliso	18	46.15 %	1.4615	1.4032	0.2104+0.2376	0.2978+0.3323
Ganji	15	38.46 %	1.3846	1.2472	0.1423+0.1967	0.2115+0.2834
Boji chokorsa	15	38.46 %	1.3846	1.2934	0.1607+0.2154	0.2322+0.3058
Ayira	16	41.03 %	1.4103	1.2954	0.1617+0.2131	0.2361+0.3012
Yubdo	17	43.59 %	1.4359	1.3042	0.1729+0.2098	0.2533+0.3011
Mean	16.33	41.88%	1.7949	1.4314	0.2544+0.1881	0.3865+0.2568

I = Shannon's information index, *NPB* = number of polymorphic bands, *PPB* = percentage of polymorphic bands

The analysis of the genetic structure between inter and intra populations showed that 0.2544 ± 0.0354 and 0.1701 ± 0.0196 respectively (Table 8). The genetic differentiation among *C.abysinica* populations (Gst) was 0.3312 with effective gene flow observed between populations(Nm=1.0096). According to Nei, Gst is classified as high when Gst > 0.15. Thus, the Gst coefficients of *C. abysinica* is high.

Table 8 Nei's analysis of gene diversity in subdivided populations

	Ht	Hs	Gst*	Nm*
Mean	0.2544	0.1701	0.3312	1.0096
St. Dev	0.0354	0.0196		

* Nm estimate of gene flow from Gst. Nm = 0.5(1-Gst)/Gst, *h* Nei's gene diversity, Gst=1- (Hs/Ht)

The analysis of molecular variance (AMOVA) test revealed significant molecular difference (P=0.05) among the studied species. It revealed that highest genetic diversity occurred within population (90%), while the genetic diversity among populations was 10% with the fixation index (FST) of 0.01854 (Table 9). This highest genetic diversity within populations indicate that anchot*e* is a relatively outcrossing species.

Table 9 Analysis of molecular variance among six populations of anchote without grouping

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variations	Fixation index (F_{ST})	*P* value
Among the populations	5	24.125	0.45708Va	10	0.01854	0.05
Within populations	18	80.750	4.11375Vb	90		
Total	23	104.875	4.57083	100		

4.2.3 Cluster analysis

Genetic similarities were obtained with UPGMA algorithm using Jaccard's similarity coefficients. Jaccard's similarity coefficient ranged from 0.649-0.785 (Table 10). The highest similarity was observed between Ganji and Ayira populations (0.785). The second highest similarity was between Boji Chokorsa and Ganji populations (0.767). The pair wise comparison of Jaccard's similarity coefficient also showed that Yubdo and Lalo Asabi are the most distantly related populations with 0.649 similarity coefficient.

Table 10 Jaccard pairwise similarity coefficient between populations

Population	LA	Gu	Ga	BC	Ay	Yu
Lalo Asabi	***					
Guliso	0.725	***				
Ganji	0.693	0.735	***			
Boji Chokorsa	0.758	0.734	**0.767**	***		
Ayira	0.682	0.674	**0.785**	0.744	***	
Yubdo	**0.649**	0.729	0.723	0.672	0.725	***

The genetic distance using cluster analysis by the neighbor joining (NJ) method in Free Tree based on Jaccard similarity coefficient showed two main groups (Fig. 5). The first cluster (A) contained individuals from Yubdo and Ayira whereas the second cluster (B) contained individuals from Ganji, Guliso, Boji chokorsa and Lalo Asabi. Both main clusters (A and B) branched into two sub-clusters (I, II, III and IV). Group A consisted two clusters; Cluster I consisted of three individuals (Y1, Y3, and Y4) whereas sub-cluster II consisted of four individuals (A1, A2, A4 and Y2).

Group B cluster consisted of two sub-clusters (III and IV); sub-cluster IV comprised of eleven individuals (L1, L2, L3, L4, Bc1, Bc2, Bc3, Bc4, Gu1, Gu3, and Gu4) of three populations (Lalo Asabi, Boji Chokorsa and Guliso), each formed their own clear and separate cluster. The remaining cluster (III) tended to form separate cluster dominated by individuals from the Ganji populations with some intermixing from other populations (Ayira, and Guliso).

