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*Full Length Research Paper*

# Genetic Diversity of Coffee (*Coffea arabica* L.) Landraces from Southern Ethiopia as Revealed by Inter Simple Sequence Repeat Marker

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Despite its importance, the genetic variability of landraces *Coffea arabica* L. from Southern Ethiopia has not yet been examined well with molecular markers. Hence, in this study the genetic diversity of 87 coffee germplasm from Southern Ethiopia was studied using five Inter Simple Sequence Repeats (ISSRs) primers. The proportion of polymorphic bands within populations varied from 20 % - 100 %, with a mean of 62.8 %. The Shannon's diversity index varied from 0.11 to 0.55 with an overall mean of 0.33 and 0.47 for the entire data. The Nei's gene diversity ranges from 0.07 to 0.38 with the overall mean values across population and the mean for the entire data of 0.22 and 0.3, respectively. Analysis of Molecular Variance revealed 78.05% within while 21.95% variation is among population variation at ( $P=0.00$ ). Neighbor Joining and Unweighted Pair Group Method using Arithmetic Averages (UPGMA) analysis showed most of the individuals fails to cluster on the basis of their respective populations while few individual grouped with others. UPGMA analysis of the populations revealed two major groups (Debub Omo and Gamo Gofa). Principal coordinate analysis showed most of the individual accessions that represent different populations spread all over the plot except few from Dawuro and Debub Omo that showed some tendency of grouping based on geographic origins. The data clearly indicated the existence of higher diversity that deserves conservation attention. Some populations are also observed to be more uniform which could be caused by specialty coffee extension program.

**Keywords:** *Coffea arabica*; germplasm; Jaccard coefficient; polymorphism; repeat motifs; ISSR; Shannon's index.

## ABBREVIATIONS

IBC Institute of Biodiversity Conservation; ICGN International Coffee Genome Network; ICO International Coffee Organization; PCO Principal Coordinate Analysis

## INTRODUCTION

Coffee is the world's most widely traded tropical agricultural commodity ICO (2011), surpassed only by oil (Prakash et al., 2002; Vega, 2008). It account for nearly

half of total exports of tropical products FAO (2009). In many producing countries, besides contributing a tremendous amount to the foreign exchange currency as a main cash crop, it serves as a means of livelihood for millions of people (Steiger et al., 2002).

Presently coffee genetic resource is under greatest threat mostly due to deforestation of its natural habitat for timber and crop production, replacement of farmers' variety by a few high yielding and disease resistant varieties, establishment and expansion of modern plantation and illegal and legal settlements (Gole, 2003; Tesfaye, 2006). To minimize the loss of coffee genetic resources, collecting germplasms from different geographic locations and conserving at *in situ* and *ex situ* conservation sites is the best strategy for conservation and sustainable utilization. To design any conservation strategy, analyzing the genetic diversity using different marker systems are vital for sustainable use and conservation strategy.

Different molecular markers have been in use since 1980s, which helps to overcome the drawbacks associated with morphological and isozyme markers (Li et al., 2008). Evaluation and characterization of coffee germplasm using molecular markers such as RAPD, ISSR, AFLP, and SSR has been the subject of several studies (Aga et al., 2005; Masumbuko and Bryngelsson, 2006; Tesfaye, 2006; Yigzaw et al., 2008; Mishra et al., 2011). However, because of availability and its cost effectiveness ISSR marker were used in this study.

Despite its economic and social importance for numerous countries around the world, coffee has received very little attention with respect to molecular genetics and genomics research. The ability to capture and conserve genetic diversity and effectively utilize germplasm in traditional coffee breeding programs will be vital for future sustainable coffee production (ICGN, 2009). The genetic diversity analysis of Ethiopia's coffee landrace with better representative sampling using molecular marker has not received much attention till recent years. Hence, the present study is initiated with the objectives to assess the genetic diversity of coffee (*C. arabica*) landraces of southern Ethiopia using ISSR markers. The study further

aimed at determining diverse populations which deserve conservation attention and identify genetically distant populations for future utilization in coffee breeding programme.

## MATERIALS AND METHODS

### Sources of plant materials

The research was conducted in the Genetic Laboratory of the Faculty of Life Sciences, Addis Ababa University, Addis Ababa. The samples were collected from Choche field gene bank of the IBC (Ethiopian Institute of Biodiversity Conservation). The samples were originally assembled from different geographic locations of southern Ethiopia and maintained for several years at the *ex situ* gene bank of IBC for conservation.

