GENETIC DIVERSITY IN ANCHOTE (*Coccinia abyssinica* (Lam.) Cogn) USING MICROSATELLITE MARKERS

MSc. THESIS

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ADVISORS' APPROVAL SHEET

(Submission Sheet-1)

This is to certify that thesis entitled "**Genetic Diversity in Anchote (*Coccinia abyssinica* (Lam.) Cogn) Using Microsatellite Markers**" submitted in partial fulfillment of the requirements for the Degree of **Master of Science** with specialization in **Plant Biotechnology**, the Graduate Program of School of **Plant and Horticultural Sciences**, Collage of Agriculture, and has been carried out by **Zerihun Teshome Melaku** ID. No SGS/PB/006/08, under our supervision and no part of a thesis have been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore; we recommend that it be accepted as fulfilling the thesis requirements.

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We, the undersigned, members of the Board of Examiners of the final open defense by **Zerihun Teshome Melaku** have read and evaluated his thesis entitled "**Genetic Diversity in Anchote (*Coccinia abyssinica* (Lam.) Cogn) Using Microsatellite Markers**" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree **Masters of Science** in Plant Science with specialization in **Plant Biotechnology.**

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Final approval and acceptance of the thesis is contingent up on the submission of the final copy of the thesis to the School of Graduate Studies (SGS) through the Department/School of Graduate Committee (DGC/SGC) of the candidate's School.

DEDICATION

I dedicate this thesis to my:

Father Teshome Melaku and

Grandfather Mamo Usman

STATEMENT OF AUTHOR

I declare that this thesis is my work and all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc. degree at the Hawassa University College of Agriculture School of plant and Horticultural science and is deposited at the University Library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Signature: _____

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ABBREVATIONS AND ACRONYMS

AMOVA	Analysis of Molecular Variance
BIC	Bayesian Information Criterion
CSA	Central Statistical Agency
CTAB	Cetyltrimethyl Ammonium Bromide
DAPC	Discriminant Analysis of Principal Components
dNTPs	Deoxy Nucleoside Triphosphate
DZARC	Debre Zeit Agricultural Research Center
EBI	Ethiopia Biodiversity Institute
EDTA	Ethylene Diamine Tetra Acetic Acid
EST-SSRs	Expressed Sequence Tag- Simple Sequence Repeat
FAO	Food and Agriculture Organization of the United Nations
GD	Genetic Diversity
GDMG	Geographic Distance Matrix Generator
ISSR	Inter Simple Sequence Repeat
MST	Minimum Spanning Tree
NJ	Neighbor-joining
NUS	Neglected and Underutilized Species
PAGE	Polyacrylamide Gel
P_{Ar}	Private Allelic Richness
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PPL	Percentage of Polymorphic Loci
PVP	Polyvinylpyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
rpm	Round Per Minute
SNNPR	Southern Nations, Nationalities and People's Region
SRAP	Sequence-Related Amplified Polymorphism
SSR	Simple Sequence Repeat
TBE	Tris- Borate- EDTA
TE	Tris-EDTA
TEMED	Tetramethylethylenediamine
UPGMA	Unweighted Pair Group Method With Arithmetic Mean
USDA	United State Department of Agriculture

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Abstract

Anchote (*Coccinia abyssinica (Lam.) Cogn*) *is an endemic crop species of Ethiopian origin mainly cultivated for its nutritious tuberous roots and tender leaves. Limited molecular marker resources hinder breeding and genetic studies for improvement, conservation and management of anchote genetic resources. The use of cross-species/genera transferable SSR markers is considered cost-effective strategy to ensure availability of markers in genetically understudied crops such as anchote. In this study we aimed to assess cross-transferability of cucurbit SSR markers to C. abyssinica and measure the genetic diversity of anchote and of its populations; across the cultivation range in Ethiopia. A total of 102 SSR primer pairs from cucurbit species were screened for transferability to anchote and polymorphic SSRs were identified. A total of 45 germplasm accessions collected from Western parts of Ethiopia were studied along with three cultivars of related species in Cucurbitaceae family using the polymorphic microsatellite markers. Out of 102 screened SSR primer pairs 46 (45.1%) were found transferable as they amplified scrabble band on anchote; of which only 24 primers pairs (23.5%) amplified polymorphic loci. Most of (53.6 %) of the SSR markers were transferred to anchote from Cucumis sativus* L. *All the analyzed 24 loci were highly polymorphic and detected a total of 354 alleles among all population, with an average of 15 alleles per locus. In the total germplasm collection the average genetic diversity, as quantified by the expected heterozygosity, was 0.88±0.06 per locus. Nei's gene diversity index revealed that the genetic diversity of East Wellega anchote maintained in situ in the farmers' field and ex situ in the DZARC gene bank was comparable, and retained the highest (I = 1.93) of all populations. Using discriminant analysis of principal components (DAPC), four clusters including outlier groups were detected. The DAPC analysis indicated that the most closely related populations were geographically occurred in closer proximity to each other. AMOVA attributed 95% of genetic variation to within population and only 5% to between populations. The results provide important genetic information in C. abyssinica to drive improvement, management and conservation decisions efforts. Analysis of genome wide functional factors and evaluation of agromorphological traits in additional germplasm set including wild anchote and other Coccina genetic resources would add necessary genetic information for improvement of the crop.*

Keywords: Anchote Germplasm; *Coccinia abyssinica;* Cucurbitaceae, Genetic diversity; Genetic structure; Microsatellites, SSR cross-transferability, West Ethiopia

1. INTRODUCTION

The Cucurbitaceae is widespread family of about 1000 species with 97 genera (Simpson, 2010). Anchote (*Coccinia abyssinica* (Lam.) Cogn) belongs to the genus *Coccinia* which comprises 30 species and ranks the 11th largest of the 97 genera of the Cucurbitaceae. All the *Coccinia* species are native to sub-Saharan Africa (Holstein and Renner, 2011; Schaefer and Renner, 2011) and ten of the species occur in Ethiopia (Thulin, 2009; Hora, 1995; Jeffrey, 1995). Anchote is the sole cultivated member of the genus with high diversity and endemism in Ethiopia (Bekele *et al.,* 2014; Polhill and Westphal, 1975; Getahun, 1973). Anchote is diploid (2n = 2x = 20) plant (Bekele, 2017).

In Ethiopia, anchote plays an important role in the local diet of rural and peri-urban communities (Wayessa, 2018). The crop is an indigenous domesticate cultivated for its nutritious tuberous roots and tender leaves since ancient times (Parmar *et al.,* 2017; Getahun, 1973). The anchote tubers are rich in essential macro- and micro-nutrients (eg. *Ca* = 7.78mg, *Fe* = 5.23mg and *Zn* = 2.05 mg/100g tuber) (Parmar *et al.,* 2017; Mitiku, 2016; Getahun, 1973). The tubers and leaves of anchote have long been prescribed in traditional medicine for various ailments. Juice prepared from anchote has saponin as an active substance and is used to treat gonorrhea, tuberculosis and tumor cancer among local community in the western part of Ethiopia. It is also used to treat bone fractures and displaced joints among traditional communities in western Ethiopia (Hora, 1995; Abebe and Hagos, 1991; Getahun, 1985). Recently, tuber extract of *C. abyssinica* has been used to synthesize Zinc oxide nano particles (Safawo *et al.,* 2018) and thermoplastic starch (TPS) (Abera *et al.*, 2020) for biomedical and industrial applications. Despite all the importance, the

crop is considered neglected and underutilized species (NUS) as compared to its potential that needs further exploration (Olango *et al.,* 2013; Ruth *et al.,* 2013).

Anchote is grown on small-scale (< 0.5 ha) by individual smallholder farmers in Ethiopia with major production in Oromia region; the crop has sporadic cultivation in Tigray, Amhara, and Southern Nations, Nationalities and People's (SNNP) regions. Anchote mainly grows at altitudes ranging from 1300 – 2800 masl (Hora 1995; Getahun 1973), but it is also reported from altitudes as low as 550 masl in Gambela region (Bekele, 2017). In these agro-ecological regions anchote shows remarkable biological diversity and endemism. The existence of wild and cultivated forms and sexual and asexual reproduction systems favored high diversity in the anchote genetic resources (Wayessa, 2018; Edwards, 1991; Getahun, 1974). Unique seed management and exchange systems are believed to have contributed for its temporal and spatial diversity in the country (Wayessa, 2018). Although little explored, genetic diversity in NUS such as anchote, could result in the discovery of novel genes for countering pressures to agricultural production (Gepts, 2006). Anchote is adapted to varying climate conditions and serves as an insurance nutritious food source in times of other crop failures. These versatile properties make anchote an interesting crop for a detailed characterization of its germplasm.

Understanding the extent and pattern of genetic diversity in crops is a prerequisite to efficiently manage and utilize germplasm. This knowledge is derived often from nutritional, morphological, molecular and biochemical marker studies. However, researches involving *C. abyssinica* thus far have focused mainly on yield improvement through conventional selection of cultivars and agronomic managements (Mengesha *et al.,* 2012; Abera and Guteta, 2007) while molecular studies seriously lag behind. For inferring the genetic diversity in anchote, use of morphological

traits (Tilahun *et al.,* 2014), assorted combination of phenotypic markers and nutritional composition (Desta, 2011) and morphological and molecular markers (Bekele, 2017; Bekele *et al.,* 2014) have been reported from handful of earlier studies. Arguably, anchote is one of genetically understudied indigenous crop of Ethiopia with less developed molecular and genomic resources. To date, with exception of nine ISSRs (Bekele *et al.,* 2014) and eight EST-SSRs (Bekele, 2017) limited studies have reported the use of molecular markers in anchote.

Studies shading light on understanding the genetic diversity and population structure of *C. abyssinica* are imperative for better utilization and conservation of the genetic resources. Microsatellite or, simple sequence repeat (SSR) markers are known for their high information content and versatility as molecular tools in germplasm characterization (Mondini *et al.,* 2009). They are favored for their co-dominant inheritance, high reproducibility, frequent occurrence in most eukaryotes, and high cross-transferability between related taxa (Agarwal *et al.,* 2008; Mohan *et al.,* 1997). However, for species with little to no genomic resource and sequence information, as is the case for anchote, the development of SSR markers is quite expensive (Powell *et al.,* 1996). Nevertheless, the use of cross-species/genera SSR markers is considered cost-effective and time saving strategy to ensure availability of markers in genetically understudied species such as anchote (Csencsics *et al.,* 2010).

The present study was carried out with major objective of screening cross-transferable SSR markers from cucurbit to anchote and characterizes microsatellite loci in anchote accessions. Specifically the study is aimed at:

- assessing transferability of cross cucurbit species/genera SSR markers to anchote,
- characterizing genetic diversity in anchote accessions using cross-transferred SSR markers, and
- determining population genetic structure and differentiation of anchote across cultivation range and *in situ* and *ex situ* germplasm management approaches.

2. LITERATURE REVIEW

2.1. Origin of Anchote

Based on molecular technique, the center of origin and diversification of *Coccinia* species was estimated to be in eastern and southern Africa around 6 - 7 million years ago (Holstein and Renner, 2011). The genus *Coccinia* (Cucurbitacea) includes 30 species all native to sub-Saharan Africa (Holstein and Renner, 2011; Schaefer and Renner, 2011). Anchote (*Coccinia abyssinica* (Lam.) Cogn) is one of the ten species that occur and believed to have originated in Ethiopia (Thulin, 2009; NBSAP, 2005; Hora, 1995; Jeffrey, 1995; Getahun, 1985). The crop is found both as cultivated and wild plant in the country (Edwards, 1991). Oral accounts and ethnoarcheological evidence indicate that anchote culture is deep-rooted in antiquity among the West Wellega Oromo culture and support the domestication of the crop in West Wellega, (Wayessa, 2018, NBSAP, 2005, Getahun, 1985).

2.2. Ecology and Distribution of Anchote

Anchote mainly grows at altitudes ranging from 1300 – 2800 m asl (Hora 1995; Getahun 1973), but it is also reported from altitudes as low as 550 masl in Gambela region (Bekele, 2017). The crop prefers black, loam, and deep to shallow soil with soil of pH of 4.5 to 7.5. Mean minimum and maximum temperature required for growth of the plant is 12^{o} C and 28^{o} C respectively. With average annual rainfall of 762 to 1016 mm (Mengesha *et al*., 2012; BARC, 2004). According to Lock (2001) anchote is grown as a root crop only in the west, south west Oromia and SNNPR regions of Ethiopia. But currently it is distributed to Amhara, Tigray, and Gambela of the regions (Bekele, 2017; Holstein, 2015; Samberg *et al*., 2013).

2.3. Taxonomy of Anchote

Anchote (*Coccinia abyssinica* (Lam.) Cogn) belongs to the order Cucurbitales, family Cucurbitaceae and genus *Coccinia* (Hora, 1995). Cucurbitaceae is widespread family of about 1000 species in 97 genera (Simpson, 2010). The anchote genus, *Coccina*, belongs to the sub tribe Beninccaseae of the tribe Benincasinae. These genus includes 30 species all native to sub-Saharan Africa (Holstein and Renner, 2011; Schaefer and Renner, 2011) and ten of the species occur in Ethiopia (Hora, 1995; Jeffrey, 1995; Thulin, 2009). *Coccinia abyssinica* (Lam.) Cogn. is sole cultivated member of the genus with edible tubers (Bekele *et al.,* 2014; Edwards *et al.*, 1995; Polhill and Westphal, 1975; Getahun, 1973). The most widely used vernacular names of cultivated *Coccinia abyssinica* (Lam.) Cogn. are, "Anchote" in Oromo, "*Ushushu*" in Wolaita, "*Shushe*" in Dawuro, and "*Ajjo*" in Kafa (Hora, 1995).

2.4. Plant Morphology

Anchote (*Coccinia abyssinica* (Lam.) Cogn) is perennial climbering vine tuber crop and it is the only root-bearing crop in the genus *Coccinia* and family Cucurbitaceae (Zeleke *et al.,* 1992) Anchote produces many branched stems, large above ground biomass, spherical to cone-shaped tubers and simple tendrils (Abera and Guteta, 2007). The vines elongate up to 5 m and covered with more or less dense, articulate, dirty-white to yellowish trichome while petioles length is 1.5–14cm long (Holstein, 2015). Anchote leaves are different in shape and size with the difference in accessions. Shape of the leaf includes rounded, kidney shaped, heart shaped, trilobular, lobbed, and almost divided. Flowers are solitary or in long-pedicelled, few-flowers are racemes and common peduncle of raceme is 2.5–10 cm long while pedicel of flowers in racemes grow up to 1.5 cm long. Its calyx lobes and corolla are 2–4 mm and 1.4 cm long respectively.

Female flowers are solitary and pedicel grow up to 3.5 cm long (Holstein, 2015). Anchote has spherical to cone-shaped tubers (Getahun, 1973). A plant could develop one to six roots clustered together. Size of the roots developed from one plant could be uniform, slightly variable, and/or moderately variable. Surface of the roots is mostly smooth. Color of flesh and skin color is highly variable among accessions (Desta, 2011). Anchote fruit develop and mature within two and half months after flowering with nine to eleven white strip lines on its outer surface. The color of immature fruits is green and light yellowish when matured. The size of fruits could range from 5 cm to 9 cm long and 27 mm to 61 mm width. Variability in size is greater among the accessions. A fruit, regardless of its difference in size, contains six locules and each locule could contain 11 to 33 seeds (Desta, 2011). Fruits 5.5–6 × 3.5–4 cm, short elliptical, glabrous, and orange-red sometimes with yellow longitudinal mottling (Holstein, 2015).