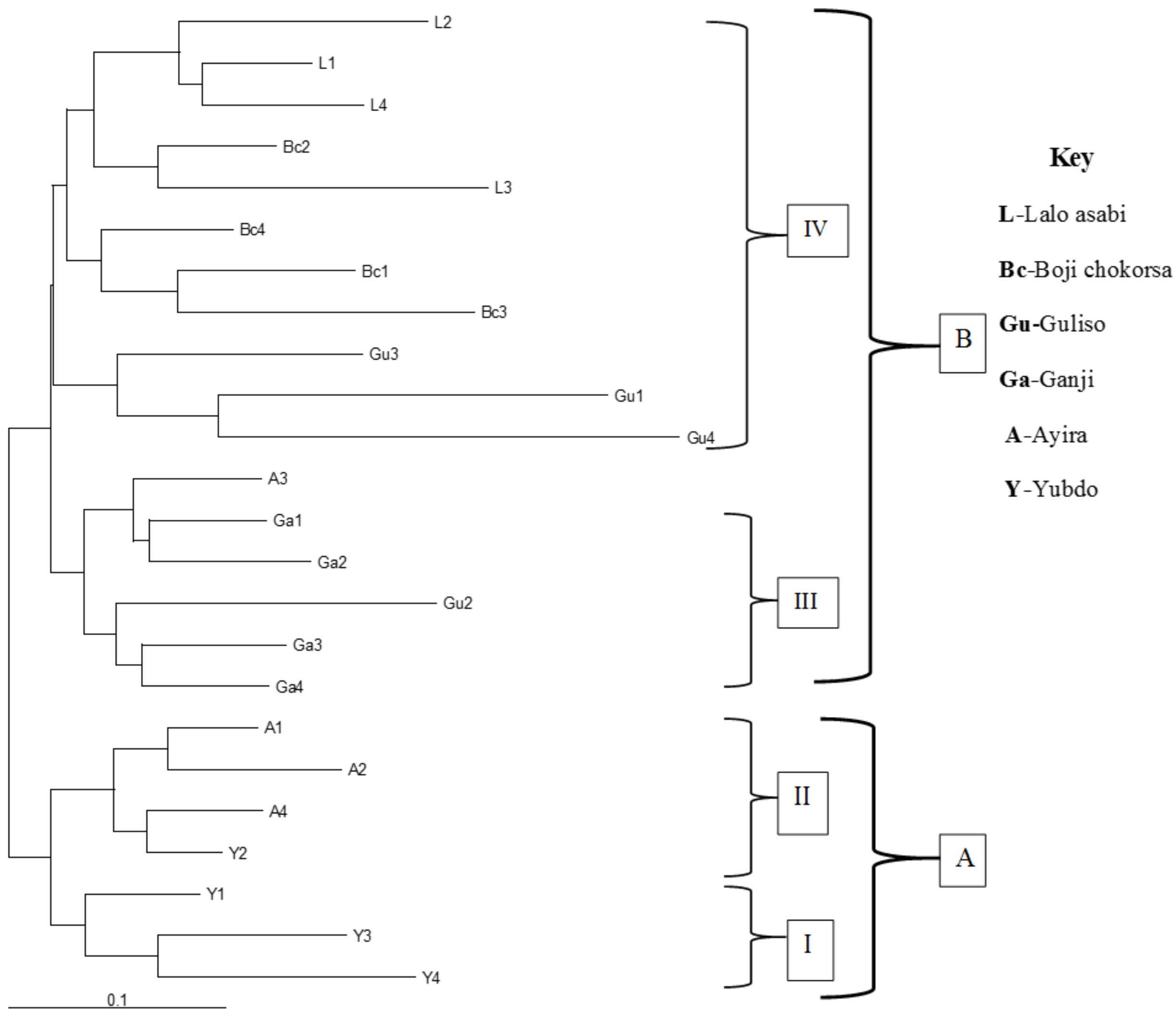


Figure 5. NJ based analysis of 24 genotypes of anchote using Jaccard's similarity coefficient

The dendrogram of six populations using cluster analysis by UPGMA method in the NTSYS-pc showed two distinct major clusters (Fig. 6). The first cluster contained populations of Yubdo, Ayira and Ganji. The second cluster contained populations of Guliso, Boji chokorsa and Lalo Asabi.