Young leaf samples from 87 coffee (*C. arabica*) individuals, each individual represents an accession, were collected from Choche field gene bank and dried using silica-gel. The leaves were collected from a plant which is randomly selected from five individuals of an accession. All collections in the IBC gene bank from southern Ethiopia were included and samples from Debub Omo, Gamo Gofa and Dawuro were taken using proportional sampling technique among zones and woredas (district). However, all available samples from some areas such as Basketo, Sidama and Gedeo of southern Ethiopia, were taken as whole.

### DNA Extraction

Genomic DNA was extracted following the modified CTAB method following methods described in Borsch et al. (2003). For optimum amount of DNA for ISSR-PCR reaction the second extraction was used, taken for polymerase chain reaction (PCR). About 50mg Silica-gel-dried leaves for each accession were ground with sterile mortar and pestle using clean sand (SiO<sub>2</sub>). The samples were stored at 4°C until electrophoresis and subsequent dilution and PCR reaction.

### Quantification of Extracted DNA and Purity Checking

The yield of DNA isolated was measured using a NanoDrop ND-8000 UV spectrophotometer at Holeta Biotechnology Research Institute, Molecular Biology Laboratory, Holeta. The concentration of total genomic DNA ranges from 30 ng/μl to 300ng/μl. The concentration was adjusted to a concentration of ca. 20 ng/μl. Moreover,

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the purity and concentration of DNA was also determined by agarose gel electrophoresis by running the samples on 1 % TAE agarose gel, depending on the intensities of band when compared with lambda DNA marker (1kb).

### Primer Selection and Optimization

Ten representative samples from diverse geographic locality, two from each geographic location, were selected to screen the primers. A total of 10 primers obtained from the University of British Columbia (primer kit UBC 900) were initially tested and screened for their polymorphism and reproducibility. Five polymorphic and reproducible primers (810, 812, 818, 824 and 873) were finally selected for the analysis. These primers were also used by Tesfaye (2006) to study the genetic diversity of wild arabica coffee in Ethiopia.

### PCR Conditions, gel electrophoresis and ISSR fragment visualization

A primer concentration of 0.6  $\mu$ l (20 pmol/ $\mu$ l) was used in each PCR reaction, which was carried out in a total volume of 25 $\mu$ l containing 11.05 $\mu$ l H<sub>2</sub>O, 5.6  $\mu$ l (5mM) dNTPs, 2.6 $\mu$ l Taqbuffer (10 times reaction buffer S), 1.25 $\mu$ l (50mM) of MgCl<sub>2</sub>, 2.4 $\mu$ l betien, 0.5 $\mu$ l (0.3 u/ $\mu$ l) *Taq* DNA polymerase and 1 $\mu$ l of genomic DNA.

Amplification was performed using biometra 2008 version 3.10 TPersonal using 48 well plates under the following conditions: preheating and initiation of PCR at 95°C for 2 min; followed by a regular cycling event of 40 cycles of 20 seconds denaturation at 94°C, with 1 min annealing at 45°C/48°C (based on primers used), 1 min and 30 sec elongation at 72°C. The last cycle was followed by an additional 7 min of product extension at 72°C. The PCR products were stored at 4°C until gel electrophoresis.

Compact Line Horizontal Electrophoresis system (model S and L) was used for electrophoresis of PCR products. The amplification products were checked first on test gel using 1% agarose gel using 1xTBE for the presence of ISSR-PCR products with model S. The amplified products were run on to ISSR gel using 1.67% agarose, with 1xTBE using model L gel electrophoresis chamber. The ISSR gel (1.67 % TBE) was prepared using 300ml TBE mixed with 5.01 gram agarose using 500 ml Erlenmeyer flask and then boiled in micro oven for 3 minutes. After it was cooled for about 20 minutes at room temperature, 12 $\mu$ l Ethidium Bromide (10mg/ml) was added and the gel was poured on casting system and allowed to solidify for more than 2 hrs. Eight micro litter ISSR amplification products and 2 $\mu$ l (6x) loading dye was mixed thoroughly and loaded on the gel. A 100 base-pair ladder was used to estimate the

molecular size of the DNA fragments. The electrophoresis was run for 3 hours with at constant voltage of 100V. The ISSR profiles was then visualized and photographed under UV light using Zenith Geldoc system.