2.5. Propagation and Mode of Pollination

Anchote is propagated sexually by seed and asexually by tuber. But the sexual, true botanical seed, method is an easy technique and commonly employed by local farmers. Vegetative propagation is performed by planting either the whole tuber or by slicing it into two or more pieces, each piece having rootlets and an external covering (Bekele, 2007; Hora, 1995). Desta (2011) reported that anchote has bisexual or perfect floral morphology (stigma shorter than anther) and self-pollination type where flower is opened after the pollination process is completed, suggesting likely self-pollination of the plant. Other reports indicate anchote is an out-cross plant due to male and female flowers occur on separate nodes and bloom at different time (Edwards *et al*.,1995). The pollination in the crop is mainly by bees or flies (Sargent and Otto, 2004; Edwards *et al.,* 1995).

2.6. Importance of Anchote

Anchote has good nutrient composition with a good supplement of vitamins and minerals compared to other tuber crops and regarded as a leading protein rich root crop with high calcium content (Aga and Badada,1997). Thus, it is potentially important crop to counter protein deficiency in developing countries such as Ethiopia (Hora, 1995). Parmar *et al.* (2017) reported that the calcium content of anchote is five to three times higher than that of cassava and sweet potato, whereas the magnesium content of red and white tissue anchote is two times higher than that of cassava and sweet potato. Raw anchote contain two to three times greater phosphorous than that of sweet potato and cassava. Anchote is a moderate supplier of micro minerals such as Cu and Zn. The Fe content of red and white anchote tubers in comparison to cassava, anchote has a three times higher Fe content. Abebe and Hagos (1991) reported that juice prepared from anchote has saponin as an active substance and is used to treat gonorrhea, tuberculosis and tumor cancer among local community in the western part of Ethiopia. It is also used to treat bone fractures and displaced joints among traditional communities in western Ethiopia (Hora, 1995) Moreover, Anchote has been in use among local people to prepare a variety of food items for traditional ceremonies, special food for guests and animal fattening (Bekele, 2007; Gelmesa *et al.,*2010; Hora, 1995).

2.7. Production and Productivity of Anchote

According to Getahun (1973) anchote is potentially suitable for wide range agroecologies from arid to cool highland areas. There is limited information in national level area coverage of anchote production in Ethiopia. Central Statistical Agency report does not include anchote in the crop production area reports. Nevertheless, other individual scholars report indicate that among

the major producing area, western Oromia zones, anchote is produced on several hectares of land (Abdissa, 2000). Getahun (2003) reported that a farmer in western parts of Wellega usually allocate 400 to 600 square meters of land for anchote production mainly for home consumption. According to (Amsalu *et al.,* 2008) anchote is produced on hectares of 440.75 and 440 in East and West Wellega zones in 1998/99 cropping season.

The productivity of anchote varies based on genotypes, soil fertility level, location and cultural practices applied. Under farmer condition anchote can yields 20 to 30 t ha^{-1} (BARC, 2004; Hora, 1995). However, under research condition it has a potential to yield up to 73 t ha^{-1} (Desta, 2011) and 76.45 t ha^{-1} (Mengesha *et al.*, 2012). Mengesha *et al.* (2012) reported on ten accessions of anchote studied in two locations (Jimma and Ebanta) that showed highest storage root yield compared to major root crops of Ethiopia. The anchote accessions yielded 42-76 t/ha which is higher than 9 t/ha for Taro (9 t/ha) and 45 t/ha for Yam (Amsalu *et al.,* 2008),15-34 t/ha for different variety of Sweet potato (Amsalu *et al.,* 2008; Million, 2008) and 22-27 t/ha for released variety of potato (Gebremedhin *et al.,* 2008).

2.8. Anchote Genetic Resources

Plant germplasm collections are considered important to conserve genetic variation within and between species and provide a source of material for exploitation. Currently there are several research centers and universities maintaining collections of different species in Ethiopia and they are overseen by Ethiopian Biodiversity Institute (formerly the Institute for Biodiversity Conservation).

Documentation and collection of the *Coccinia* species is not well established in the country. From a total of 10 *Coccinia* species that have been reported to occur in Ethiopia, eight were recorded in the Flora of Ethiopia and Eritrea (Edwards *et al.,* 1995) and two more species, *C. ogadensis* Thulin described by Thulin (2009) and *C. microphylla* Gilg by Holstein and Renner (2011). Among the eight species found in the Flora of Ethiopia and Eritrea, only five were fully named (*C. schliebenii* (Harms 1932), *C. adoensis* (Hochst. Ex. A. Rich.) Cogn., *C. abyssinica* (Lam.) Cogn., *C. megarrhiza* C. Jeffrey, and *C. grandis* (L) Voigt (syn. C. indica Wight & Arn.) according to the standard rules for giving scientific names to plants. Those not fully named are: *C. sp* = Bally 12989, *C. sp.* = Burger 2947A, and *C. sp.* = Gilbert & Jones 129 (Edwards *et al.,* 1995). Except for some sporadically organized collection for *C. abyssinica*, there are no established collections for other *Coccinia* species in the country. Even for anchote comprehensive collections including all its cultivation ranges including both cultivated and wild germplasm are missing. Great majority of the anchote accessions collected from different parts of Ethiopia were collected not for medium and long term maintenance but by scholars and researchers for academic requirements assessing morphological, nutritional and molecular variation (Bekele, 2017; Desta, 2011; Edwards *et al.,* 1995; Tilahun *et al.,* 2014). Institutional collections of anchote germplams are found in Debre Zeit Agricutural Research Center (DZARC) and Bako Agricultural Research Center (BARC) (Tilahun *et al.* 2014). The DZARC and BARC collection of anchote germplasm holds about 49 and 20 accessions respectively mainly collected from Western Ethiopia.

2.9. Diversity in Anchote Germplasm

Anchote exhibits a great range of diversity in its morphology (Tilahun *et al.,* 2014; Desta, 2011; Gelmesa *et al.,* 2010), nutritional composition (Parmar *et al.*, 2017; Desta, 2011) and molecular markers (Bekele, 2017; Bekele *et al.,* 2014). The knowledge of diversity in germplasm is derived often from nutritional, morphological, molecular and biochemical marker studies in crops.

Morphological descriptors are the most commonly and easily observable markers to classify and evaluate different trait of interest in crop plants (Singh *et al.*, 2015). According to Bekele (2017) about 149 accessions of anchote showed significant variations in several morphological and agronomic features of anchote including days to 50% emergence , primary lobe, growth habit , vine pubescence, stem color and days to male flower opening. However, some fruit and seed traits similar performances and comparable variation among accessions. Similarly (Desta, 2011) reported, no variation in number of locules per fruit, number of sepals and number of petals among 36 anchote accessions. Overall, morphological markers generally are characterized by insufficient polymorphism,and are affected by environmental conditions at different developmental stages (Vienne, 2003).

The nutritional composition of anchote is comparable to most food crops cultivated in Ethiopia in the amount of energy and fat content and excels most root and tuber crops in its protein content (FAO, 1990). There is high infra-specific variation of anchote accessions for, macro-micronutrients, and anti-nutrient contents (Parmar *et al.*, 2017; Beruk *et al.*, 2015; Yenenesh A., 2015). Nutrient composition of anchote accessions varies with tuber flesh color, with red colored tubers roots having higher macro- and micro-nutrients than white flesh once (Parmar *et al.*, 2017). Yenenesh A. (2015) reported variation among different anchote plant organs for

composition of proteins and carbohydrates, with leaf found to be rich in crude protein content while roots showed superiority in utilizable carbohydrates.

2.9.1. Exploitation of Molecular Markers in Anchote

Molecular diversity refers to any variation in nucleotides, genes, chromosomes or whole genomes of organism. Assessment of genetic variability is important for conservation and utilization of genetic resources (Lefebvre *et al.,* 2001). Molecular based genetic diversity and population structure studies of a crop plant have great importance for germplasm collection, breeding programs, genetic resource conservation and management of the crop (Lu *et al.,* 2011; Wen *et al.,* 2010). Thus far, only two studies have applied molecular markers for characterization of anchote accessions. The first study was genetic diversity study of 10 populations of anchote based on nine ISSR markers (Bekele *et al.*2014) where as the second one used eight EST–SSR markers to characterize 146 anchote accessions (Bekele, 2017). Both results showed the presence of genetic variability in anchote accessions with insignificant geographic population differentiation.

2.9.2. Simple Sequence Repeats (SSR) as Markers of Choice

Microsatellites are referred to as simple sequence repeats or simply, SSRs. SSRs are any one of a series of very short (2-6bp), repetitive, tandem arranged, highly variable DNA sequences dispersed throughout genomes (Heletjaris and Burr, 1989). It is not certain how these tandem repeats arose in genomes. Initially, it was thought that the occurrence of unequal crossing-over between repeat units during meiosis and DNA replication slippage accounted for the length variation in SSRs (Strand *et al*., 1993). SSR markers are found to be informative, and abundantly

present in eukaryotic genomes (Heletjaris and Burr, 1989). .They are particularly abundant in plants, occurring on average every 6-7 kb (Cardle *et al.,* 2000).

Although no molecular marker technique is ideal for every situation. Various molecular marker techniques have been successfully employed to study the genetic relationships among cucurbit species such as RAPD, SRAP, ISSR and SSR (Inan *et al.,* 2012; Gong *et al.,* 2008; Ferriol *et al.,* 2003; Youn and Chung 1998). SSR markers have turned out to be an effective tool for germplasm characterization and genetic diversity studies harboring many desirable features. SSR markers are known for their high information content and versatility as molecular tools in germplasm characterization (Mondini *et al.,* 2009). They are favored for their co-dominant inheritance, high reproducibility, frequent occurrence in most eukaryotes, and high cross-transferability between related taxa (Agarwal *et al.,* 2008; Mohan *et al.,* 1997).

2.9.3. Categories of SSR markers

SSR markers are generally classified into two categories based on the origin of sequences used for development of SSRs: genomic SSRs (gSSR) and expressed sequence tag SSRs, (EST-SSRs or eSSRs). *Denovo* development of genomic SSR markers is labor, money and time intensive (Zane *et al.,* 2002). On the contrary, EST-SSRs identified from transcribed RNA sequences are more conserved than non-coding sequences more often can be developed from sequences of related species. EST-SSRs are becoming more and more widespread, not only because they are potentially linked with particular transcriptional regions that contribute to agronomic phenotypes (Varshney *et al.,* 2005) but also because they have high transferability among closely-related species (Sun *et al.,* 2017; Eujayl *et al.,* 2001; Peakall *et al.,* 1998).

2.9.4. Cross-amplification of SSRs

For isolated SSRs for which primers can be designed, SSR marker systems have many merits over other marker systems. Often, genomic and molecular resources are available only for economically important species. Species that attract less financial resources such as anchote might not have sufficient sequence information to design SSR primers. In such scenario, SSR primers identified for one species can be used in closely related species either in the same or different genus, termed SSR cross-transferability or cross-amplification (Yong-Jin *et al.,* 2009). The transferability of SSR markers has aided to comparative analysis within cucurbits (Yildiz *et al.*, 2015) and deemed useful to clarify genome evolution of different species and genus.

Successful SSR transferability between species within a genus has been reported in several genera other than in Cucurbitaceae family, for example, Olea (Rallo *et al.,* 2003), Limonium (Palop *et al.,* 2000), Prunus (Wuensch and Hormaza, 2002; Dirlewanger *et al.,* 2002), Vitis (Di Gaspero *et al.,* 2000). Among cucurbits extensive cross-genus and cross-species transferability of SSRs have been reported. Weng (2010) reported that cross-species transferability of the 42 cucumber SSRs across melon, watermelon, and squash with rate of 83.3%, 45.0%, and 50.0% transferability respectively. A total of 86 SSRs isolated from cucumber (*Cucumis sativus*, L) amplified on gherkin (*C. anguria,* L) (Yuichi, 2012). About 27 SSRs developed on *Cucurbita pepo* cross-amplified *Cucurbita moschata, Cucurbita maxima,* and *Cucurbita ecuadorensis* (Stift *et al.,* 2004). In addition several account of cross transferability between/among several species of cucurbits including melon (*Cucumis melon* L.), gherkin (*Cucumis angural* L.)*, Cucurbita moschata , Cucurbita ecuadorensis* and *Cucurbita pepo* were reported (Zhu *et al.,* 2016; Yildiz

et al., 2015; Yuichi, 2012; Gong *et al.,* 2008). The transferability of each the SSR marker varies across species and genus.

SSRs from coding regions are more successful in cross-species/genus amplification. A total of 82 EST-SSRs identified in *C. pepo* showed 84.14% transferability in *C. moschata* (Mao *et al.,* 2014). Similarly transferability of 28 EST-SSR makers that were developed form *C. sativus* and examined for transferability on four cucurbit species including melon, watermelon, pumpkin and gourd showed 92.9%, 57.1%, 53.6% and 60.7% rate of transferability respectively (Hu *et al.,* 2010).

The genomic SSR and EST-SSR differ in assessing genetic diversity of a species since they come from different regions of the genome. Possible selection against alterations in the conserved coding sequences in EST-SSRs limits their variation but favors their cross amplification. Accordingly, higher allele variations for genomic SSR than EST-SSR markers was also reported in cucumber (Hu *et al.,* 2011) and other plant species (Parthiban *et al.,* 2018; Wen *et al.,* 2010; Chabane *et al.*, 2005, Eujayl *et al.,* 2001).

2.9.5. Biological function of SSRs

SSRs were generally believed to be evolutionarily neutral. However, numerous evidences have indicated that distribution of SSRs in eukaryotic genome is not random (Tóth *et al*., 2000). It is estimated that 14% of the genes in eukaryotic species contain repeated sequences. Incorporation of repeat sequences in eukaryotic genomes may confer an evolutionary advantage of adaptability to new environments (Wren *et al.,* 2000). Debates on the functional roles of the SSRs on species adaptation and survival have been well documented (Li *et al*., 2002 and Li *et al*., 2004). Some

SSRs on the untranslated regions are deemed to be involved in the regulation of expression of nearby genes in animals. Although biological roles for SSRs in plants have not been reported as yet, similar roles are expected for these molecular markers in plant genes.

2.9.6. Application of SSRs

The large number of genome wide SSR markers developed from plant species including cucurbits genome provides a valuable resource for genetic map construction, QTL exploration, map-based gene cloning and marker-assisted selection. Microsatellites show a high level of polymorphism. As a consequence, they are very informative markers that can be used for many population genetics studies, ranging from the individual level (e.g. clone and strain identification) to that of closely related species. Microsatellites are also considered ideal markers in gene mapping studies(Hearne *et al,.* 1992; Jarne and Lagoda 1996). Molecular markers have proven useful for assessment of genetic variation in germplasm collections (Mohammadi and Prasanna 2003). Expansion and contraction of SSR repeats in genes of known function can be tested for association with phenotypic variation or, more desirably, biological function (Varshney *et al.,* 2005). Several studies have found that genic SSRs are useful for estimating genetic relationship and at the same time provide opportunities to examine functional diversity in relation to adaptive variation (Russell *et al.,* 2004; Eujayl *et al.,* 2001).