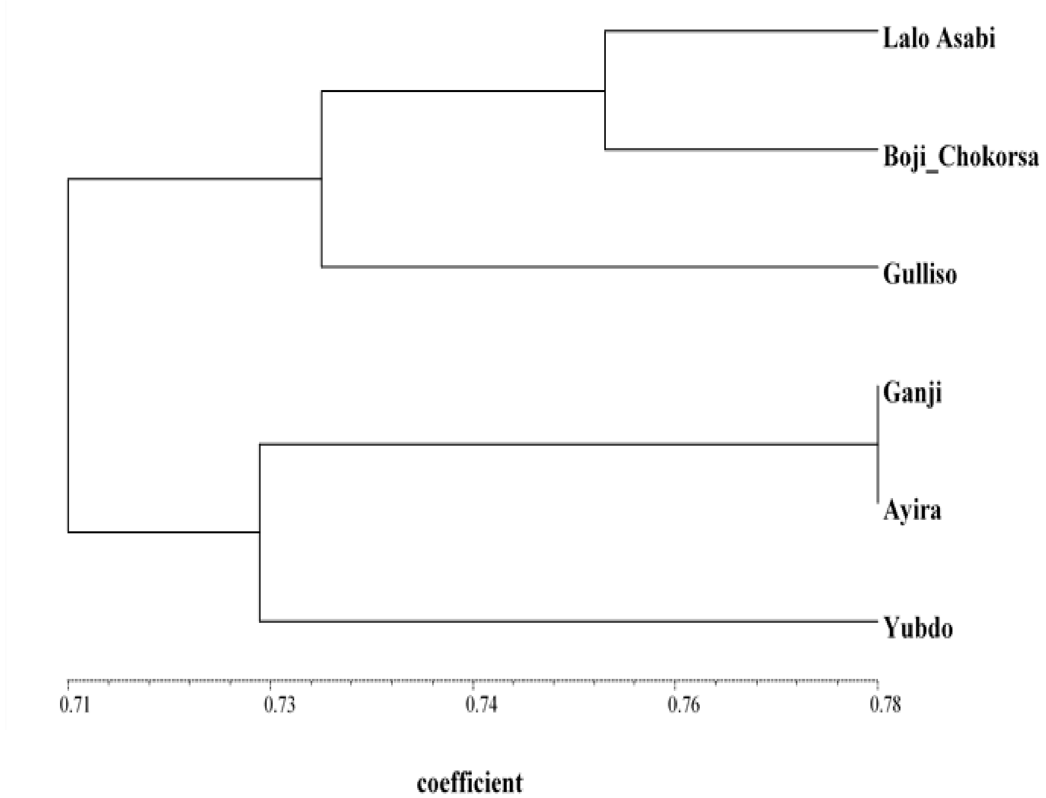


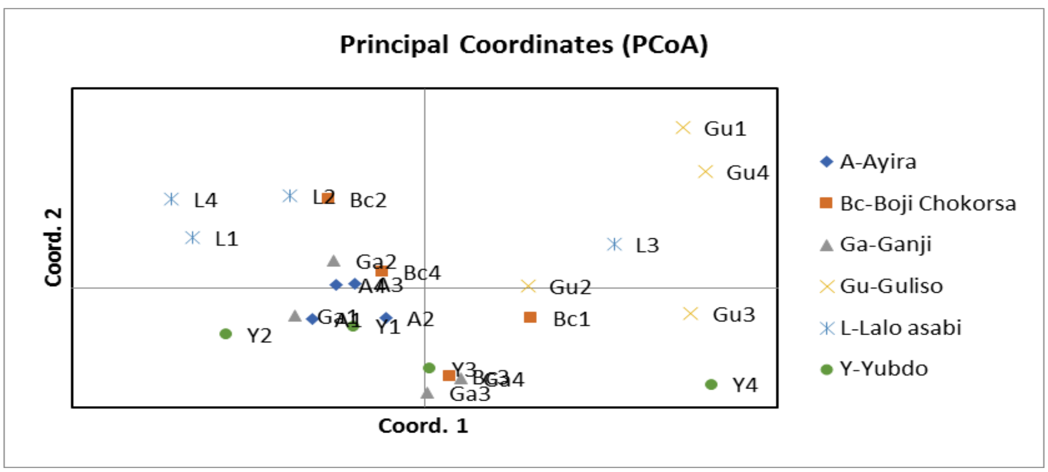
Figure 6 UPGMA based dendrogram for six populations of anchote

PCoA was performed for ISSR data in order to establish the relationship among populations and comparison to cluster analysis using the first three coordinates having 3.06, 3.01 and 2.56 Eigen values that accounted for 14.14%, 13.77% and 11.72% variation respectively. Results showed that distribution patterns of samples were mainly similar to the results from cluster analysis. The proportion of variation explained by the three axes of PCoA was 39.63. Negative side of the first coordinate consists patterns of some individuals from Yubdo (Y1, Y2), Ayira (A1, A2) and Ganji (Ga1) populations. Similarly, the positive side consisted intermixing of individuals from Ganji (Ga3, Ga4), Boji chokorsa (Bc1, Bc3), Yubdo (Y4, Y3) and Guliso (Gu3) populations. The rest individuals of populations were dispersed all over the coordinate.