### Data Scoring and Statistical Analysis of Diversity

ISSR bands were scored as present (1), absent (0) and missing data (?) and entered into a binary matrix representing the ISSR profile of each sample. The 0/1 matrix was used to calculate the Jaccard (1908) coefficient for all possible pairs of samples using Free Tree 0.9.1.50 Pavlicek et al. (1999) and NTSYS-pc version 2.02 Rohlf (2000) software. The resulting similarity matrices were employed to construct Neighbor Joining (NJ) and UPGMA-based phenogram (Sneath and Sokal, 1973; Saitou and Nei, 1987; Studier and Keppler, 1988). Analysis of molecular variance (AMOVA) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier, 2006). Gene diversity and measures of population differentiation, and Shannon's information index (King and Schaal, 1989) were computed with POPGENE software 1.32 (Yeh et al., 1997). The matrix of genetic similarity was also used in a principal coordinate analysis (PCO) to resolve the patterns of clustering among the genotypes based on Jaccard's coefficient Jaccard (1908). The calculation of Jaccard's coefficient was made with PAST software version 1.18 (Hammer et al., 2001). The first three axes were used to plot the three dimensional PCO with STATISTICA version 6.0 software (Hammer et al., 2001; statsoft Inc., 2001).

## RESULTS AND DISCUSSION

### Banding patterns of the ISSR primers

Five primers, four dinucleotides (810, 812, 818 and 824) and a tetranucleotide (873), which gave clear banding pattern were selected and used in this study (Table 1). The size of the fragments amplified using the primers were in the range of 100 bp to 900bp. A total of 50 clear and scorable bands were recorded from 87 coffee accessions representing six populations. The highest scorable fragments were recorded for primer 810 while the least was recorded for 818 with 13 and 8 fragments, respectively. However, Tesfaye (2006) has got the highest reproducible and scorable band for CoIS001, 844 and 812 primers with 31, 22, 22 fragments in ISSR fingerprint done on both wild and cultivated coffee from Ethiopia. The higher number of fragments recorded from this author might be due to the use of higher samples number from wild and cultivated coffee from all over coffee producing regions of Ethiopia.

**Table 1.** Banding patterns generated using the five primers, their repeat motifs, amplification patterns and number of scored bands.

Primers	Repeat Motif	Amplification Pattern	Number of scored bands
810_H	(GA) <sub>8</sub> T	Good	13
812_H	(GA)8A	Good	9
818_H	(CA)8G	Good	8
824_H	(TC)8G	Good	10
873_H	(GACA)4	Good	10
Total			50

**Table 2.** No. of polymorphic loci and percent polymorphism using all five primers.

Population	No. of polymorphic Loci	Percentage of polymorphic Loci	Mean	
			*h	*I
Debub Omo	47	94	0.38	0.55
Gamo Gofa	50	100	0.36	0.54
Dawuro	32	64	0.20	0.31
Basketo	18	36	0.09	0.15
Sidama-*Gedeo	10	20	0.07	0.11
Mean	31.4	62.8	0.22	0.33
Entire data	50	100	0.30	0.47

\*Gedeo- as No. of samples from there is low (two), POPGENE analysis was obtained with its adjacent location, Sidama.

\*h = Nei's (1973) gene diversity.

\*I = Shannon's Information index.

All the scored fragments per primers were found to be 100% polymorphic for the five populations evaluated (Table 2). However, Tesfaye (2006) obtained 11.1%, 25%, 6.3% polymorphism with 810, 812, 818, respectively, for coffee landraces collected from limited localities in south, southwestern and eastern Ethiopia. The higher percent of polymorphism detected in this study could be because of large number of samples from several zones in the southern Ethiopia, whereby known landraces and cultivars are being introduced for several years.