The cross-species transferable SSR markers have practical uses in many applications in species like in Cucurbitaceae family, for most of which the whole genome sequences are not yet available (Zhu *et al.,* 2016). Highly transferable SSR markers thus facilitate the development of an integrated or composite Cucurbita map by merging different maps (Wang *et al.,* 2020; González-Martínez *et al.,* 2004). The transferability of SSR markers deemed important for

comparative genetic studies within genus and elucidate the evolution of different species (Weeden and Robinson, 1986).

2.9.7. Limitation of SSR and cross transferable SSR markers

One of the main drawbacks of microsatellites is that high development costs are involved if adequate primer sequences for the species of interest are unavailable, making them difficult to apply to unstudied minor groups of crops. Although microsatellites are in principle codominant markers, mutations in the primer annealing sites may result in the occurrence of null alleles (no amplification of the intended PCR product), which may lead to errors in genotype scoring. The potential presence of null alleles increases with the use of microsatellite primers generated from germplasm unrelated to the species used to generate the microsatellite primers (poor "cross species amplification"). Null alleles may result in a biased estimate of the allelic and genotypic frequencies and an underestimation of heterozygosity. Furthermore, the underlying mutation model of microsatellites (infinite allele model or stepwise mutation model) is still under debate. Homoplasy may occur at microsatellite loci due to different forward and backward mutations, which may cause underestimation of genetic divergence. A very common observation in microsatellite analysis is the appearance of stutter bands that are artifacts in the technique that occur by DNA slippage during PCR amplification. These can complicate the interpretation of the band profiles because size determination of the fragments is more difficult and heterozygotes may be confused with homozygotes. However, the interpretation may be clarified by including appropriate reference genotypes of known band sizes in the experiment (Dinolfo *et al*., 2014; Schlag and McIntosh, 2012)

3. MATERIALS AND METHODS

3.1. Plant Materials

The anchote germplasm used in the present study comprises 45 accessions (Table 1). Seeds for thirty of the accessions were obtained from Debre Zeit Agricultural Research Center (DZARC), Ethiopia. For the remaining fifteen accessions (described as *in situ* and named in order from HU – 01 to HU – 15 in Table 1) seeds were collected from farmers field of Western Ethiopia. In addition, two commercial varieties of related species in Cucurbitaceae family, watermelon (*Citrullus lanatus* var. c*rimson sweet* (Thunb.) Matsum and Nakai) and cucumber (*Cucumis sativas* var. marketer L.) as well as one local cultivar of cucumber were included as an outlier for comparative evaluation. The 45 anchote accessions were originated from six zones of Oromia Region (East Wellega, West Wellega, Horro Guduru, Kelem Wellega, Jimma, Ilubabor), East Gojam Zone of Amhara Region and Keffa Zone of Southern Nations, Nationalities and Peoples' Region (SNNPR) in Ethiopia (Figure 1). Agro ecologically, all the accessions represented midland altitude (*Woiana Dega,* 1747 – 2452 masl).

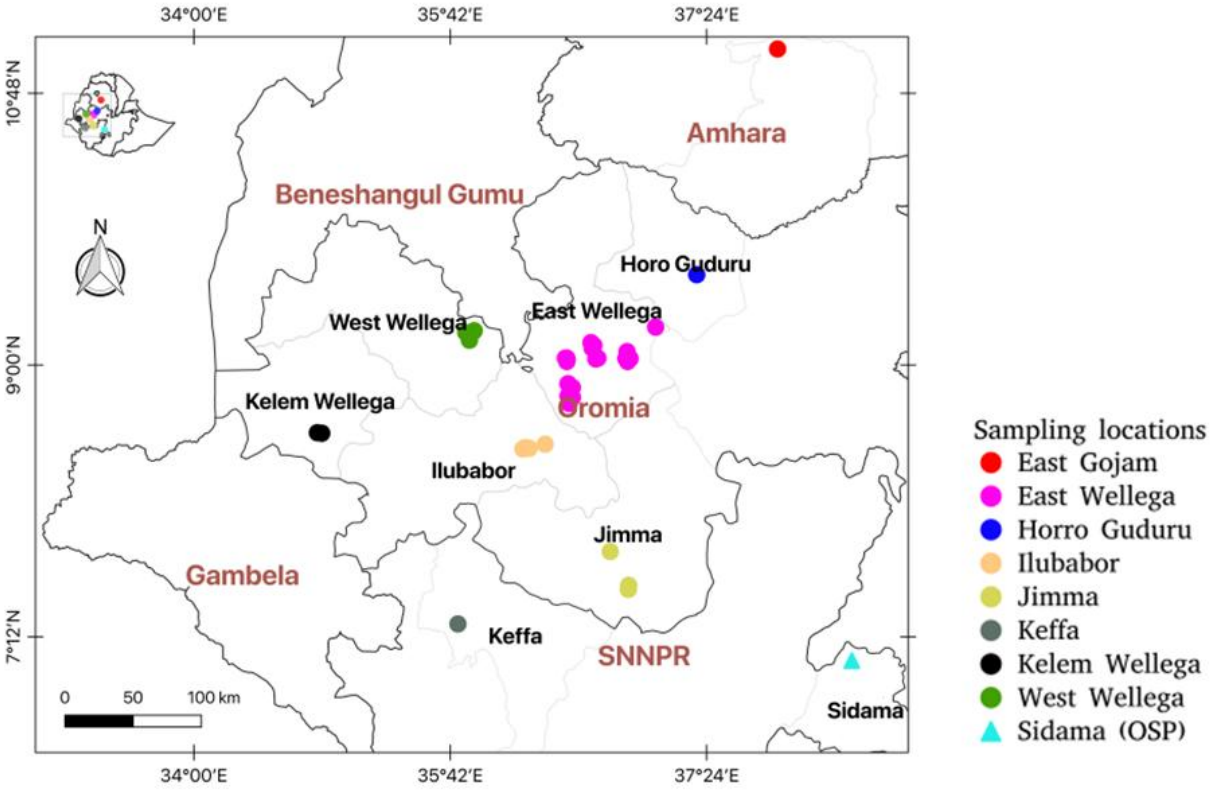

Figure 1. Geographic location of collection sites for anchote accessions included in this study. Different geographic zones for the anchote accessions are indicated with colored dots.

Table 1. The origin of anchote accessions investigated in the study with their respective geographic locations (Latitude and Longitude) in nine administrative zones of Ethiopia

No.	Populations/ Accession Number	North	East	Altitude	No.	Populations/ Accession Number	North	East	Altitude
	East Gojam (n = 1)					**Horro Guduru (n = 1)**			
1	229702	$11^0$04'13.88"	$37^0$51'42.45"	2399	29	90802	$9^0$34'38.91"	$37^0$19'31.42"	2277
	East Wellega *ex situ* (n = 12)								
2	223086	$9^0$01'47.75"	$36^0$27'21.33"	2197		**Ilubabor (n = 5)**			
3	223092	$9^0$01'32.76"	$36^0$51'53.17"	1798	30	223108-1	$8^0$26'02.25"	$36^0$10'49.24"	1976
4	223093	$9^0$01'40.32"	$36^0$51'56.73"	1810	31	223109	$8^0$25'44.86"	$36^0$12'15.76"	1986
5	223096	$9^0$07'38.91"	$36^0$37'54.25"	2040	32	223110	$8^0$26'42.42"	$36^0$11'32.24"	1949
6	223098	$9^0$06'52.06"	$36^0$38'42.02"	2153	33	223108-2	$8^0$25'56.93"	$36^0$12'0.18"	1998
7	223099	$8^0$46'43.68"	$36^0$29'45.2"	2362	34	223112	$8^0$27'29.73"	$36^0$19'56.11"	1950
8	223100	$8^0$47'11.00"	$36^0$29'40.70"	2282		**Jimma (n = 3)**			
9	223101	$8^0$47'22.28"	$36^0$28'50.19"	2443	35	223104	$7^0$31'20.72"	$36^0$52'35.37"	2047
10	DIGGA1	$9^0$02'00.38"	$36^0$26'18.99"	2181	36	223105	$7^0$29'26.93"	$36^0$52'52.17"	2244
11	KICHI	$9^0$02'05.13"	$36^0$40'05.13"	1896	37	223113	$7^0$44'30.93"	$36^0$44'10.91"	2092
12	223096	$9^0$06'39.84"	$36^0$38'03.48"	2373		**Keffa(n = 1)**			
13	DIGGA2	$9^0$01'28.55"	$36^0$28'36.08"	2240	38	240407	$7^0$30'01.57"	$35^0$28'45.11"	2304
	East Wellega *in situ* (n = 15)					**Kelem Wellega (n = 2)**			
14	HU-01	$8^0$50'54.47"	$36^0$28'49.18"	2422	39	DD1	$8^0$31'06.89"	$34^0$48'36.48"	1792
15	HU-02	$8^0$51'21.80"	$36^0$28'15.39"	2452	40	DD2	$8^0$32'53.21"	$34^0$49'03.94"	1747
16	HU-03	$8^0$52'12.24"	$36^0$28'02.44"	2439		**West Wellega (n = 5)**			
17	HU-04	$9^0$14'07.21"	$37^0$02'12.92"	1899	41	223087	$9^0$09'58.16"	$35^0$49'31.18"	1878
18	HU-05	$9^0$01'26.89"	$36^0$27'03.61"	2195	42	223088	$9^0$11'30.97"	$35^0$47'16.60"	1838
19	HU-06	$9^0$01'47.75"	$36^0$27'21.33"	2197	43	223090	$9^0$12'10.59"	$35^0$50'20.52"	1837
20	HU-07	$9^0$01'26.08"	$36^0$27'28.64"	2205	44	GM	$9^0$12'28.80"	$35^0$50'43.66"	1804
21	HU-08	$9^0$01'53.52"	$36^0$39'44.27"	1926	45	230566	9^{01}2'48.42"	$36^0$51'11.23"	1833
22	HU-09	$8^0$49'05.92"	$36^0$29'06.57"	2399					
23	HU-10	$9^0$01'00.92"	$36^0$52'24.77."	1790		**Other species (n = 3)**			
24	HU-11	$9^0$01'25.08"	$36^0$52'22.17"	1814	46	Cucumber (*Cucumis sativas* var. *local cultivar*)			
25	HU-12	$9^0$02'49.40"	$36^0$52'56.20"	1868	47	Cucumber (*Cucumis sativas* var. *marketer* L.)			
26	HU-13	$9^0$03'09.85"	$36^0$51'24.64"	1889	48	Watermelon (*Citrullus lanatus* var. c*rimson sweet*)			
27	HU-14	$8^0$44'31.78"	$36^0$28'52.55"	2318					
28	HU-15	$8^0$44'21.31"	$36^0$28'53.45"	2329					

3.2. Genomic DNA Isolation

Leaves collected by using ice box from Hawassa university experimental field were immediately brought to Hawassa University Plant Cell Laboratory and kept in deep freezer at -20^{o}c until it will be extracted. Genomic DNA was isolated from leaflets of three weeks old seedlings following the CTAB (Cetyl Tri-methyl Ammonium Bromide) method (Doyle and Doyle, 1987) with some modifications as in (Brown *et al.,* 1998). For DNA extraction, 0.5 g young and healthy leaf samples were grinded into fine powder using pre-chilled mortar and pestle and the powder was transferred into the 2 mL microcentrifuge tube with 1.3 mL DNA extraction buffer (2 M NaCl, 0.5/50 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1% PVP). DNA samples were quantified with a Genova Nano JENWAY^{TM} Spectrophotometer (Bibby Scientific, UK) using absorbance at 260/280 nm. The quality and integrity of DNA was checked using 1% agrose gel electrophoresis stained with Ethidium bromide and the bands were visualized using Doc^{TM} EZ Imager (BIO-RAD, California, USA). The concentration of DNA isolated from different samples varied starting from 105.24ng/µl to 5687.1ng/µl with an average of 2002.23ng/µl. The final working DNA concentration was adjusted to 50 ng·µL $^{-1}$.The molecular analysis was conducted in 2019 at Plant Cell Laboratory of Hawassa University, Ethiopia.

3.3. PCR and SSR Transferability

A total of 102 SSR markers earlier designed on different Cucurbitacea species including *Cucumis sativus* L. (Cavagnaro *et al.,* 2010; Ren *et al.,* 2009; Fukino *et al.,* 2008), *Cucurbita pepo* L. (Mao *et al.,* 2014; Gong *et al.,* 2008) *Cucumis melo* L (Chiba *et al.,* 2003), *Cucurbita moschata* Duchesne (Gong *et al.,* 2008) and *Momordica charantia* L. (*Wang et al., 2010*) were screened for transferability to anchote (*Coccinia abyssinica* (Lam.) Cogn) (Appendix 1). The

markers were selected based on polymorphic information content (PIC), allelic richness and history of transferability to related species. After optimizing the amplification conditions for each primer pair in a BIO-RAD T100^{TM} Thermal Cycler Gradient System (BIO-RAD, California, USA), PCR was carried out in a volume of 10 µL consisting of 30 – 50 ng genomic DNA, 1× PCR buffer with 1.5mM $MgCl_2$, 0.5 µ M of each primer, 0.2 mM each dNTP and 0.5 U Taq DNA polymerase (Top-Bio, Vestec, Czech Republic). The amplification conditions included initial denaturation at 95°C for 3 min, followed by 35 cycles of 94 °C for 30s, annealing at optimal temperature (Table 2) for 30s, and 72°C for 60 s, and a final 10 min elongation step at 72 °C. The amplicons were mixed with 6x blue DNA loading dye (Thermo scientific corp, Lithuania) and separated on a 2% agarose gels (Duchefa, Netherlands) stained with ethidium bromide (EBr) in a 0.5x TBE buffer. The separated amplicons were visualized on a Gel Doc^{TM} EZ Imager gel visualization system (BIO-RAD, California, USA). A panel of six randomly selected anchote accessions (223093, GM, 223086, HU-11, 223090 and 223108) along with watermelon (*Citrullus lanatus* var. c*rimson sweet* (Thunb.) Matsum and Nakai) and cucumber (*Cucumis sativas* var. *marketer* L.), were used for optimization of PCR amplification condition and testing the reproducibility of PCR product pattern before genotyping all the accessions.