On the second coordinates, most individuals from Lalo Asabi population (L1, L2, L4) and Boji Chokorsa (Bc2, Bc4) populations grouped together on the second coordinate toward positive sides. Individuals of Guliso pupation that have high genetic diversity grouped together (Fig.7A).

Principal coordinate analysis resulted in some patterns of clustering correlating to their geographic location. Lalo Asabi and Boji Chokorsa populations grouped together on the positive side of both coordinates whereas Ganji and Ayira populations grouped together on the negative side of coordinates. Guliso and Yubdo populations separately grouped on positive side of the first coordinate and second coordinate respectively (Fig. 7B). From PCoA two main groups of populations were observed. Yubdo, Ayira and Ganji poulations were gouped together on the first coordinate while Guliso, Boji Chokorsa and Lalo Asabi populations grouped on the second coordinate. Generally, the distribution patterns of studied populations were agreed with result of cluster analysis by using UPGMA.

A



B

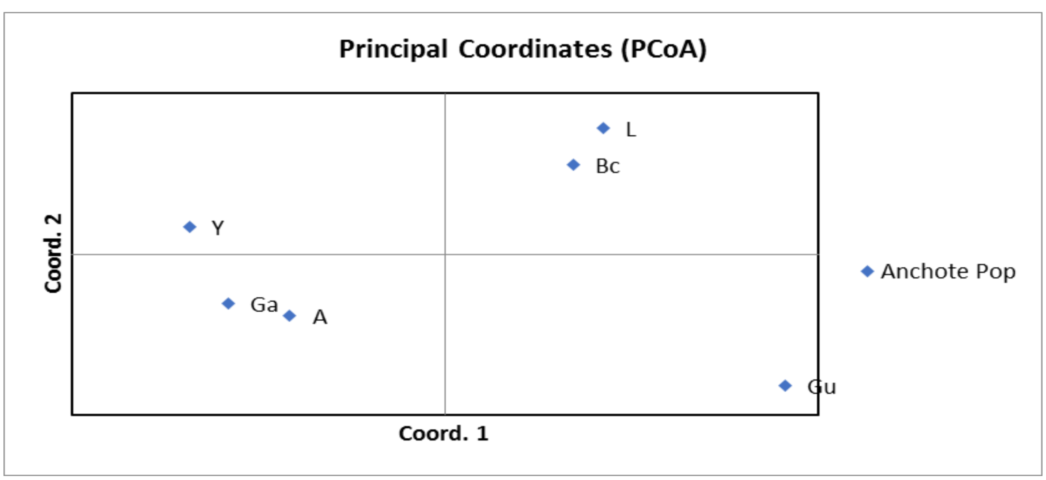


Figure 7 PCoA for 24 individuals (A) and 6 populations (B) of anchote analyzed using ISSR marker

5 DISCUSSION

5.1 Local use of anchote study

5.1.1 Indigenous classification method and cultivation status of anchote

Local people/farmers of the study area in general, elders and others knowledgeable community members in particular have well developed knowledge about anchote crops. The farmers' knowledge and anchote have been coevolving together. This has resulted in the prevalence of rich indigenous knowledge of farmers. However, the indigenous knowledge transfer on uses of anchote plants to the younger generations seems to be poor. The older informants were observed to be more knowledgeable than the younger ones, as they knew much more about the local cultivars and values of use.

Indigenous people gave name anchote for *Coccinia abyssinica* and classify anchote as Red (Diimaa; in Oromo) and White (Adii; in Oromo) in the study area. In spite of various vermicular names for *Coccinia abyssinica*, Ushushu (Welayita), Shushe (Dawaro), Wushish (Tigrinya), Ajjo (Kafigna) (Wolde Michael, 1987), only anchote, spelt ''Ancootee'' in Afan Oromo was attributed for *Coccinia abyssinica* in west Wollega, Oromia region of Ethiopia. This result in agreement with anchote endemicity to the Ethiopia when it comprises various vernacular names and farmers knew much more about uses and managements of anchote.