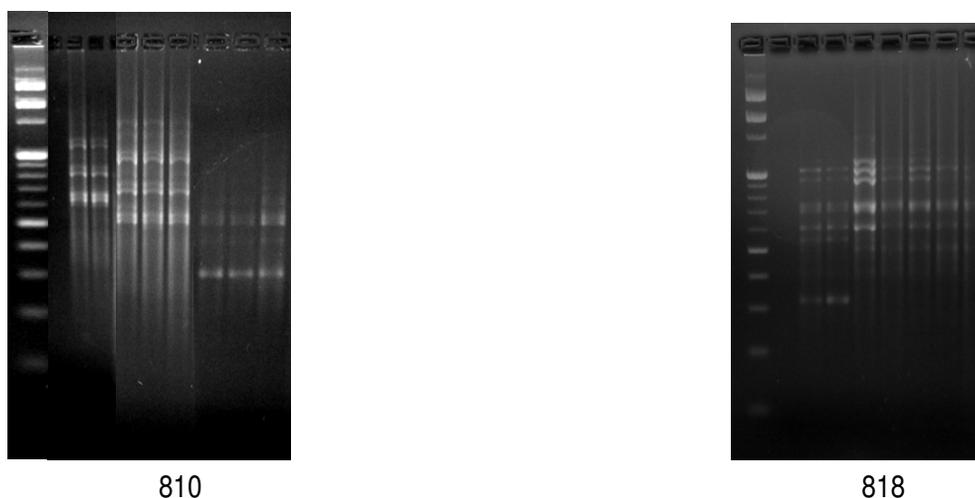
The gene diversity and Shannon's index value ranges from 0.45-0.22 and 0.64-0.36 for primers 812 and 824, respectively (Table 3). However, Tesfaye (2006) has got the second highest number of polymorphic fragments for primer 812 for coffee landraces; while Balemi (2007) finds the third least percent polymorphism (22.22%) using primer 812 for wild coffee from Hareenna forest. The variation on sample size and difference on the status of domestication (wild vs. landrace) could be the main

reason for different levels of polymorphism revealed by the same primers. Figure 1 shows the amplification pattern of the primers used in the study. The first and the second lanes represent molecular size (100 bp DNA ladder) marker and negative control respectively while the rest lanes represent randomly selected coffee accessions in the present study.

In terms of number of percent polymorphism per class of primer, both tetra nucleotide and dinucleotides were found to be equal. This is in contrast to the patterns reported by Tesfaye (2006), Oljira (2006) and Balemi (2007) that detect high levels of polymorphisms (100%) among wild and cultivated coffee collected from Ethiopia using tetra nucleotides primers such as ColS001. Although it is likely that the major factor in this difference is the length of the repeat unit and type, the possibility that the sequence composition plays a role cannot be ruled out from present study because the number of dinucleotides and tetranucleotide is not equal (four vs one). All primers

**Table 3.** Number of scored band, number of polymorphic band, percent polymorphism, gene diversity Shannon index for each primers.

Primer	No. of scored bands	No. of polymorphic loci	Percentage of polymorphic loci	Mean	
				*h	*I
810	13	13	100	0.30	0.46
812	9	9	100	0.45	0.64
818	8	8	100	0.32	0.49
824	10	10	100	0.22	0.36
873	10	10	100	0.25	0.41

**Figure 1.** ISSR fingerprint generated from accessions of *C. arabica* from primers: 810 and 818.

employed in this analysis of genetic diversity showed unique patterns where almost all individuals were observed to have different banding patterns and a specific band, a band which only present between the 100bp and 200bp, was observed for the accession 824 from primer 824. The absence of this fragment in other accessions indicates the possibility of this fragment to be linked to trait that could have adaptive role. However, further studies, that are amplification of more samples with these and other primers along with confirmed accessions with such trait and sequencing of the fragments followed by database search using bioinformatics tools, are to be performed.

ISSR markers are observed to be highly variable within the species and reveal clear polymorphisms among individuals in a population since they use longer primers that allow more stringent annealing temperatures (Fang et al., 1997; Wolfe et al., 1998; Claudia and Lucia, 2008). The current fingerprint analysis and others (Tesfaye,

2006; Oljira, 2006; Balemi, 2007; Mishra et al., 2011) showed the importance of ISSR markers to reveal diversity within and among cultivated and wild populations of coffee.