Table 2. SSR markers used in the current study and PCR conditions

SSR locus	Source species	Primer sequence (5′–3′)	Repeat motif	Ta (°C)	Size Range (bp)	Reference
SSR00219[z?]	*C. sativus*	F: GTGAATGAGAGCCGTTGGAT R: GAGACGCATTCAATTCCTGC	$(GAA)_{10}$	60.3	221	a
SSR02384	*C. sativus*	F: AAAAATCCGACAAATCGTGC R: GGTCAAATGTTGCCTTTTGC	$(AT)_{14}$	56	142	a
SSR07100	*C. sativus*	F: CACACCATTTACGGTTATGGG R: CATTTGGTTCAGAAAGGGGA	$(CT)_{16}$	60	192	a
SSR10335	*C. sativus*	F: AAATGGAACCCCAACAATCA R: TAGATTAATTTGGGCAGCCG	$(CAG)_{13}$	60	185	a
SSR04570[y]	*C. sativus*	F: AAAGGTTGCCAAAGGGAAAT R: TCGCGTAAAGAAACAGAGGC	$(TGA)_8$	60.5	298	a
SSR01012	*C. sativus*	F: TCCAAAAATCGCGACCTAAA R: GTGAGCCGTTGATTTCTCGT	$(ATA)_9$	56	200	a
SSR01178[z]	*C. sativus*	F: AATTGACTTTTGGGCCTCAG R: TGCTCTCAGTTGAAAAATGCTC	$(AG)_{14}$	58	173	b
SSR01566z	*C. sativus*	F: TGGAAAAGCTCAGTTTGTTTGA R: TGAGTGCGAACCAACCTACA	$(AG)_{14}$	58	176	b
CSN270	*C. sativus*	F: TTGCCTTCTCTCTCCTCTTCCAAA R: AGTGGCTAAAAAGGCAGCAAGATG	$(TA)_5(TC)_3(CT)_3(TC)_6(TC)_6$	59.5	291-294	c
CMTp193	*C. pepo*	F: GGTGACGGCAAGAAAAGCTA R: GCTGACCCTCTCTCCCTCTC	$(GA)_{18}$	60	186	d
CMTp210	*C. pepo*	F: GTGGAAGTTACTGCGATTGG R: GCAAAGAATGTCCTCAGCAG	$(GTGTGC)_6$	58	117	d
CMTp132	*C. pepo*	F: CCATTTCCATTTCCATTTCA R: AGGTTAGAAACAGGGGGAATC	$(GAT)_{12}$	58	151	d
CMTp158	*C. pepo*	F: CCGTAGAGATGTCAGAGACAAGG R: AGGGATGCTCATCACACCTC	$(GCAG)_7$	60	134	d
PU000705	*C. pepo*	F: AAAGAAAAGCTCGCTTGGTG R: TCCATGCCCACATCAACTAA	$(CGA)_7$	56	122	e
PU002147	*C. pepo*	F: GCTCTGTGAAGGAGGACGAC R: CCGCTGCATAGGGAATGTAT	$(GAAGAG)_5$	60.5	239	e
PU000007	*C. pepo*	F: GGAGACCCCATTTTCCTGTT R: AAGCACTCACTTCCCCTTCA	$(CCG)_5$	58	105	e
PU001491	*C. pepo*	F: CACAGACGCAGAAACAGAGG R: AAGGACAAGATCCATGGGG	$(CTT)_5$	58.5	158	e
CMMS15-4	*C.melo*	F: GTCCGCCATCGCCACTACAAATCAA R: CTCCGTAAAACCTTCTTCCTCTCTC	$(GA)_{14}$	66.5	116	f
CMMS30-3	*C.melo*.	F: TTCCCACCAGCCCAACGGACACACT R: GAGATACAGAAACGACGACTAACCT	$(GA)_{16}$	67.5	271	f

Table 2. SSR markers used in the current study and PCR conditions (Continued)

CMTm83	C. moschata	F: GGCATTTCTGAGAACAGCTT R: ACGTTAGTTATGCTATTTTGTAGGC	$(AG)_{19}$	57	111	d
CMTm252	C. moschata	F: CCCATTCCCCATTTCTTTCT R: CAAATTCGATCGGCTTTACAC	(CT)13	60	101	d
S13	M. *charantia*	F: TTGGTTGTGGTGCTGAGTTC R: GATGTAGGGGTTGGGTTGAT	$(TC)_{15}$	57	270-281	b
S15	M. *charantia*	F: GGGTAGTGGAATGATGGGTT R: TAGTGTTTTCGTGAGGGAGG	$(AG)_6(AG)_{12}$	57	232-247	b
S26	M. *charantia*	F: GAACGCCCTGTGACTTTAGC R: TTTCGTCTTCCAATGAGCC	$(GA)_{13}$	58	193 - 198	b

References: a=Ren *et al*., 2009; b=Wang *et al.,* 2010; c=Fukino *et al.,*2008; d =Gong *et al.,* 2008; e = Mao *et al.,* 2014; f = Chiba *et al.,* 2003

3.4. Polyacrylamide Gel Staining and Genotyping

Amplified products were electrophoresed in 6% (w/v) Polyacrylamide Gel (PAGE) in 0.5xTBE buffer and SSR bands were detected by silver nitrate staining following the earlier described protocol (Huang *et al.,* 2018). For genotyping 10µl of the PCR product was mixed with 3 µl of 6x loading buffer (0.4% (w/v) bromo-phenol blue, 0.4% (w/v) xylene cyanole and 5ml of glycerol. The band sizes for each locus were estimated in comparison with DNA ladder pUC19 DNA/Mspl marker of 26 –500 bp (Sangon Biotech, Shanghai). The gel images were documented and the band size was measured using UVITEC (UVITEC, Cambridge, UK) software. The genotyping was carried out at the Molecular Laboratory of Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia.

3.5. Data Analysis

Microsatellite marker genotyping data were used to assess locus based diversity indices including the number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He) or gene diversity (*GD*) using GenAlEx v.6.501 (Peakall and Smouse, 2012). PowerMarker 3.25 (Liu and Muse, 2005) was used to estimate major allele frequency (*MAF*), and polymorphic information content (*PIC*).

Population genetic diversity parameters including mean number of alleles per population (*A*), number of unique alleles, effective number of alleles (*Ne*), average observed heterozygosity (*Ho*), average expected heterozygosity (*He*), sample-size weighed expected heterozygosity (*UHe*), fixation index (F_{IS}) were computed in GenAlEx v.6.501 (Peakall and Smouse, 2012). Population allelic richness (A_r) and private allelic richness (P_{Ar}) estimates were rarefied to allow correction for uneven sample size and estimated in HP-Rare 1.0. (Kalinowski, 2005). Estimates of genetic differentiation (*PhiPT*, Φ) were computed by Analysis of Molecular Variance (AMOVA) to partition total genetic variation into within and among population subgroups using GenAlEx 6.501 (Peakall and Smouse, 2012).

The pattern of genetic relationships among all individual anchote accessions and three related cultivars was assessed based on the neighbor-joining method using shared allele distance computed with PowerMarker 3.25 (Liu and Muse, 2005) and visualized using MEGA 6 (Tamura *et al.,* 2013). At population level, genetic relationship and structure were further examined using unweighted pair-group method with arithmetic mean (UPGMA) tree based on Nei's genetic distance (Nei, 1972) and a non-model-based multivariate approach, the Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.,* 2010) implemented in the adegenet package

version 1.4.153 in R.(Jombart, 2008). DAPC is an adequate analysis of genetic clustering for clonal or partially clonal organisms and does not require the populations to be in Hardy-Weinberg equilibrium (Jombart *et al.,* 2010). The method required a priori clustering algorithms to infer the optimal number of genetic clusters describing the data using the Bayesian Information Criterion (BIC). After selecting the optimal number of genetic clusters associated with the lowest BIC value, DAPC was performed retaining the optimal number of PCs as recommended in adegenet. Minimum Spanning Network (MSN) was used to visualize relationships among individuals as implemented in R package poppr 2.8.3 (Kamvar *et al.,* 2014). Geographic distances (D_G) between each individual and sub-population pairs were computed with the Euclidian distance using their coordinates as implemented in Geographic Distance Matrix Generator (GDMG version 1.2.3) (Ersts, 2020). The Mantel test based on 999 permutations was performed to identify correlations between geographical (GGD) and genetic distances (GD) using GenALEx version 6.501 (Peakall and Smouse, 2012).

4. RESULTS

4.1. Microsatellite Transferability

A total of 46 out of 102 SSR primer pairs (45.1%) were found transferable as they amplified scrabble band on anchote. However, only 24 of the 46 cross transferable primers pairs (23.5%) amplified polymorphic loci on anchote whereas the remaining 22 primers were discarded as they amplified either monomorphic or non-specific multiple bands. While most (53.6 %) of the SSR markers from *Cucumis sativus* were transferable, 42.86 % of *Cucumis melo*, 41.67 % for each of *Cucumis moschata* and *Momordica charantia* and 34.78 % of *Cucurbita pepo* were transferable to anchote (Table 3).

Table 3. Percent cross-transferability of cucurbit microsatellites to anchote, watermelon and Cucumber

SSR origin species	Total number of markers used	Number of markers Amplified	Transferability (%)	Polymorphism (%)
Cucumis melo	14	6	42.86	2(14.3)
Cucumis moschata	12	5	41.67	2(16.7)
Cucumis sativus	41	22	53.66	9(22)
Cucurbita pepo	23	8	34.78	8(34.8)
Momordica charantia	12	5	41.67	3(25)
Total	102	46	45.1	24(23.5)

The 24 cross-transferable SSR markers were screened on panel of randomly selected six (223093, GM, 223086, HU11, 223090 and 223108) anchote accessions along with watermelon and cucumber cultivars produced clear and unambiguous band on agarose gel (Figure 2 A). These 24 markers (Table 2) were genotyped for allelic polymorphism and genetic diversity analysis on polyacrylamide gel (Figure 2 B)

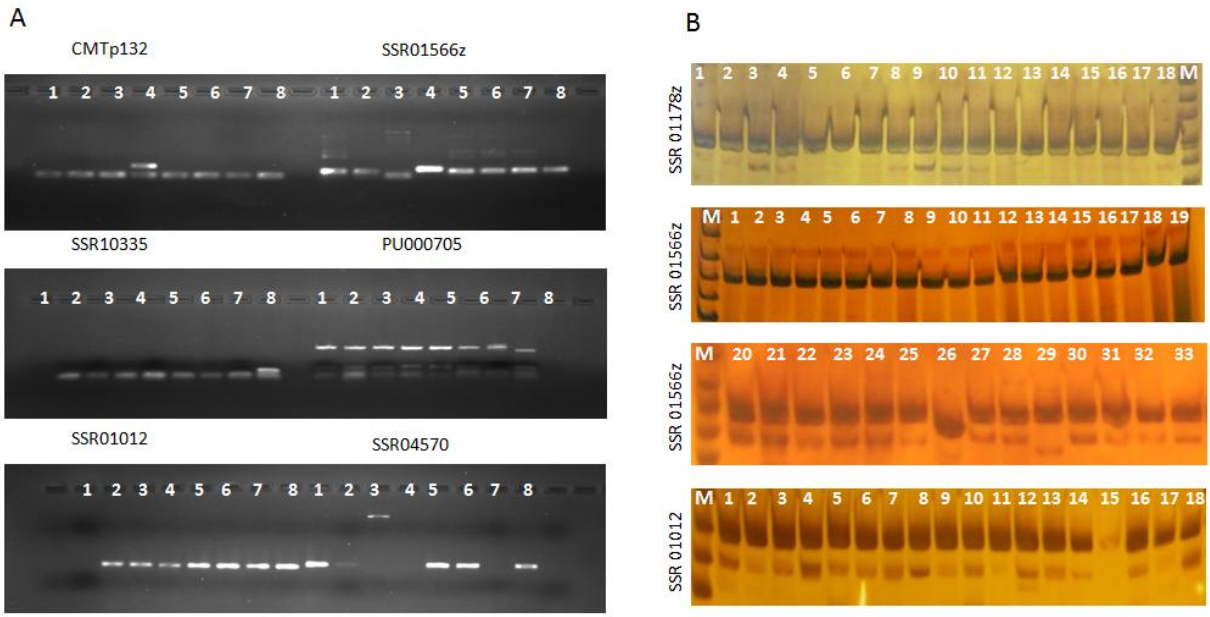

Figure 2. Sample agaros gel picture of primer cross-transferability screening (A) and polyacrylamide gel picture used for genotyping (B). The genotypes lebeled from 1 – 8 in the agaros gel picture are 223093, GM, Watermelon, Cucumber, 223086, HU11, 223090 and 223108. Genotyping gel picture showing PCR products of genotypes with their corresponding codes indicated in appendix III.

4.2. Microsatellite Polymorphism

The 24 SSR markers revealed a total of 354 alleles across the 45 anchote accessions, two cucumbers (*C. sativas* var. marketer and local) and one watermelon (*C. lanatus* var. crimson sweet) varieties (Table 4). The allelic richness per locus varied widely among the markers, ranging from 5 (CMTp210 and PU002147) to 29 (CMTp132) alleles, with an average of 14.75±6.69 alleles. Rare alleles (with frequency < 0.05) comprised 52% of all the detected alleles while intermediate alleles (with frequency 0.05–0.50) accounted for the remaining 48%. Locus CMTp132 was informative providing relatively large amounts of genetic information and its Shannon's information index (I) was 3.15. The average observed heterozygosity (Ho = 0.13±0.24) was lower than the expected heterozygosity/gene diversity (GD = 0.88±0.06); in connection the fixation index (F_{IS}) value was considerably high, F_{IS} = 0.85±0.27 implying high inbreeding among the anchote accessions. Polymorphic Information Content (PIC) values representing allelic diversity at specific locus ranged from 0.67 (CMTp210) to 0.95 (CMTm83 and CMTp132) with an average of 0.86±0.08 implying all the 24 markers are highly informative (PIC ≥ 0.5). The detail of the microsatellite marker polymorphism parameters is provided in Table 4.