Anchote (*Coccinia abyssinica*) is more cultivated and highly valuable in western Wollega, Oromia region, Ethiopia. Most farmers have been growing and producing anchote. According to Daba Mengesha *et al.* (2012) reported that anchote is high growth and yield performance root crop produced and widely used in west Wollega Zone, Oromia Regional state of Ethiopia. The crop is accounted as important food security crop of the country regarding its nutritional content's compared to other root crops (Abreham Bekele *et al.*, 2014). In contrast, the current cultivation status of the crop is low based on farm land size in hectare for each farmer in the study area. Therefore, national and regional agents should be taking attention and responsibility for the improvement of agricultural production and productivity for this food security crop.

Anchote can easily grow in field and home garden but needs good cultivation practices and soil fertility. Most farmers cultivate anchote with maize which supports the tendril of the plant and the plants are grown on it. Farmers perceive that unless anchote climb up on maize or trees, it will not produce root. Plant height affects yield and other traits because it is directly contributing to root storage processes or photosynthesis (Bekele Serbessa, 2017). Therefore, according to traditional knowledge of the farmers, to achieve good and quality yield of anchote crop, its cultivation process is important.

Almost all farmers in the study area have principal mechanism for anchote seed management and conservation. Anchote mother plant or Gubo (in Oromo) is planted in home garden and maintained through its generation to generation. According to indigenous knowledge, mixing the seeds with ash is important for two reasons. The first reason is prevention of seeds from germination. The second reason is to remove water from the seed. According to Reyes and Casal (2004) reported that the ash produced by forest fires has an inhibiting effect on seed germinations and little effect on development of seedling. In similar case seed germination of many species responded to ash (Tessa, 2014). This result implies that anchote species may be responded to ash. However, further study is required to confirm this.

5.1.2 Use categories and pattern use of anchote

Anchote is multi-purposely utilized, indigenous root crop to Ethiopia (Abera Hora *et al.*, 1995). Five use categories (Food, cash income, medicinal and cultural value and other purposes) were recorded for anchote in the study area and all farmers used anchote for various purposes whether they cultivated it or not. According to Yosef Yambo and Tileye Feyissa (2013), anchote has nutritional, medicinal, economic, and social importance. The finding of present study agrees that anchote crop has strong link with socio-economic and cultural, and medicinal purposes than any other crop in the study area.

Most farmers of the study area have appreciable knowledge on the utilization of anchote crop. Different parts of anchote are used in nutrition, medicine, and fodder for animals. The roots were the most used part by local people for food, medicine, cash income, and cultural purposes and prepared in different ways. In most cases the roots were boiled, then peeled, ground as "lanqaxa"; in Afan Oromo (finely ground form of root after boiled and peeled) or chopped into pieces after boiling and prepared with butter and eaten with enjera.

According to local people, anchote roots are used in treatments of injured bone, swelling body, malaria, tuberculosis, gonorrhea, and overall health of child born women and lactation.

Particularly the roots of mother plant or Gubo (in Oromo) that is preserved in soil for more than four years is used for medicinal purpose. Interview with farmers in Ganji woreda indicated that two individuals whose legs and hands injured were treated with anchote root that survived in the soil for seven years. In addition, swelling body was treated with the roots of gubo anchote from Lalo Asabi woreda. The present study agrees that the nutrient content of anchote root with high calcium and protein (Habtamu Fufa and Kelbessa Urga, 1997; Desta Fekadu, 2011; Abreham Bekele *et al*., 2014).Therefore, because of its calcium content, it may help in fast mending of broken/fractured bones and displaced joints.

According to indigenous knowledge, the mode of preparation for medicinal purpose is either as Lanqaxa (Oromo) or other special way that root dried and ground to powder and prepared as broth with butter, onion and ginger. The broth preparation mode is effective to treat injured bone, swelling body, defend against malaria, tuberculosis, gonorrhea, and mothers for lactation. These all-medicinal values may be the result of calcium and protein content as different scholars have confirmed its nutrient contents. In its nature, calcium is essential for normal developments (bones and teeth) and functioning of the body or many metabolic processes including nerve function, muscle contraction, and blood clotting. Since the roots survived for generation to generation it may accumulated its nutrient contents. In general, anchote is used for medicinal purpose in very special way from food purpose accordingly indigenous knowledge of population in the study area. Such knowledge is very important to increase use values of anchote in different areas of the country and improving food insecurity program with such multipurpose crops.