#### **Polymorphic loci and percent polymorphism within population**

All scored fragments were observed to be polymorphic (100%) for all populations evaluated in this study. The number of polymorphic loci per population ranges from 10 for Sidama-Gedeo and 50 for Gamo Gofa. Accordingly, Gamo Gofa showed 100% polymorphism and followed by Debub Omo with 94%. The least percent polymorphism was observed in Sidama-Gedeo and Basketo with 20% and 36% respectively. Moderate polymorphism was detected for Dawuro population with polymorphic level 64%. The least percent polymorphism for Sidama-Gedeo populations (20%) could be due to promotion of speciality

**Table 4.** Analysis of Molecular Variance (AMOVA) of coffee accessions from Southern Ethiopia without grouping.

Source of Variations	Sum of squares	Variance components	Percentage variation	P	Fixation indices
Among populations	155.053	2.19593	21.95	0.00	0.22
Within populations	567.650	7.80666	78.05	0.00	
Total	722.703	10.00258			

coffee with limited seedling exchange among neighboring farmers. Both district agricultural bureau and coffee improvement program in Sidama-Gedeo area focused on germplasms and improved cultivars assembled from the same area, this enhanced the narrowing down of genetic base of coffee genetic cultivation in the area.

#### Genetic diversity within populations of *Coffea arabica*

Gene diversity and Shannon's index were calculated for all the five populations. Samples collected from Debub Omo were the most diverse ( $h = 0.38$ ), and followed by samples from Gamo Gofa ( $h = 0.36$ ) and Dawuro ( $h = 0.20$ ). The samples from Basketo ( $h = 0.09$ ) and Sidama-Gedeo ( $h = 0.07$ ) were the least diverse. The calculated Shannon's diversity index also followed the same patterns like that of the gene diversity. The overall gene diversity and Shannon's index values for the total population were found to be 0.3 and 0.47, respectively.

Generally, the results indicated the high levels of genetic diversity in coffee landraces in southern part of Ethiopia. However, Tesfaye (2006) observes lower Shannon's diversity index (0.17-0.22) for the landraces collected from different parts of Ethiopia this could be associated with usage of small sample size-representing different parts of coffee growing regions. The higher genetic diversity was observed for Gamo Gofa and Debub Omo populations followed by Dawuro, and the lower diversity was observed for Basketo and Sidama-Gedeo respectively, which could be attributed to the levels of management implemented and focus on limited germplasm disseminated by different stakeholders.

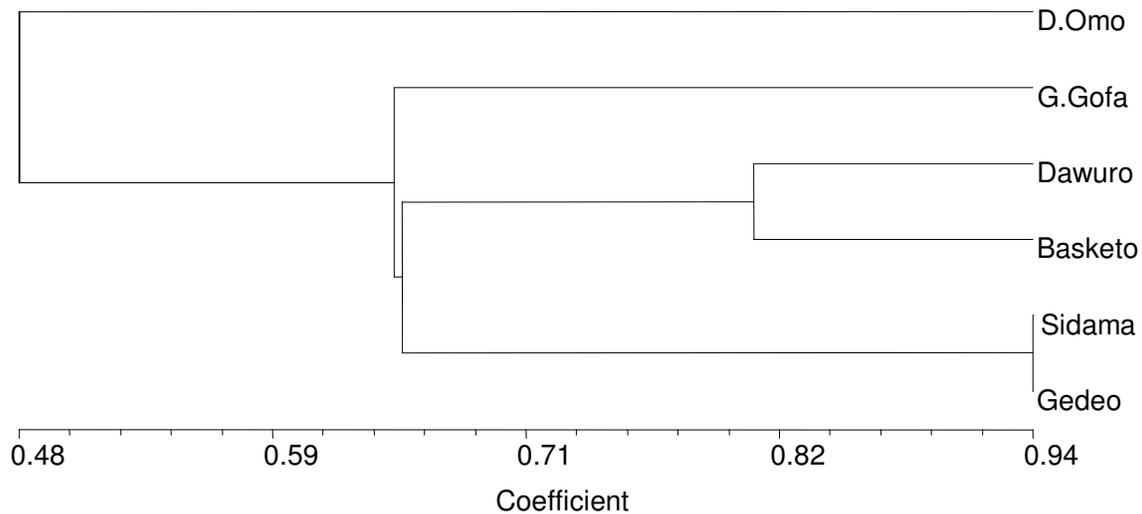
#### Analysis of molecular variance and partitioning of genetic diversity

Analysis of molecular variance was carried out on the entire ISSR data for coffee accessions included in the analysis. The analysis was carried out without further grouping the population based on its geographical proximity; this is mainly because of unequal sample size among populations.