Table 4. Genetic diversity parameters of 24 SSR markers in *C. abyssinica* accessions

Locus	*Diversity parameters								
	N_a	N_e	I	H_o	GD	uHe	MAF	PIC	F_{IS}
CMMS15-4	6	4.43	1.62	0.00	0.77	0.78	0.36	0.74	1.00
CMMS30-3	27	18.18	3.08	0.15	0.94	0.96	0.11	0.94	0.84
CMTm252	16	10.87	2.55	0.00	0.91	0.92	0.15	0.90	1.00
CMTm83	25	21.60	3.15	0.00	0.95	0.97	0.08	0.95	1.00
CMTp132	29	19.04	3.15	0.09	0.95	0.96	0.10	0.95	0.91
CMTp158	8	5.82	1.86	0.00	0.83	0.84	0.25	0.81	1.00
CMTp193	15	10.38	2.50	0.00	0.90	0.91	0.19	0.90	1.00
CMTp210	5	3.47	1.37	0.00	0.71	0.72	0.42	0.67	1.00
CSN270	23	15.23	2.93	0.00	0.93	0.94	0.16	0.93	1.00
PU000007	13	7.11	2.16	0.50	0.86	0.87	0.24	0.84	0.42
PU000705	8	6.99	2.00	0.27	0.86	0.87	0.21	0.84	0.68
PU000750	8	4.63	1.76	0.00	0.78	0.79	0.38	0.76	1.00
PU002147	5	4.16	1.49	0.00	0.76	0.77	0.29	0.72	1.00
S13	17	13.71	2.71	0.00	0.93	0.94	0.10	0.92	1.00
S15	12	8.29	2.27	0.00	0.88	0.89	0.17	0.87	1.00
S26	18	13.47	2.71	0.92	0.93	0.94	0.13	0.92	0.01
SSR00219	21	14.58	2.84	0.00	0.93	0.94	0.13	0.93	1.00
SSR01012	11	7.53	2.16	0.00	0.87	0.88	0.23	0.85	1.00
SSR01178z	14	10.29	2.45	0.35	0.90	0.91	0.17	0.89	0.61
SSR01566z	17	11.18	2.58	0.58	0.91	0.92	0.17	0.90	0.36
SSR02384	13	8.53	2.31	0.32	0.88	0.89	0.22	0.87	0.64
SSR04570	12	9.22	2.33	0.00	0.89	0.90	0.17	0.88	1.00
SSR07100	16	10.02	2.51	0.00	0.90	0.91	0.19	0.89	1.00
SSR10335	15	7.11	2.26	0.00	0.86	0.87	0.23	0.84	1.00
Mean	14.75± 6.69	10.24± 4.89	2.37± 0.5	0.13± 0.24	0.88± 0.06	0.89± 0.06	0.2± 0.09	0.86± 0.08	0.85± 0.27

* = *Diversity parameters:* - N_a: number of alleles detected per locus; N_e: effective number of alleles; I: Shannon's information index; H_o: observed heterozygosity; H_e: expected heterozygosity; uH_e: Unbiased Expected Heterozygosity; F_{IS}: Fixation Index; *MAF*: Major allele frequency; *PIC*: polymorphic information content

4.3. Genetic Diversity in Anchote Accessions and Population

Table 5 depicts the assessment of genetic diversity among the anchote populations. Overall, both the *ex situ* and *in situ* managed East Wellega anchote populations had relatively highest level of genetic diversity than other geographic regions. The e*x situ* and *in situ* populations from East

Wellega had identical value for some diversity parameter estimates (H_e = 0.83; A_p = 51 and PPL = 100) and comparable values for the others. Anchote from Ilubabor, Jimma, and West Wellega had comparable moderate genetic diversity. When comparing all ex situ collection with in situ collections from East Wellega, the in situ collections showed higher values for nearly all the diversity parameters. Observed numbers of alleles (N_a) were in the range of 1.92 – 8.63 across the anchote populations, and rarefied allelic richness (*Ar,* number of alleles corrected for sample size) was between 2.46 and 3.27. The higher H_e compared to H_o in all geographic populations of anchote indicated some inbreeding within population. At least one private allele was amplified from each of the populations. The mean percentage of polymorphic locus (PPL) across population was 95% varying in the range of 87.5–100 percent.

Table 5. Genetic diversity in the populations of *C. abyssinica* accessions based on geographic geographical origins and groups of *in situ* and *ex situ* maintained *C. abyssinica* accessions

§Populations	*Population diversity parameters										
	Na	Ar	Ne	I	Ho	He	uHe	Ap	Apr	F	PPL
East Wellega *ex situ*	8.25	3.27	6.54	1.93	0.12	0.83	0.86	51	1.20	0.86	100
East Wellega *in situ*	8.63	3.25	6.62	1.93	0.15	0.83	0.86	51	1.18	0.83	100
Ilubabor	3.75	2.71	3.38	1.20	0.12	0.66	0.73	12	0.79	0.84	100
Jimma	2.83	2.54	2.77	0.96	0.08	0.58	0.69	7	0.67	0.90	95.8
Kelem Wellega	1.92	−	1.90	0.62	0.08	0.43	0.58	6	−	0.85	83.3
West Wellega	3.42	2.53	2.85	1.08	0.16	0.62	0.69	5	0.66	0.79	100
Other species	2.88	2.46	2.73	0.91	0.19	0.54	0.64	8	0.70	0.72	87.5

* = *Population diversity parameters such as* - Na: average number of alleles detected per population; Ar: rarified allelic richness;Ne: effective number of alleles; I: Shannon's information index; Ho: observed heterozygosity; He: expected heterozygosity; uHe: Unbiased Expected Heterozygosity; Ap: Number of private alleles per population; Apr: private rarified allelic richness; F: Fixation Index; PPL: percentage of polymorphic loci. § = populations with sample size of < 2 (Horo Guduru, East Gojam, and Keffa*) were not considered and for Kelem Wellega (n = 2) rarification (correction of alleles to sample size) was not calculated due to smaller sample size*

4.4. Genetic Distances and Relationships

Neighbor-joining (NJ) based cluster analysis of 45 anchote accessions and three cultivars of related species accessed from nine different zones (Figure 1) resulted in six major clusters (Figure 3). Each of the six clusters comprised individual plants from different collection zones (geographic regions).The clustering pattern has shown little tendency of association with geographic origin of the germplasm. Although there is considerable admixture, individual accessions from East Wellega were grouped together at sub-cluster level. In one of the sub-clusters, two anchote accessions (HU – 14 and 229702), were grouped together with the outlier related species. In fact, accession 229702, collected from minor growing area in East Gojam ($11^0$04'13.88" E and $37^0$51'42.45" N) shared more number of alleles with cucumber and watermelon cultivars than anchote accessions.

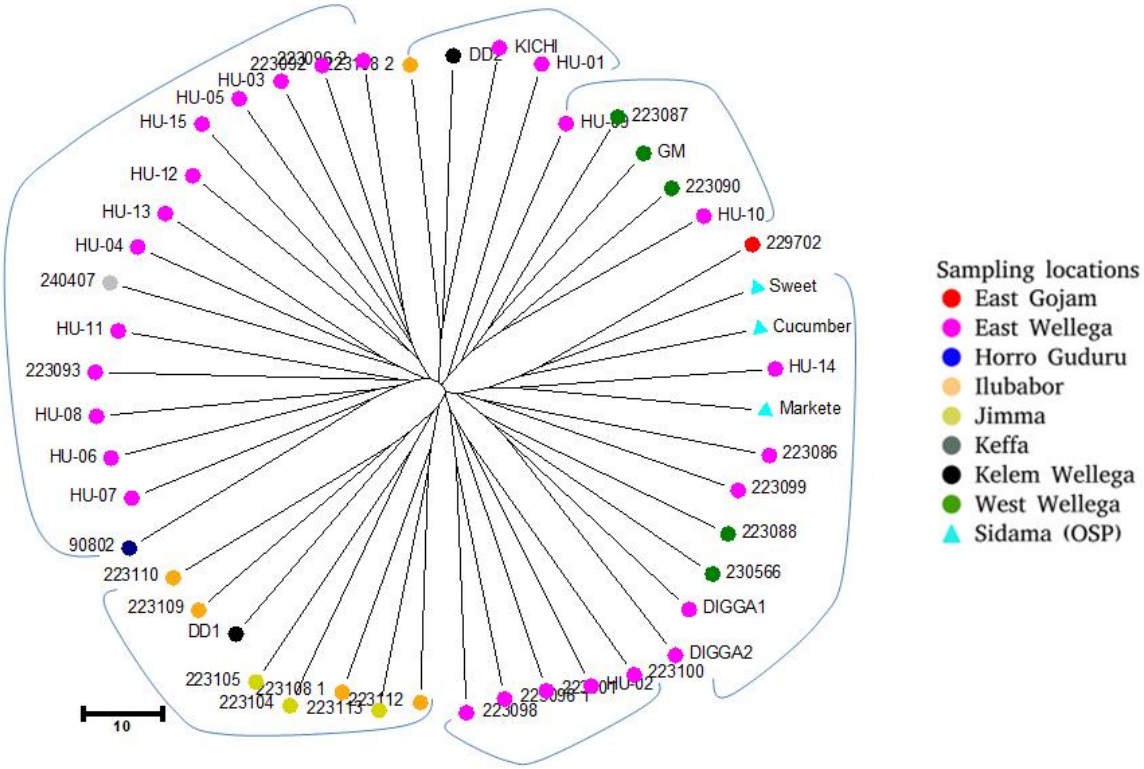

Figure 3. Unrooted neighbor-joining (NJ) tree based on sharedallele distance and 24 SSRs. The key relates the color of the sampling location to accessions.

The pairwise Nei's genetic distances and *Fst* values for all the anchote populations representing growing regions and *ex situ* and *in situ* management pools are shown in Table 5. Nei's genetic distance ranged from 0.357 between East Wellega *in situ (*EWi) and East Wellega *ex situ* (EWe) to 1.648 between Kelem Wellega (KW) and other species (OS). The *Fst* values confirmed the pattern, with the highest value 0.325 for the pair of Kelem Wellega (KW) and other species (OS) and the lowest value 0.043 for East Wellega *in situ* (EWi) and East Wellega *ex situ* (EWe). Expectedly, most of the anchote populations showed highest genetic distance from other species (OS, watermelon and cucumber) included as outlier.

Table 6. Nei's unbiased genetic distance (D_A) below diagonal and pairwise population differentiation (F_{ST}) above the diagonal among anchote growing geographic areas

*Populations	EWe	EWi	OS	IA	JM	KW	WW
East Wellega *ex situ* (EWe)	−	0.043	0.149	0.094	0.132	0.189	0.110
East Wellega *in situ (*EWi)	0.357	−	0.148	0.101	0.129	0.172	0.106
Other species (OS)	0.828	0.884	−	0.228	0.257	0.325	0.228
Ilubabor (IA)	0.588	0.736	1.304	−	0.166	0.212	0.164
Jimma(JM)	0.826	0.809	1.361	0.636	−	0.261	0.211
Kelem Wellega (KW)	0.938	0.720	1.648	0.688	0.856	−	0.281
West Wellega(WW)	0.710	0.667	1.093	0.976	1.218	1.560	−

*= Populations with less than two samples (Horo Guduru, East Gojam and Keffa) were not included in the analysis.

A phylogenetic tree based on Nei's genetic distances using UPGMA (Figure 4), resulted in grouping the 10 populations into five distinct clusters. The first cluster consisted of accessions from East Wellega (EW *in situ* and EW *ex situ*), Ilubabor (IA), Kelem Wellega (KW) and Jimma (JM). Keffa(KF) and Horo Guduru (HG) formed clusters of single accession, while the West Wellega (WW) accessions formed solitary cluster. The other species (OS) including cucumber and watermelon clustered together with anchote accession from East Gojam (EG) with a bootstrap value of 97.4%. Hierarchical cluster analysis showed that populations from Southern (JM, IA and KW) and Eastern (EW) parts of West Oromia were grouped together according to their geographical proximity. The phylogenetic tree infers a close relationship between *ex situ* and *in situ* managed East Wellega anchtoe with a bootstrap value of 98.1%. At the same time, the distinctly different individuals from SNNPR and Amahara region were very closely related to the outlier group and distantly related to anchote accession from Oromia region (Figure 4).

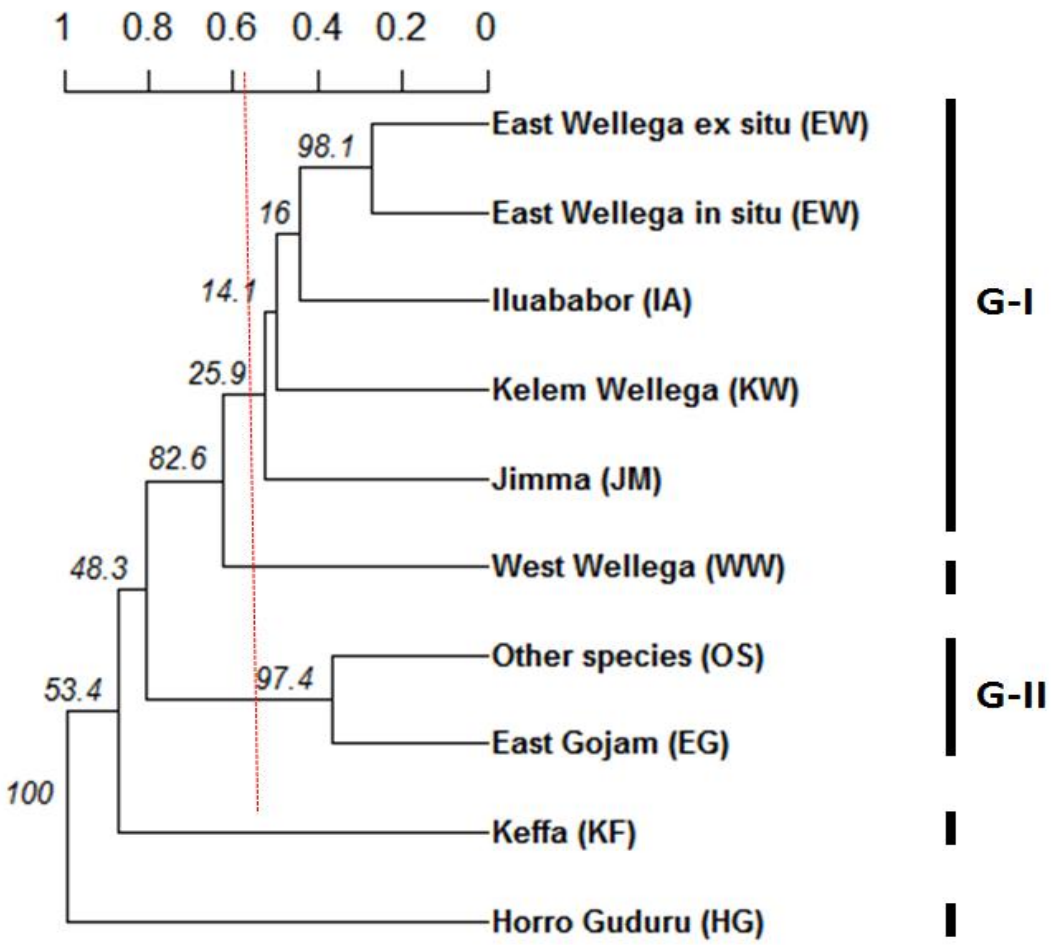

Figure 4. Relationship of anchote populations from different growing administrative zones using unweighted pair-group method with arithmetic mean (UPGMA) tree based on Nei's genetic distance.

Minimum spanning network (MSN) also revealed slight subdivision of population according to geographic origins. In general, accessions originated from the same geographic locations were genetically closer, although considerable admixture is evident (Figure 5). Similar to the UPGMA tree the MSN showed close relationship of East Gojam anchote accession (dot 01 in red) to the gourp of other cucurbit species.