The crop is highly linked to socio- economic and values of the people in the study area (Yosef Yambo and Tileye Feyissa, 2013).The result of the present study confirmed that socio-economic and cultural benefit of the crop but much emphasis has not given for usages of this food items. Therefore, research focuses on the improvement of this plant makes it more valuable and popular.

Anchote is popular cultural food in the area and is used during different ceremony such as “Meskel” holyday, Thanks giving, New Year or at any holyday and kinship. It is is commonly used during “Meskel” holyday and invitation of different kinship when various ceremonies occur according to cultural character of community in the study area. These uses may link to both cultural and religion order. The production of anchote has strong cultural ties with west Wollega

Oromo, since it is used as cultural food during the finding true cross locally called ‘’Meskel” festival. According to Abera Hora *et al*. (1995), roots of anchote cooked and prepared with butter for “Meskel “holyday. The result of present study confirmed that anchote dish is prepared from its roots by cooking, peeling, finely grinding tuber as locally called “lanqaxa” and prepare with butter or other pepper, then used for various ceremonies.

5.2 Molecular genetic diversity of anchote

5.2.1 Genetic diversity and population structure

Based on molecular genetic diversity analysis used in this study, the obtained result indicated that the anchote genotypes have high genetic diversity and gene differentiation. The highest genetic diversity obtained in this study is in an agreement with the finding of the previous study conducted on genetic diversity of Anchote from different parts of Ethiopia by ISSR marker (Abreham Bekele *et al*., 2014).

Based on the h and I value, Guliso population showed the highest genetic diversity whereas Ganji population showed lowest genetic diversity. Sampled individuals from Guliso population considered to be possess highest genetic variation as compared to other populations. In addition, anchote is highly cultivated in Guliso woreda and wide morphological traits were observed among anchote population during field survey of the study. The genetic diversity issue of crop is directly related to its pollination biology. Indeed, the reproductive nature of anchote plants, invites outcrosses and prevents inbreeding (Bekele Serbessa, 2017). Therefore, the high genetic variation among Guliso population and all other studied populations may be associated with existing of genetic exchange between population through pollen or seed exchange and occurrences of cross pollination in anchote species.

The analysis of the genetic structure between inter and intra populations showed that anchote populations also had high gene flow and genetic differentiation. Accordingly, Edwards *et al.* (1995) reported that anchote occurrences in its wild form provides gene flow and ensures broad genetic diversity. The high gene flow and high genetic polymorphism is used as indicator for occurring of its wild form in the study area. So, the high genetic differentiation within a population may be caused by outcrossing pollination in this species. Moreover, the center of origin, is usually considered, as center of diversity (Sebastian, 2011). In line with this finding, Abreham Bekele *et al*. (2014) reported that anchote population samples from Gimbi and Nedjo (west Wollega) showed higher genetic diversity. The finding of present studies suggested that the availability of indigenous genetic resource in the study area. Therefore, the information on such genetic based variability of anchote crop could be used as the raw materials that offers breeders a good opportunity for successful selection and for appropriate conservation measures.

AMOVA test result revealed that highest genetic diversity within populations (90%) and low genetic diversity among populations.This highest genetic diversity within populations indicate that anchot*e* is a relatively outcrossing species.

5.2.2 Genetic relationship among population and Clustering

The similarity coefficient showed certain level of close relatedness based on geographic location of the studied populations. In contrast, Guliso population is very closer to Ayira but showed lower similarity coefficient (0.674) compared to Lalo Asabi, Boji Chokorsa and Ganji populations. This result agrees with the result of Abreham Bekele *et al*. (2014) and Bekele Serbessa (2017) reported absence of clear correlation between geographical distance and genetic diversity in anchote populations using ISSR and SSR markers respectively.

Cluster analysis of anchote using the NJ and PCoA a method showed in traceable patterns of geographic origin and occasional intermixing. The grouping of genotypes does not strongly correlate with geographical location and genetic divergence (Jeena and Sheikh, 2003). According to Edwards *et al*. (1995) pollination form of anchote is highly contributed to its genetic diversity and genetic differentiation. Similarly, Abreham Bekele *et al.* (2014) reported that human activities have also strongly contributed to the genetic differentiation of anchote accessions. Therefore, this result may be due to the existence of genetic exchange between the groups.