Analysis of molecular variance revealed that higher percentage of variation (78.05%) is attributed to the within population variation while the remaining variation (21.95%) is due to the among population variation. The variation was also found to be highly significant at ( $P=0.00$ ). According to Wright (1978) the fixation indices value of 0.22 of this analysis indicate that the process of fixation of own alleles of each population is great which means genetic divergence increased gradually as time goes on. In *Coffea arabica* population, higher among population diversity was expected than within population genetic diversity as the plant is predominantly self-pollinated species (Raus et al., 2003). However, the pollination biology study was carried out in non-native habitats of arabica coffee and hence might not be representative for arabica coffee native habitat where pollinators are actively engaged in facilitating active pollen exchange among individual coffee plant in the high lands of Ethiopia (Fichtl and Adi, 1994).

Meyer (1965) observed higher variation among wild and semi-wild *Coffea arabica* populations' from montane rain forests of Ethiopia as compared to the cultivated materials and also reported 40% to 60% cross pollination in wild population of *C. arabica* around Jimma in Ethiopia. Moreover, Aga et al. (2003), Tesfaye (2006), Oljira (2006), Balemi (2007) observe high within population variation as compared to among population variation, which is in agreement with the results of this study. The high within population variation observed in this and other study on Ethiopian arabica coffee indicate that there is high gene flow through either seeds or seedlings exchange. The gene flow could be enhanced via birds, insects, wild and domestic animals by facilitating exchange of pollens and seeds. Moreover, coffee farmers could also contribute to gene flow by exchanging seeds and seedlings of enhanced landraces among nearby districts with the objective of improving productivity of coffee.

The higher within populations' genetic diversity might be accounted to two contrary reasons. *Coffea arabica* is affected by multiple evolutionary forces which operate within historical and biological context of the plant species. This includes the mating types, gene flow, mode of



**Figure 2.** UPGMA based dendrogram obtained for 6 populations using 5 ISSR primers.

Key: D.Omo-Debub Omo, G.Gofa-Gamo Gofa.

reproduction and natural selection etc. Hamrik and Godt (1989). For this reason, it could be speculated from this result that *Coffea arabica* might have mixed mating system (partial out-crossing by pollen and seed and partial selfing) for which some extent of gene flow is expected as reported by Meyer (1965) which could result in high within population genetic diversity (Loveless and Hamrik 1984; Aga et al., 2003; Tesfaye, 2006; Oljira, 2006; Balemi, 2007).

In contrary to the above, as Balemi (2007) reported it could also be speculated that the high genetic diversity observed within populations of the *Coffea arabica* might be due to preferential adaptive gene complexes adapted to environmental changes being evolved during long evolutionary period in a given region. In this case, *Coffea arabica* population uses selfing as mechanisms to prevent influx of the gene from another portion of the populations that might reduce diversity through disrupting adaptive genes Lowe et al. (2004).

#### **Clustering analysis and relationships among populations and individuals**

Based on Jaccard's coefficients UPGMA and neighbor joining (NJ) analysis were used to construct dendrogram for 50 ISSR-PCR fragments scored from 6 populations and 87 individuals. UPGMA analysis of the populations revealed two major groups (Debub Omo and Gamo Gofa). The second major cluster again forked into two sub groups; the first containing Dawuro and Basketo populations, while the second contained Sidama and