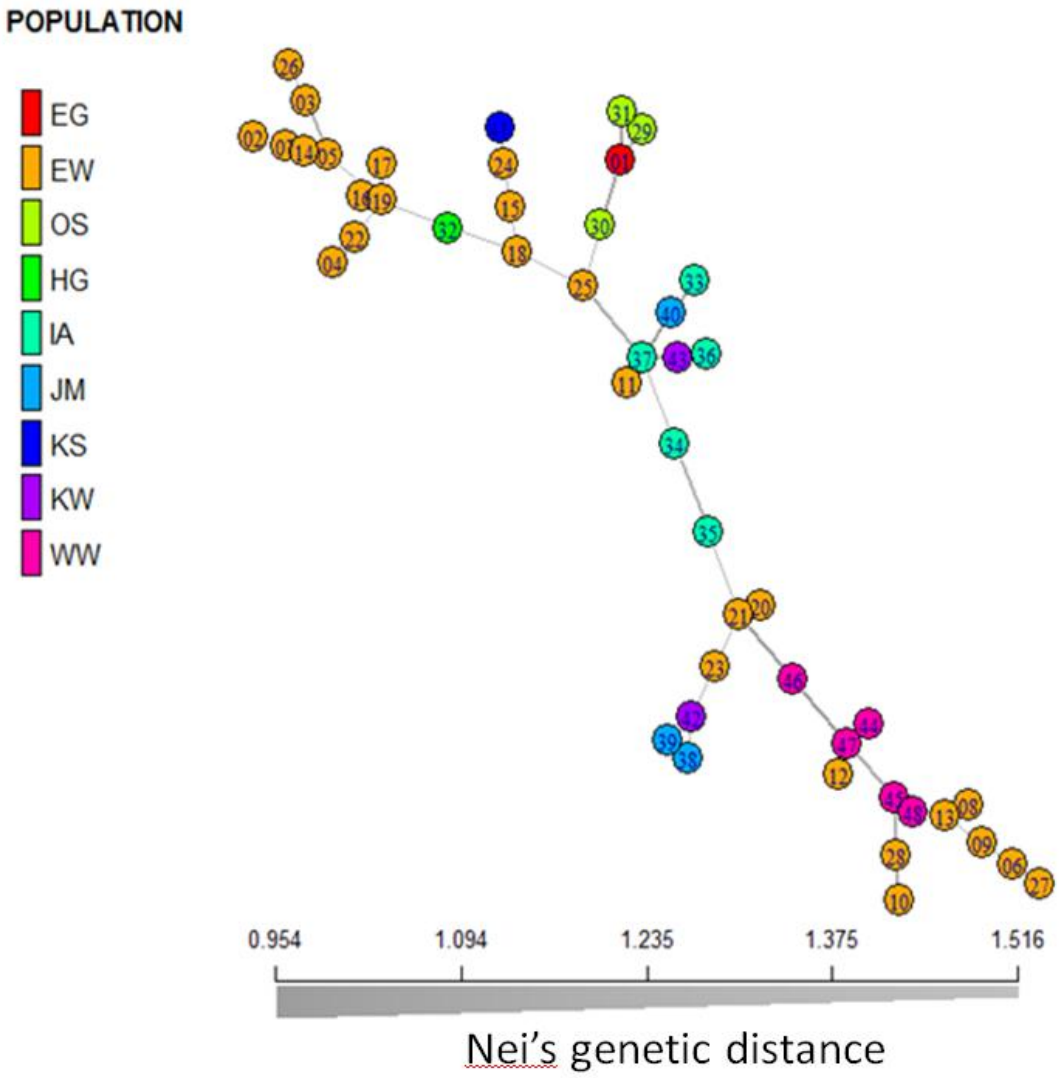

Figure 5. Minimum spanning tree (MST) of 45 anchote accessions and three related species based on 24 SSR markers.

4.5. Genetic Structure and Differentiation

Multivariate coordination method, DAPC, computed to assess structure in anchote detected four major clusters (Figure 6). These four clusters primarily corresponded to the geographic distribution of anchote populations (Figure 6). The first cluster covered all accessions from West Wellega (G-I), whereas in the second cluster (G-II) represented accessions from East Wellega. In

G-III accessions from Jimma, Ilubabor and Kelem Wellega were grouped with some overlaps. In the fourth cluster (G-IV), the related species (cucumber and watermelon) were distinctly grouped with one anchote accession from East Gojam.

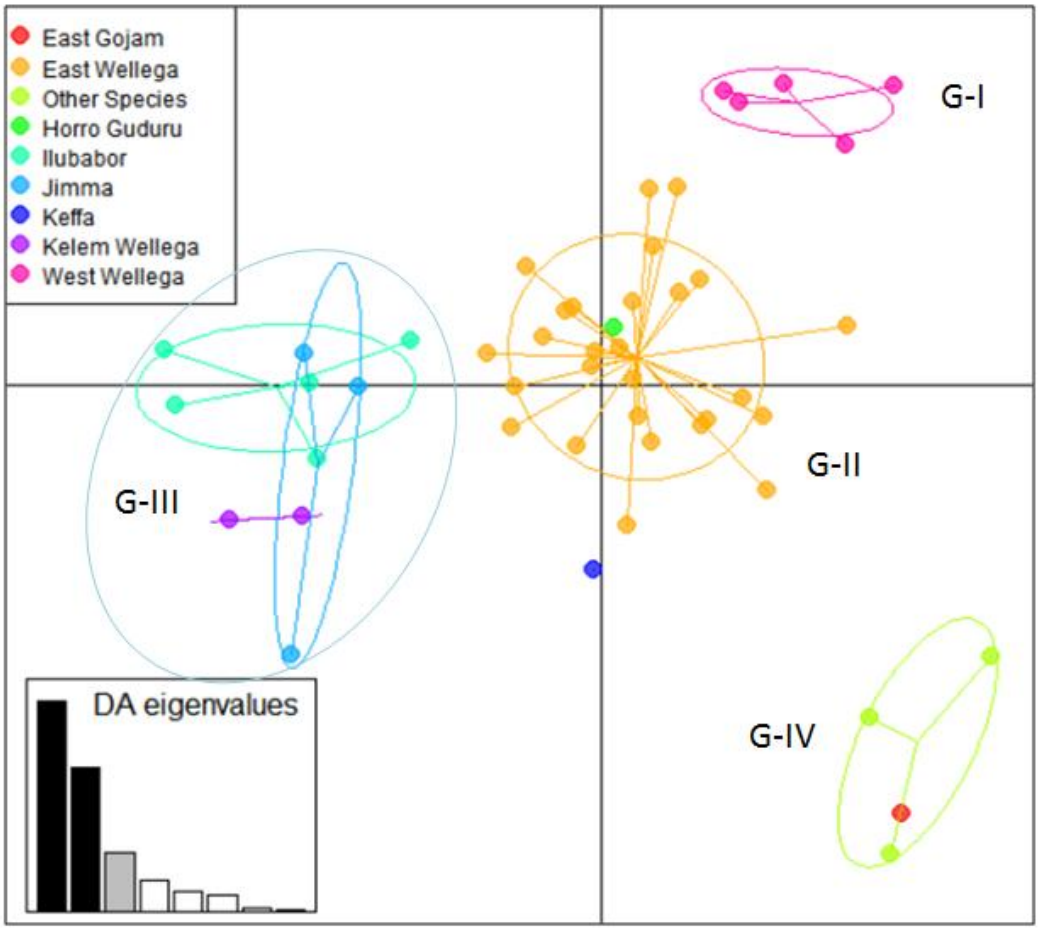

Figure 6. Scatter plot of the discriminant analysis of principal components (DAPC) of anchote accessions (points) and clusters (ellipses) in the first two axes.

As indicated in the membership probability bar plot (Figure 7), the extent of genetic admixture of individual accession from each anchote population was variable. The accessions of West Wellega had relatively less admixture. On the other hand, accessions from Jimma, Ilubabor and Kelem Wellega had marked degree of admixture among each other.

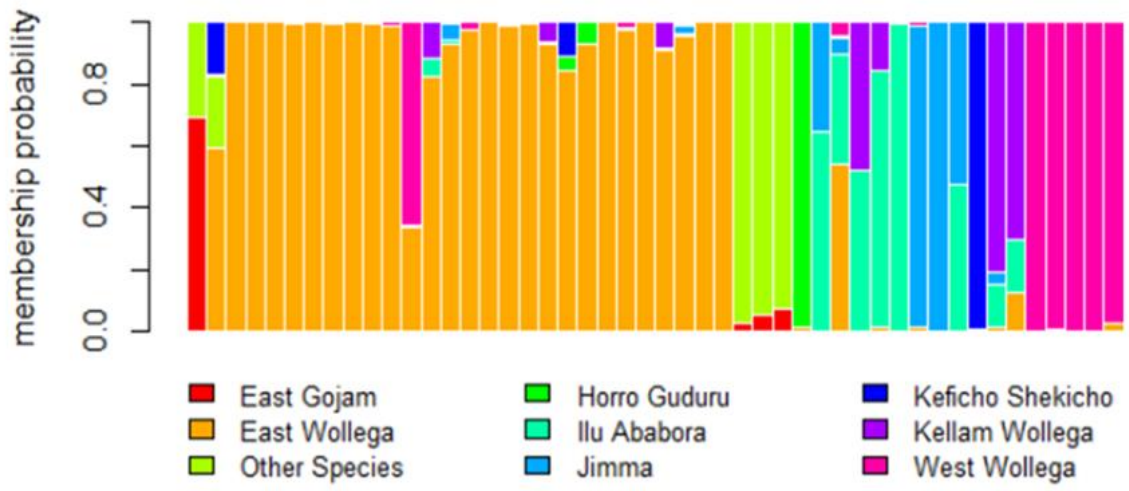

Figure 7. Bar plot representation of the individual accession membership to clusters together with their population of origin.

Patterns of genetic divergence by AMOVA analysis showed that most (95%) of the genetic variation among anchote populations is explained within the cultivation regions, and only 5% of the variation is due to differences among regions ($p < 0.001$, Table 7). Additional AMOVA grouping based on germplasm management (*in situ* and *ex situ*) found similar results to the grouping of geographic regions, with a greater percentage of the variation (98%) ascribed to within rather than between the management approaches. However, there was insignificant differentiation between *in situ* and *ex situ* maintained anchote germplasm. The results of the Mantel test showed lack of correlation between genetic divergence and geographic distances ($Rxy = 0.109$; $p = 0.011$) among the studied populations, indicating absence of isolation by distance.

Table 7.AMOVA among populations of geographical origins and groups of *in situ* and *ex situ* maintained *C. abyssinica* accessions

Source	Degree of freedom	Sum of squares	Mean Squares	Estimated variance		
				Value	%	*Φ*
Cultivation regions [N = 45]						
Among regions	6	303.922	50.654	2.165	5	0.054*
Within regions	38	1442.233	37.954	37.954	95	
Total	44	1746.156		40.118	100	
Germplasm management approaches (*In situ* and *Ex situ*) [N = 27]						
Between managements	1	48.763	48.763	0.699	2	0.017
Within managements	25	986.200	39.448	39.448	98	
Total	26	1034.963		40.147	100	

* Significant $p < 0.001$. *PhiPT (**Φ**) statistics* are compared with values obtained from 1000 permutations. Populations with less than two samples (Horo Guduru, East Gojam and Keffa) were not included in the analysis.

Mantel test showed the observed correlation (R^2= 0.081) between genetic divergence and geographic distances was insignificant (*Rxy* = 0.109; p = 0.011) among the studied populations, indicating absence of isolation by distance.

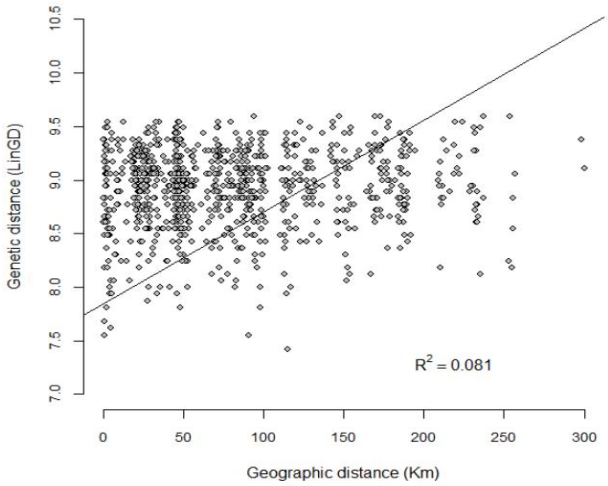

Figure 8. Scatter plot of genetic distance based on shared alleles and geographic distance for individual accessions

5. DISCUSSION

Genetic diversity and population structure studies using molecular markers for anchote are limited. The present investigation used 24 genomic SSRs after screening 102 SSRs from five species belonging to cucurbitaceae family for their cross-transferability. The 46% transferability with 23.5% polymorphism rate from the present study is moderate as compared to cross-genera SSR marker transferability in the Cucurbitacae family. Several studies showed high cross species and cross-genus SSR transferability in Cucurbitaceae, on average up to 70-88% cross-transferability rate (Yuichi, 2012; Weng, 2010; Stift *et al.,* 2004). In general, the transferability of SSR markes varies across species and genus (Yildiz *et al.,* 2015; Kumawat *et al.,* 2014) and the marker types higher for EST-SSRs (Hu *et al.,* 2011).

The number of molecular markers reported in the present study is higher than previously applied for the species (Bekele, 2017; Bekele *et al.,* 2014). The statistical parameters, such as mean number of alleles amplified per primers (Na = 15), gene diversity (GD = 0.88) and PIC value ranging from 0.67 - 0.95, reported in the present work are higher than estimates from EST-SSRs in anchote (Bekele, 2017). Conversely, the level of observed heterozygosity (H_o = 0.13) for the populations in this study was lower than anchote collections characterized using EST-SSR markers (Bekele, 2017). The genomic SSR and EST-SSR differ in assessing genetic diversity of a species since they come from different regions of the genome. Possible selection against alterations in the conserved coding sequences in EST-SSRs limits their variation. Accordingly, higher allele variations for genomic SSR than EST-SSR markers was also reported in cucumber (Hu *et al.,* 2011) and other plant species (Parthiban *et al.,* 2018; Wen *et al.,* 2010; Chabane *et al.*, 2005, Eujayl *et al.,* 2001).

Relatively lower average observed heterozygosity than the expected heterozygosity and the associated high fixation index (F_{IS} = 0.85) in our study implies high inbreeding among the assessed anchote accessions. Various factors contributing towards inbreeding in anchote populations can be hypothesized; first, mode of pollination and second, reduction in population sizes. Although contradicting reports of anchote pollination including selfing and crossing possibilities exist (Bekele, 2017; Holstein 2015; Desta, 2011), self-pollination might contribute to high F_{IS} values in the crop. On the other hand, studies show declining production size of anchote which can also lower genetic diversity and lead to inbreeding depression. This is a probable scenario in the studied population of *C. abyssinica*, which is confirmed by recent reports of the species continued production retreat from field crop to garden crop (Wayessa, 2018). Overall, future availability of descriptive and experimental data on breeding system, floral biology, and pollination mechanisms would permit to elucidate the pattern of differentiation and genetic structure in anchote populations.

Genetic relationship of accessions assessed using cluster analysis revealed groups primarily associated with geographic origins of accessions. Although the grouping pattern had coincided with DAPC population structure analysis, the number and type of accessions grouped into different clusters was variable and there are admixtures between them. Geographic pattern of genetic structure was observed with consistent distinct grouping of anchote accessions from West Wellega. Researchers hypothesize possible domestication of anchote in Wellega (Bekele *et al.*, 2014; Desta, 2011). Oral accounts and ethnoarcheology indicate that anchote culture is deep-rooted in antiquity among the West Wellega Oromo culture (Wayessa, 2018). These records support that the crop has been domesticated and therefore selected by West Wellega farmers since ancient times, which likely caused the genetic differentiation of the West Wellega anchote

in comparison to the other populations. However, earlier studies using other markers reported insufficient support of geographical population differentiation (Bekele *et al.,* 2014; Bekele, 2017). This might be related to the fact that population structure analysis is sensitive to marker type, number of loci, number of populations and individuals in a study (Evanno *et al.,* 2005). AMOVA analysis also attributed most of the variation (95%) to within group differences than between groups. Similar partitioning of variation at population level has been reported in prior studies (Bekele, 2017; Bekele *et al.,* 2014).