In general, NJ, UPGMA and PCoA indicated similar results. Neighbor joining cluster analysis revealed strong clustering patterns in which the majority of individuals of studied populations clustered together. PCoA also confirmed that many individuals of each population grouped together on the coordinates. However, some individuals of studied populations showed occasional intermixing which confirmed the existence of high gene flow in anchote populations in study area. Clustering together of individuals from different populations in the same group resulted from gene flow through pollen or seed, migration of people from place to place, short and long distance marketing of either tuber or seed (Abreham Bekele *et al*., 2014). Clustering patterns in present study is linked to high gene flow between populations that results from seed and tuber exchanges practices from one location to the other. UPGMA and PCoA analysis exhibited strong clustering patterns in majority of populations clustering according to their locations.

6 CONCLUSIONS

The result of this study showed that anchote (*Coccinia abyssinica)* is more cultivated and highly valuable in western Wollega, Oromia region, Ethiopia. Farmers have been growing and producing anchote. The farmers' knowledge and anchote have been coevolving together. This has resulted in the prevalence of rich indigenous knowledge of farmers.

Anchote can easily grow in field and home garden but needs good cultivation practices and soil fertility. The young anchote plant cannot produce more or fertile seed fruit. The one grown from "gubo" (root mother plant) only can produce more and fertile seed fruit or in other way mother plant (gubo) conserved for seed regeneration.

Based on local use assessment, the present study finding proved utilization of anchote tuber is more popular in the localities and utilized over other food for different socio-cultural purposes. In addition to useful nutrient contents, anchote tuber has great utilities for socio economic improvement of society. It is universally utilized for preparation of traditional medicine to repair injured bone, swelling body, mothers for lactation and defend against malaria, tuberculosis, and gonorrhea.

Different parts of anchote are used for various purposes. Particularly anchote root is the most commonly used for preparation of anchote dishes and anchote root mother plant or Gubo (in Oromo) prepared in special way that root dried and ground to powder and prepared as the broth with butter, onion and ginger and then used as broth for medicinal purpose.

Based on molecular genetic diversity analysis used in this study, the obtained result indicated that the anchote genotypes have high genetic diversity and gene differentiation. The high genetic differentiation, mainly within populations, may be caused by outcrossing phenomena. Therefore, the genetic diversity of the crop will be maintained through occurrence of cross population among the populations.

Cluster analysis of anchote using the NJ, PCoA and UPGMA method showed in traceable patterns of geographic origin and occasional intermixing. This is because of high gene flow among the groups. Moreover, UPGMA based dendrogram of cluster analyses of populations grouped Lalo Asabi and Yubdo populations distantly, most probably due to their geographic distance.

The obtained information on genetic variation among individual plants and populations can be used for future, germplasm collection, conservation and breeding program of the crop.

7 RECOMMENDATIONS

Based on the results of the present study, the following points are recommended.

- The naming and classification knowledge of indigenous people complements morphological, biochemical and molecular marker study and maintenance of the creative dynamics of traditional knowledge and transmission of such knowledge are important for better conservation, preservation and sustainable use of anchote crop.
- Anchote is more useful in many aspects of nutritional contents availability and highly linked to socio economic and culture of the people. Therefore, focuses on improvement of this crop and its promotion is needed.
- Anchote is multi-purposely utilized indigenous root crop to Ethiopia. Since Ethiopia has problem of food and nutritional security gap, diversification and improvement of underutilized and neglected such crop considered as possible solution.
- The information on the genetic diversity and structures of populations helps to design appropriate management program to conserve the species. The result of present study on anchote showed that the presence of wide genetic variation in collection area. Therefore, better conservation is needed to protect or reduces the risk of losing these genetic variants due to different factors.
- The ISSR analysis in this study displayed high level of genetic variability among anchote populations, indicating the potential resource for the use of this germplasm in many selection breeding programs. For better understanding of the genetic diversity of anchote, future studies should focus on a large number of populations and accessions collected from more geographical ranges with different molecular markers.

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9 APPENDICES

A**ppendix 1** Data Collection Sheet

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL &COMPUTATIONAL SCIENCES

DEPARTMENT OF BIOLOGY

Ethnobotany and molecular genetic diversity studies of anchote (*Coccinia abyssinica*) in west Wollega,Oromia Region of Ethiopia

Data Collection Sheet

Dear respondents, this questionnaire is prepared to make a survey on the ethnobotanic and molecular genetic diversity of anchote (*Coccinia abyssinica*). The study is aimed to investigate ethnobotany and molecular genetic diversity of anchote population cultivated by the farmers of west Wollega Zone of Oromia Regional state, Ethiopia.