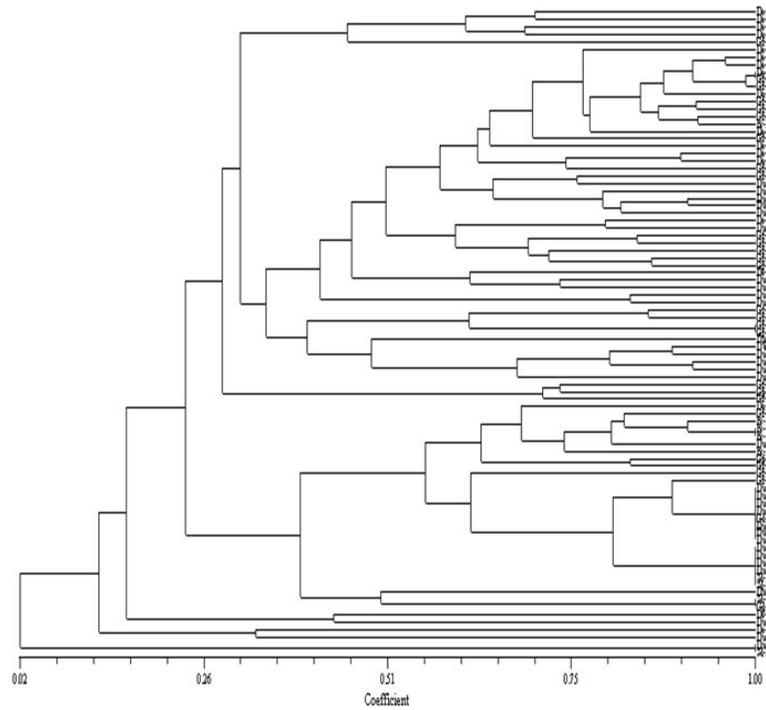
Gedeo populations (Figure 2). Sidama and Gedeo populations were clustered at the same coefficient level. The population grouping follows geographical proximity which shows the presence of extensive gene flow among neighboring regions or zones.

The UPGMA analysis of the individuals in each accession revealed that the individuals were distributed and inter-mixed with individuals of another population except that some accessions were tending to form cluster in their own population (Figure 3).

Like that of the UPGMA analysis of the individual accession the dendrogram derived from neighbor-joining analysis of the whole ISSR data were not showing a clear grouping (Figure 4). Few accessions from Dawuro, Sidama, Basketo and Gamo Gofa were tended to form their own cluster while some of the accessions distributed all over the tree i.e. individuals of all populations were inter-mixed. Generally, the dendrogram analysis using *Coffea arabica* individual plant form inter-mixed cluster between populations since high levels of genetic variation is detected in almost all populations investigated.

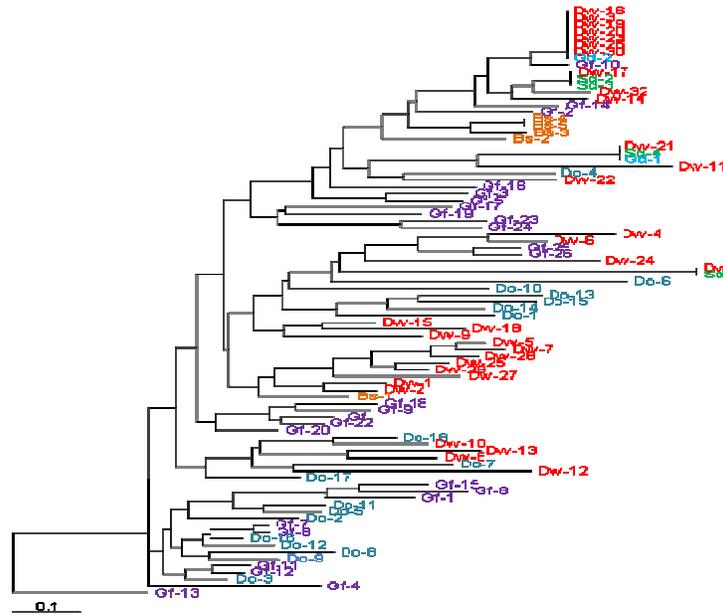
#### **PCO analysis and individual coffee tree variability on three dimension plot**

All the data obtained using 5 ISSR primers were used for PCO analysis using Jaccard's coefficients of similarity. The first three coordinates of the PCO having eigen values of 10.95, 5.10 and 4.34 with variance of 14.70%, 6.84% and 3.82%, respectively, used to show the grouping of individuals using two and three co ordinates



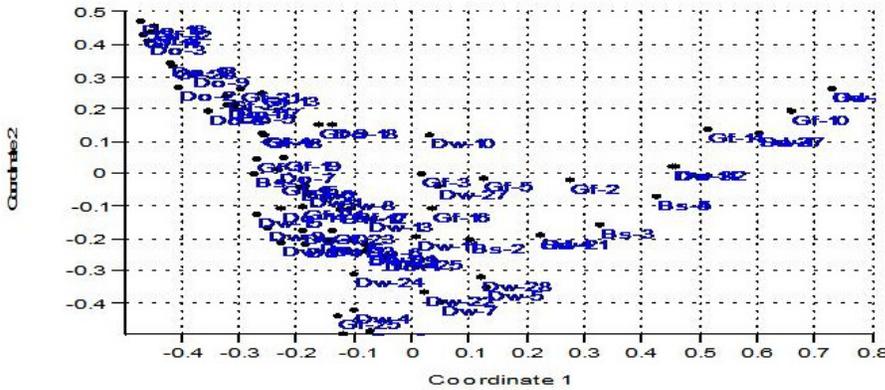
**Figure 3.** Dendrogram showing genetic relationships among 87 *Coffea* plants constructed by UPGMA clustering analysis using 5 ISSR markers.