The study has implications for improvement and management of anchote genetic resources. Currently, anchote germplasm collections as well as genetic diversity studies are biased towards cultivated accessions from major growing areas of Western Ethiopia (Bekele, 2017; Bekele *et al.*, 2014). The extent and population genetics of anchote wild relatives, related *Coccina* species and cultivated anchote from minor producing areas are virtually unknown. For instance, accession 229702, collected from minor growing area in East Gojam showed unique allelic variation at all analyzed microsatellite loci. Such unique genetic resource pools can broaden the genetic base for improvement of anchote and merit greater consideration for further characterization. *In situ* collections showing higher values of diversity parameters also demonstrate the need for special emphasis to the genetic resources on farmers' fields and necessitate the collection and conservation of these and additional genetic resources in *ex situ*. In wider context, further knowledge on the African Cucurbitaceae genetic resources, particularly on the native *Coccinia,* might provide sound basis for improvement of cucurbits globally particularly in the era of climate change.

6. SUMMARY AND CONCLUSION

The study assessed the extent of genetic diversity in anchote accessions and population structure across major cultivation ranges in Ethiopia. A total of 45 anchote germplasm accessions collected from different parts of Ethiopia were studied along with three cultivars of related species in Cucurbitaceae family. The first set of cross-transferred 24 SSR markers from several species of cucurbits showed high level of genetic diversity with medium differentiation maintained within the anchote accessions. While higher allelic diversities are suggestive of presence of rare alleles imparting a broad genetic base to the *C. abyssinica* populations, the narrow population structure is of concern as the individual populations may suffer from dramatic decline due to genetic drift. Seed collections from many individuals and geographic agroecologies need to be deployed in order to stoke seeds in seed and gene banks. Observed heterozygosity is less than expected heterozygosity and associated high fixation index is a concern that may cause high inbreeding/genetic drift. To overcome the above issue broader collections and reintroduction to major producing areas will be conducted. Studies are biased towards cultivated anchote from Western parts of Ethiopia. To determine the unique properties of anchote at molecular level, farther studies will be done on wild anchote and other native *Coccina* (8 species) as well as anchote from other areas by sequencing the crop and designing new primers from the generated sequence information. Genetic diversity should be monitored at genome level focusing on functional factors and agro-morphological traits of improvement concern. This knowledge of population structure and genetic diversity of *C. abyssinica* accessions is crucial for future studies using genomic selection, marker assisted selection and geonome wide association studies.

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Appendix

Appendix I. List of SSR primer pairs used to test cross transferability

Source	SSR Primers	Forward primer	Reverse Primer	SSR motif	Expected size
C. moschata	CMTm209	attagaatcccttggagaacg	gcaatcgtttttctgttggt	(ct)14	152
C. moschata	CMTm68	cgccatcgttgttcttcac	agatccacagcgatttcgtc	(ttc)12	109
C. moschata	CMTm83	ggcatttctgagaacagctt	acgttagttatgctattttgtaggc	(ag)19	111
C. moschata	CMTm144	acatgggcatacctcgaatc	cacctggctgttttgtctga	(ag)11	150
C. moschata	CMTm224	gtgggtcgctccttaaa	atagcggataaagtggactg	(ct)10	120
C. moschata	CMTm219	ttcagaggacatctgccatt	atagttctcgctgggcttg	(ag)10	112
C. moschata	CMTm120	gccaaaggttccaaatgaca	tgatttgcgcacaaacaaac	(ct)13	121
C. moschata	CMTm252	cccattccccatttctttct	caaattcgatcggctttacac	(ct)5+8	101
C. moschata	CMTm84	tatcaccaaacgtgcat	aggttttgaagaacaaattc	(ag)13	122
C. moschata	CMTm66	acgcgttgttgttagtgtgg	ggggatttatgaacccaacat	(ag)8+4	126
C. moschata	CMTm111	ctccattcccatggcttc	ccatgagcttgagagaggtg	(cct)5	110
C. moschata	CMTm207	ggaagagagagattgtaagcaca	ccctacacaacagtggctct	(ag)5+8	108
Cucumis melo L.	CMMS004	gcccaacggacacactcactcacac	gagggagtaagaataagaagaagaa	(GA)16(GA)12	190
Cucumis melo L.	CMMS1-3	ttgaatgattggagggaagataacg	caaatattgatggatttaatatatt	(GA)13(AAT)6	259
Cucumis melo L.	CMMS2-2	ctcttttgcattataataattaacc	ggggccaacgaaatccagtcataga	(GA)18(AAG)5	323
Cucumis melo L.	CMMS3-1	aaatataagcaaaccaaagttgacc	ccgggatatacggacatacacacac	(GT)25	190
Cucumis melo L.	CMMS3-2	agtttttgggcatttttagttggat	ggcttatctcccacccaaccattct	(GA)19	420
Cucumis melo L.	CMMS11-3	tgccatattctgtgctgattgaaac	atgtaatcaattctctctctctctc	(GA)15	168
Cucumis melo L.	CMMS12-4	gatgcggtgagaaagagttgagaga	agagggagagagtttgtaaaaaaat	(GA)15(GT)11	347
Cucumis melo L.	CMMS14-1	cattgctactattgtcgtcgttgct	tttctttctttttccgtatccatttt	(GA)15(GA)9	400
Cucumis melo L.	CMMS15-4	gtccgccatcgccactacaaatcaa	ctccgtaaaaccttcttcctctctc	(GA)14	116
Cucumis melo L.	CMMS30-3	ttcccaccagcccaacggacacact	gagatacagaaacgacgactaacct	(GA)16	271
Cucumis melo L.	CMMS33-1	tgtaataggatgaccaaggggagtt	ttcaggagctacaacaagatttcaa	(GT)13	420
Cucumis melo L.	CMMS34-8	tttcttacttttggtttggttctg	ggcgctgtggtgagtgtcgggagag	(GA)13	480

Appendix I. List of SSR primer pairs used to test cross transferability (continued)

Source	SSR Primers	Forward primer	Reverse Primer	SSR motif	Expected size
Cucumis melo L.	CMMS35-3	cggagaagaaggaagggttttaaga	attcgtagttcatactctctttctc	(GA)14	312
Cucumis melo L.	CMMS35-4	acggatacatcgaggagacttcatg	gtcagcttcaacccttttacttttttc	(GT)11	105
Cucumis sativus L.	SSR00219	gtgaatgagagccgttggat	gagacgcattcaattcctgc	(GAA)10	202
Cucumis sativus L.	SSR00647	cgattttccagcattgatgt	tagttttgggctttcgatgg	(GAA)10	202
Cucumis sativus L.	SSR01115	attccaatcccaaaaaggt	ctcctcctccaatgagcaag	(TACA)15	215
Cucumis sativus L.	SSR01254	atcttcggcaaaaagcaaac	atactcaccccaccgtcact	(GA)16	
Cucumis sativus L.	SSR02384	aaaaatccgacaaatcgtgc	ggtcaaatgttgccttttgc	(AT)14	142
Cucumis sativus L.	SSR03514	tagggtccccttccctcata	gggtacccaaaagcaagtga	(TC)14	154
Cucumis sativus L.	SSR04534	ggttcaaaccccctcaactt	gggggttgggagacagttat	(TA)27	151
Cucumis sativus L.	SSR07100	cacaccatttacggttatggg	catttggttcagaaagggga	(CT)16	192
Cucumis sativus L.	SSR10335	aaatggaaccccaacaatca	tagattaatttgggcagccg	(CAG)13	185
Cucumis sativus L.	SSR00004	ttcattgcaaagcacacaca	tgaaaagagggaacaaaagca	(AT)15	216
Cucumis sativus L.	SSR00710z	gagaacccatctcaaagatgaa	ataaatgggtttcggttccc	(AT)13	
Cucumis sativus L.	SSR00931y	ggacatccccattgaatttg	acgtggggtcgacgtaataa	(TA)21	
Cucumis sativus L.	SSR01012	tccaaaaatcgcgacctaaa	gtgagccgttgatttctcgt	(ATA)9	200
Cucumis sativus L.	SSR01178z	aattgacttttgggcctcag	tgctctcagttgaaaaatgctc	(AG)14	173
Cucumis sativus L.	SSR01191	ttctttcaaatgcccatcaa	aaagatatgggtgggagcaa	(TG)15	166
Cucumis sativus L.	SSR01379	tgttactgctggcaatgctt	tcagaaatcaggagctggaga	(TC)18	212
Cucumis sativus L.	SSR01552	cacgacttgccattaaccct	cccaaactttcccctcaaat	(TCT)8	135
Cucumis sativus L.	SSR01566z	tggaaaagctcagtttgtttga	tgagtgcgaaccaacctaca	(AG)14	
Cucumis sativus L.	SSR01903	cattgtcctgatggggaaag	acccttctcagcggctatt	(AT)12	209
Cucumis sativus L.	SSR04570	aaaggttgccaaagggaaat	tcgcgtaaagaaacagaggc	(TGA)8	
Cucumis sativus L.	SSR07248	cgattggaaaatatcggcac	cgaatcgccttcagttcttt	(AAG)9	141
Cucumis sativus L.	EC13	gcaatgaatcatgacctcca	ctgagaattgggaagggaca	(TTC)9	244
Cucumis sativus L.	EC15	accaaaaacagacccctatg	gaaagggaaaacaaacgagg	(AGA)6	267

Appendix I. List of SSR primer pairs used to test cross transferability (continued)

Source	SSR Primers	Forward primer	Reverse Primer	SSR motif	expected size
Cucumis sativus L.	EC18	tgccatttcatcgactcttc	gcatttctgctgtggcttag	(CCT)6	218
Cucumis sativus L.	EC24	acaacacaaccgcttctcgt	tgagcccaagcacataacag	(TTA)7	234
Cucumis sativus L.	EC27	gttggaaggcacacaaagtc	cgagatgattggaggatgatg	(ATC)7	287
Cucumis sativus L.	EC31	ctaaccagcagaacccaatg	gtatcctgtttccagcgaga	(TTC)7	164
Cucumis sativus L.	EC34	gatccccatcataatcaccc	caaagggctacaataacaaac	(TA)15	191
Cucumis sativus L.	EC39	ccaagtttaagttatttaggag	gaagaggacgataaagatga	(TCA)7 N8 (CTT)5	155
Cucumis sativus L.	EC41	agcatgtggaggagaaagca	ttcatcatcgagtgggtctg	(AGA)5	234
Cucumis sativus L.	EC47	cgatctttgtcatccgacct	agaacgagcacgttttgagc	(CT)8	258
Cucumis sativus L.	EC49	cgtgttttctcagatttccca	cacttcccttatcaacccca	(TCTTTC)6	219
Cucumis sativus L.	EC54	ttcatcacccctttccctt	aaacacgatttcccaacacc	(CTT)10	267
Cucumis sativus L.	EC57	ttcttcacagcttcctttgg	gcaaacaaagcaacaaaacg	(GAA)9	249
Cucumis sativus L.	CSN080	gggtattaattaggatgtgaagcga	gggaattcgattgtttagccttgt	(ag)27, (tct)4	104-126
Cucumis sativus L.	CSN221	gagaaccacttttccggcaaataa	caaaaggttggatcttgttgtcca	(ag)12	256-339
Cucumis sativus L.	CSN263	attacaaccacaagtggcgagaca	agctgatttcaccacagcttcaaa	(tc)15	275-291
Cucumis sativus L.	CSN270	ttgccttctctctcctcttccaaa	agtggctaaaaaggcagcaagatg	(ta)5(tc)3,(ct)3(tc)6(tc)6	291-294
Cucumis sativus L.	CSN287	agggagatagtatgacaagatttcctc	agtggggttgagcaagttgaagac	(ag)14	194-306
Cucumis sativus L.	CSN288	cctttgaaaattaagaggctgctca	gcagaagcctaagaaaacgagagaga	(ag)24	278-297
Cucumis sativus L.	CSN306	tttcctccccttttccttcattctc	caacccaaatgcttagagaaccca	(tg)12	294-301
Cucurbita pepo	PU000007	ggagaccccattttcctgtt	aagcactcacttccccttca	(CCG)5	105
Cucurbita pepo	PU000396	cctgcgattgtaagatgcaa	cagacctcgaaagccaagtc	(TCT)5	221
Cucurbita pepo	PU000705	aaagaaaagctcgcttggtg	tccatgcccacatcaactaa	(CGA)7	122
Cucurbita pepo	PU000750	aaagttgtccaaaaccgacg	ggctcgagaatctgaaaacg	(CCTTT)5	228
Cucurbita pepo	PU001491	cacagacgcagaaacagagg	aaggacaagatccatgggg	(CTT)5	158
Cucurbita pepo	PU002147	gctctgtgaaggaggacgac	ccgctgcatagggaatgtat	(GAAGAG)5	239
Cucurbita pepo	CMTp68	cacacccatttcattttgacc	attgattgggacgtgaggaa	(tc+ggcttc)10+6	189
Cucurbita pepo	CMTp193	ggtgacggcaagaaaagcta	gctgaccctctctccctctc	(ga)18	186
Cucurbita pepo	CMTp201	aggagtggtgggctaatacg	tgaaattgagggagggagag	(ct)18+7	110

Appendix I. List of SSR primer pairs used to test cross transferability (continued)