Therefore, dear respondents, your genuine response will help us to:

- To assess the indigenous knowledge on utilization and conservation of anchote
- To identify use categories, purpose, method of preparation, and usable parts of anchote
- To document farmers folk knowledge on use, naming, classification, conservation and management of anchote
- To assess molecular genetic diversity among and within anchote populations using ISSR marker

Dear respondents, I prepared this questionnaire which have two parts in mind of the above objectives. Thus, I request you to answer it accordingly and I guarantee you that the answer you give will be confidential and used only for the mentioned purpose. So, feel free and answer it. I thank you a lot for your cooperation in advance.

NB: If your preference is more than one, you can mark’’ X’’ in the checkbox as your choice.

PART I BACKGROUND INFORMATION

1. Name of respondent: ________________________ woreda: __________ kebele_______
2. Sex: male ☐ female ☐
3. Age: below 20 ☐ 21-37 ☐ 38-48 ☐ Above 49 ☐
4. What is your total family size? __________
5. 5. Religion: Orthodox ☐ Protestant ☐ Muslim ☐ Others ☐
6. For how long have you lived in the area?

   Since birth ☐ for the last 20 years ☐ for the last 10 years ☐ for less than 10 years ☐
7. Educational background (what is the last grade attended?) ________________________

PART II ETHNOBOTANICAL DATA

8. What is the local name of plant?
9. Do you cultivate Anchote? Yes ☐ No ☐
10. How many Anchote seed plants do you have? __________________________________
11. How you can classify Anchote plants do you have? ______________________________
12. What is the total size of your anchote cultivated land in hector?
13. What part of anchote plant used for cultivation? _________________________________
14. How do you conserve and cultivate anchote plant? _______________________________
15. What is your primary objective of Anchote production?

    Food ☐ Cash income ☐ Medicinal ☐ Cultural ☐ Other(specify)

    ____
16. If your answer for question #15 is "**Cultural/historical value**"Describe it.____________
17. If your answer for question #15 is "**medicinal** "what are the disease treated by anchote in your area (give local name) ____
18. If your answer for question #15 is "**medicinal** "What part of it is used? Leaf (L), Root (R), Bark (B), Stem (St), Flower (Fl), Fruit (Fr), Seed (Se), Latex (Lx), and Whole plant (WP).
19. Do you prefer Anchote food than others? Yes ☐ No ☐

    Why____
20. For what purpose you used anchote in your area? _______________________________
21. If you have any other information about habits, uses, conservation and management status of anchote specify it! ____

Thank you!

Appendix 2 List of households and their background information

s/n	Farmer ‘s Code	Sex	Age	Education	Religion	district	kebele
1	WG	F	60	-	Adventist	Lalo Asabi	Hatusi siban
2	MA	M	40	2	Adventist	Lalo Asabi	Hatusi siban
3	MT	M	36	9	Protestant	Lalo Asabi	Jarso Damota
4	TD	M	65	-	Protestant	Lalo Asabi	Jarso Damota
5	CE	M	42	6	Protestant	Guliso	Guji Worabu
6	GA	M	56	-	Orthodox	Guliso	Guji Worabu
7	BD	M	45	3	Protestant	Guliso	Seda Birbir
8	WS	M	50	-	Protestant	Guliso	Seda Birbir
9	EB	M	60	-	Protestant	Ganji	Sage Guji
10	HF	F	35	4	Protestant	Ganji	Sage Guji
11	LD	F	7	-	Protestant	Ganji	Kapi Guracha
12	SD	M	70	-	Protestant	Ganji	Kapi Guracha
13	SI	M	37	6	Protestant	Boji Cokorsa	Charaki kobara
14	BJ	M	45	5	Protestant	Boji Cokorsa	Charaki kobara
15	AF	M	44	2	Protestant	Boji Cokorsa	Gombo kobara
16	SH	M	37	3	Protestant	Boji Cokorsa	Gombo kobara
17	BA	F	50	-	Protestant	Ayira	Hojin Gudina
18	WM	M	45	9	Protestant	Ayira	Hojin Gudina
19	QM	M	40	3	Protestant	Ayira	Lalisa birbir
20	DB	M	38	6	Muslim	Ayira	Lalisa birbir
21	MO	M	40	7	Orthodox	Yubdo	Alaku Gabrel
22	SC	M	65	2	Orthodox	Yubdo	Alaku Gabrel
23	AA	M	65	2	Orthodox	Yubdo	Alaku Decha
24	BT	F	48	1	Orthodox	Yubdo	Alaku Decha

Appendix 3 Cultivation of anchote in home garden