Source	SSR Primers	Forward primer	Reverse Primer	SSR motif	Expected size
Cucurbita pepo	CMTp131	gcacttgaatcttcgtcaac	cgagaaagaattaacgagca	(ccg)7	151
Cucurbita pepo	CMTp174	gccggaaccagacttctc	ccctcccttcccattaaac	(tc+t)6+5+11	176
Cucurbita pepo	CMTp125	cttgttccgcagcatcag	agtgagagggagacgcaaag	(tc)8+6	115
Cucurbita pepo	CMTp210	gtggaagttactgcgattgg	gcaaagaatgtcctcagcag	(gtgtgc)6	117
Cucurbita pepo	CMTp132	ccatttccatttccatttca	aggttagaaacaggggaatc	(gat)12	151
Cucurbita pepo	CMTp158	ccgtagagatgtcagagacaagg	agggatgctcatcacacctc	(gcag)7	134
Cucurbita pepo	CMTp182	cacgaagatttgatggcctta	ggattgggatggtgaagatg	(CGT)11	138
Cucurbita pepo	CMTp138	aaaggtttccacatccttg	gaaaaggaaaaagtgttcaaag	(T)18	103
Cucurbita pepo	PU000007	ggagaccccattttcctgtt	aagcactcacttccccttca	(CCG)5	105
Cucurbita pepo	PU000396	cctgcgattgtaagatgcaa	cagacctcgaaagccaagtc	(TCT)5	221
Cucurbita pepo	PU000705	aaagaaaagctcgcttggtg	tccatgcccacatcaactaa	(CGA)7	122
Cucurbita pepo	PU000750	aaagttgtccaaaaccgacg	ggctcgagaatctgaaaacg	(CCTTT)5	228
Cucurbita pepo	PU001491	cacagacgcagaaacagagg	aaggacaagatccatgggg	(CTT)5	158
Cucurbita pepo	PU002147	gctctgtgaaggaggacgac	ccgctgcatagggaatgtat	(GAAGAG)5	239
Momordica charantia	JY001	ggctcagaactggcacag	tatcacccatccattcac	(CT)5(CTT)17	402
Momordica charantia	JY004	gtcaactgccatcggtac	agggaagaagaagaagaag	(CTT)22	105
Momordica charantia	JY005	tttatagcaaacggctca	gaacatatcgcaaacctta	(AG)6(CTT)7	330
Momordica charantia	JY006	tttccagaggagcaga	gctcagaactggcaca	(CTT)16(CTT)6	294
Momordica charantia	JY009	taaacaacaaaaccac	ctcagagtcagagcaa	(CT)23	298
Momordica charantia	S26	gaacgccctgtgactttagc	tttcgtcttccaatgagcc	(GA)13	193-198
Momordica charantia	N1	gtcttccaggttgggaacag	atctggttcctcgggagatt	(GA)11(A)9	145-160
Momordica charantia	N6	gggaattctcaaagagccaga	tggcacactctgcatgaaat	(GA)14	140-164
Momordica charantia	N12	cagaggggtggttcctcttt	ccacatggatgatcgagaga	(AG)9(GA)11	169-190
Momordica charantia	S9	ttcccattcacagatcactcc	ccaccaaattcaagaacccac	(TC)6(TC)11	332-350
Momordica charantia	S13	ttggttgtggtgctgagttc	gatgtaggggttgggttgat	(TC)15	270-281
Momordica charantia	S15	gggtagtggaatgatgggtt	tagtgttttcgtgagggagg	(AG)6(AG)12	232-247

Appendix II Twenty four SSR markers used for genotyping with accessions and scoring result

Accession No	CMMS15-4	CMMS30-3	CMTm252	CMTm83	CMTp132	CMTp158	CMTp193	CMTp210	CSN270	PU000007	PU000705	PU000750
90802	120/120	271/271	105/105	159/159	229/229	126/126	187/187	129/129	0/0	131/131	128/128	239/239
223086	122/122	291/291	99/99	92/92	266/266	150/150	181/181	117/117	293/293	125/125	125/125	221/221
223087	120/120	275/275	111/111	0/0	276/276	142/142	160/160	111/111	302/302	134/134	131/131	227/227
223088	122/122	0/0	109/109	0/0	272/272	142/142	163/163	111/111	0/0	125/125	128/128	227/227
223090	122/122	283/283	111/111	0/0	272/272	134/134	169/169	123/123	294/294	131/131	122/122	233/233
GM	122/122	267/267	119/119	103/103	273/249	138/138	166/166	0/0	302/302	134/134	131/131	227/227
223092	116/116	265/265	105/105	118/118	0/0	134/134	178/178	123/123	290/290	136/129	122/119	251/251
223093	118/118	271/271	103/103	124/124	229/229	130/130	175/175	129/129	299/299	128/113	122/122	245/245
223096	116/116	261/261	93/93	120/120	230/230	134/134	175/175	123/123	285/285	122/113	125/122	263/263
223098	114/114	271/261	89/89	98/98	237/237	142/142	178/178	117/117	292/292	122/116	128/122	227/227
223099	0/0	261/261	91/91	112/112	236/236	134/134	172/172	117/117	293/293	125/113	134/125	227/227
223100	120/120	255/255	95/95	120/120	240/240	122/122	181/181	111/111	290/290	125/113	137/128	239/239
223101	122/122	257/257	95/95	103/103	248/248	130/130	178/178	117/117	291/291	125/113	134/128	239/239
223104	120/120	259/259	97/97	126/126	256/256	126/126	178/178	111/111	293/293	122/113	137/137	233/233
223105	116/116	255/255	99/99	129/129	264/264	122/122	184/184	117/117	294/294	128/121	137/137	233/233
DD	124/124	245/245	97/97	0/0	263/263	134/134	193/193	117/117	285/285	128/119	137/137	233/233
223108	116/116	299/255	97/97	130/130	259/259	122/122	190/190	117/117	291/291	128/119	128/128	227/227
223109	124/124	274/255	95/95	0/0	263/263	130/130	187/187	123/123	285/285	128/119	128/128	221/221
223110	124/124	261/243	97/97	0/0	263/263	126/126	187/187	129/129	292/292	128/119	134/134	227/227
223112	122/122	259/247	101/101	136/136	264/264	130/130	190/190	123/123	255/255	125/119	140/140	227/227
223113	122/122	287/287	101/101	137/137	266/266	122/122	190/190	117/117	301/301	131/119	140/140	233/233
229702	118/118	275/275	107/107	114/114	264/264	142/142	166/166	117/117	293/293	128/128	134/122	221/221

Appendix II. 24 SSR markers used for genotyping with accessions and scoring result (Continued)

Accession No	PU002147	S13	S15	S26	SSR00219	SSR01012	SSR01178z	SSR01566z	SSR02384	SSR04570	SSR07100	SSR10335
90802	227/227	256/256	218/218	192/184	251/251	191/191	177/177	164/142	160/160	246/246	180/180	173/173
223086	245/245	254/254	236/236	204/196	306/306	197/197	201/185	170/156	150/150	249/249	180/180	179/179
223087	227/227	250/250	230/230	204/192	267/267	176/176	185/177	172/148	146/146	252/252	188/188	173/173
223088	239/239	246/246	230/230	204/190	273/273	173/173	185/177	172/148	152/152	249/249	186/186	179/179
223090	239/239	248/248	230/230	204/192	267/267	179/179	185/177	164/148	152/150	246/246	188/188	176/176
GM	239/239	248/248	230/230	204/190	267/267	176/176	183/175	170/148	154/148	246/246	186/186	176/176
223092	233/233	262/262	234/234	194/182	260/260	176/176	175/175	164/142	164/164	240/240	180/180	167/167
223093	233/233	256/256	230/230	196/184	260/260	182/182	173/173	166/142	166/166	234/234	186/186	167/167
223096	227/227	248/248	234/234	190/180	263/263	185/185	175/175	166/142	164/164	264/264	202/202	173/173
223098	233/233	240/240	238/238	186/174	269/269	182/182	171/171	174/174	158/158	255/255	206/206	182/182
223099	227/227	244/244	236/236	186/174	263/263	179/179	173/173	172/142	168/158	264/264	204/204	179/179
223100	233/233	242/242	240/240	186/176	273/273	182/182	177/177	158/158	156/156	258/258	200/200	185/185
223101	239/239	254/254	234/234	192/182	260/260	182/182	175/175	150/150	158/158	255/255	204/204	194/194
223104	233/233	254/254	234/234	196/184	276/276	185/185	177/177	148/148	160/160	261/261	212/212	200/200
223105	233/233	260/260	234/234	198/186	282/282	185/185	181/181	170/170	154/154	267/267	212/212	206/206
DD	233/233	260/260	234/234	208/192	285/285	194/194	195/195	144/144	160/160	255/255	180/180	179/179
223108	239/239	254/254	234/234	200/188	303/303	191/191	191/191	148/148	160/160	264/264	174/174	179/179
223109	239/239	252/252	234/234	186/186	273/273	188/188	187/187	150/150	156/156	249/249	178/178	179/179
223110	239/239	246/246	232/232	200/188	285/285	188/188	187/187	146/146	154/154	255/255	176/176	182/182
223112	239/239	244/244	238/238	204/186	282/282	191/191	191/191	146/146	0/0	261/261	178/178	179/179
223113	239/239	246/246	236/236	204/188	300/300	191/191	191/191	148/148	152/152	255/255	178/178	182/182
229702	245/245	234/234	238/238	194/182	279/279	176/176	189/189	170/148	0/0	243/243	184/184	173/173

Appendix II. 24 SSR markers used for genotyping with accessions and scoring result (Continued)

Accession No	PU002147	S13	S15	S26	SSR00219	SSR01012	SSR01178z	SSR01566z	SSR02384	SSR04570	SSR07100	SSR10335
DIGGA	245/245	238/238	238/238	194/186	276/276	173/173	187/179	160/160	148/148	243/243	188/188	173/173
230566	233/233	246/246	236/236	200/188	276/276	173/173	187/179	168/148	148/148	249/249	186/186	179/179
240407	227/227	232/232	224/224	198/198	303/303	173/173	173/173	152/152	154/154	246/246	180/180	191/191
KICHI	221/221	236/236	230/230	198/186	279/279	182/182	183/183	144/144	154/154	252/252	178/178	142/142
HU-01	233/233	248/248	230/230	208/208	283/283	182/182	183/183	146/146	156/156	249/249	182/182	170/170
HU-02	233/233	242/242	230/230	210/210	300/300	179/179	177/177	150/150	154/154	243/243	180/180	182/182
HU-03	227/227	250/250	222/222	190/178	260/260	185/185	175/175	166/142	160/160	234/234	190/190	176/176
HU-04	227/227	252/252	224/224	208/198	285/285	200/200	187/179	166/144	162/154	252/252	206/206	191/191
HU-05	227/227	272/272	226/226	206/192	288/288	185/185	189/189	162/144	164/158	249/249	180/180	176/176
HU-06	233/233	260/260	220/220	198/188	279/279	185/185	181/181	162/144	164/156	249/249	178/178	179/179
HU-07	227/227	262/262	218/218	198/188	277/277	182/182	181/181	164/144	158/152	246/246	182/182	173/173
HU-08	227/227	258/258	220/220	194/184	263/263	191/191	173/173	164/164	168/162	249/249	180/180	173/173
HU-09	239/239	250/250	226/226	206/192	264/264	179/179	183/175	174/146	156/152	255/255	188/188	170/170
HU-10	239/239	248/248	224/224	206/194	267/267	188/188	185/175	166/166	156/150	261/261	190/190	176/176
HU-11	233/233	260/260	216/216	198/186	263/263	182/182	175/175	164/142	0/0	252/252	176/176	164/164
HU-12	227/227	252/252	222/222	206/194	270/270	182/182	185/175	162/148	160/156	255/255	198/198	183/183
HU-13	233/233	244/244	224/224	198/192	270/270	188/188	185/175	166/146	158/154	252/252	200/200	188/188
Cucumber	239/239	240/240	236/236	196/188	273/273	185/185	189/181	166/148	152/146	243/243	176/176	176/176
HU-14	245/245	244/244	238/238	200/190	270/270	191/191	191/183	164/152	146/146	240/240	176/176	173/173
Markete	245/245	232/232	240/240	192/184	273/273	182/182	185/175	158/146	148/144	243/243	176/176	173/173
Sweet	245/245	232/232	238/238	194/182	273/273	179/179	191/181	170/150	146/146	246/246	180/180	176/176
HU-15	221/221	250/250	224/224	194/182	257/257	176/176	173/173	164/144	160/160	234/234	192/192	170/170
223096	221/221	246/246	236/236	188/174	263/263	182/182	177/177	168/138	154/154	270/270	206/206	179/179
DD	227/227	258/258	232/232	206/186	294/294	194/194	195/195	144/144	154/154	267/267	178/178	176/176
223108	233/233	250/250	236/236	200/184	315/315	203/203	203/203	148/148	154/154	258/258	178/178	176/176
DIGGA	239/239	238/238	236/236	194/186	279/279	176/176	187/179	170/146	0/0	252/252	186/186	179/179

Appendix III. Sample with DNA concentration and quality

No	Accession number	DNA conc. in ng/µl	260/280	230/280	No	Accession number	DNA conc In ng/µl	260/280	230/280
1	**90802**	741.34	2.08	1.98	25	**229702**	1468	2.144	2.211
2	**223086**	3889.9	2.22	2.368	26	**DIGGA1**	1607.2	2.172	2.175
3	**223087**	1201.5	2.151	2.24	27	**DIGGA2**	724.45	2.04	1.96
4	**223088**	1344.5	2.188	2.241	28	**230566**	630.36	2.179	2.11
5	**223090**	1601.4	2.042	1.819	29	**240407**	2516.5	2.125	2.079
6	**GM**	5647.8	2.214	2.274	30	**KICHI**	2231.5	2.09	1.876
7	**223092**	1378.1	2.19	2.325	31	**HU-1**	2061.29	2.241	1.42
8	**223093**	5687.1	2.167	2.28	32	**HU-2**	1029.5	2.081	1.962
9	**223096**	3953	2.187	2.288	33	**HU-3**	585.25	2.106	2.09
10	**223096**	105.24	3.344	0.319	34	**HU-4**	447.85	2.133	1.997
11	**223098**	2165.2	2.08	2.093	35	**HU-5**	2100.3	2.176	2.294
12	**223099**	575.71	2.096	2.053	36	**HU-6**	2028.6	2.162	2.268
13	**223100**	539.92	2.169	2.063	37	**HU-7**	1057.6	2.157	2.227
14	**223101**	5012	2.215	2.369	38	**HU-8**	1236.5	2.174	2.251
15	**223104**	3543.6	2.202	2.367	39	**HU-9**	3191.8	2.23	2.342
16	**223105**	2273.4	2.181	2.307	40	**HU-10**	1666.5	2.084	1.947
17	**DD1**	829.49	2.095	1.984	41	**HU-11**	3176	2.17	2.212
18	**DD2**	4342.1	1.292	1.347	42	**HU-12**	1243.9	2.194	2.328
19	**223108**	1868.8	2.184	2.128	43	**HU-13**	1975.4	2.115	1.985
20	**223108**	4253.8	2.195	2.34	44	**HU-14**	1658.6	2.145	2.239
21	**223109**	303.99	2.152	1.858	45	**HU-15**	1518.3	2.188	2.304
22	**223110**	2198.3	2.157	2.228	46	**Cuc1**	2124.1	2.184	2.269
23	**223112**	1175.7	2.082	2.007	47	**WM2**	3050.4	2.222	2.138
24	**223113**	1023.9	2.165	2.038	48	**WM3**	1121.2	2.218	2.14

SKETCH OF BIOGRAPHY

The author was born in 1985 GC in East Wellega, Jimma Arjo woreda, Oromia region state of Ethiopia. He attended the primary education (grade 1-6) at Hara Gabato Elementary School. He then, joined Mekonnin Demisso Primary and Secondary School (grade 7 and 8) and completed his secondary and high school. After passing the Ethiopian university entrance examination, he joined Jimma University, College of Agriculture and Veterinary Medicine in 2002GC and graduated with the Diploma in General Agriculture in June 2004GC. After serving in governmental office for four years he re-joined Jimma University to attend Bachelor Degree in Horticulture, and graduated with great destination in June, 2011GC. Immediately after his graduation, he was employed as instructor of Horticulture by Alage Agriculture TVET College where he served for two years. In 2014 GC, he was employed by Hawassa University, College of Agriculture as a graduate assistance. After severing for two consecutive years in the College, he joined Master's Program in Plant Sciences (Specialization: Plant Biotechnology) in Hawassa University in the year 2016.