Key: Dw-Dawuro, Gd-Gedeo, Gf-Gamo Gofa, Sd-Sidama, Bs-Basketo, and Do-Debub Omo.

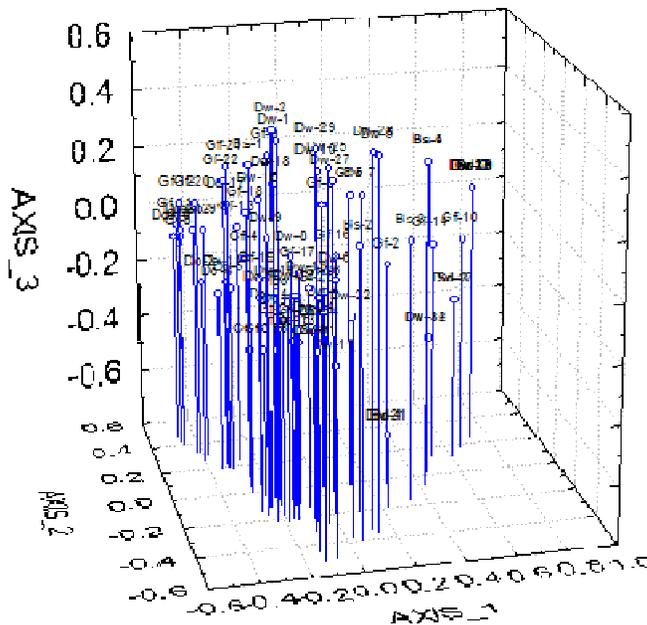


**Figure 4.** Neighbor-joining analysis of 87 individuals based on 50 PCR bands amplified by five ISSR primers.

Key: Dw-Dawuro, Gd-Gedeo, Gf-Gamo Gofa, Sd-Sidama, Bs-Basketo, and Do-Debub Omo



**Figure 5.** Two dimensional representation of principal coordinate analysis of genetic relationships among 87 individuals.  
Key: Dw-Dawuro, Gd-Gedeo, Gf-Gamo Gofa, Sd-Sidama, Bs-Basketo and Do-Debub Omo



**Figure 6.** Three dimensional representation of principal coordinate analysis of genetic relationships among 87 individuals.  
Key: Dw-Dawuro, Gd-Gedeo, Gf-Gamo Gofa, Sd-Sidama, Bs-Basketo and Do-Debub Omo.

(Figure 5 and 6) . With the exception of few accessions that come from Debub Omo, Dawuro and Gamo Gofa in 3D most of the individual accessions that represent different populations spread all over the plot. Using two coordinates except Debub Omo and Dawuro, which tends to form their own cluster, almost similar result was observed like that of three coordinates. This also clearly indicates the high diversity that exists among cultivated

coffee in Southern Ethiopia. This is in contrast with the results Tesfaye (2006), whereby individuals collected from the same locality tending to occupy the same 3D (three dimension) space on the PCO. However, the analysis of this author was carried out predominantly on wild coffee which could be the reason for deviation of the PCO result of this study.

## CONCLUSIONS

The information of the pattern and extent of genetic variation can be used to design effective germplasm conservation, for setting germplasm collection mission, to predict the risk of genetic erosion in certain region as well as for defining hetrotic patterns in hybrid breeding by relating the observed patterns with presence of certain economically important traits.

Genetic diversity can be studied using morphological, biochemical or molecular markers. Among the molecular markers, ISSR markers are important to study genetic variations in plant species, as they are effective in detecting very low levels of genetic variation (Zietkiewicz et al., 1994). This marker technique observed to generate information on genetic diversity of landraces of coffee from southern Ethiopia. Four di-nucleotide and a tetranucleotide ISSR primers were used in the present study and revealed high genetic variability within 87 *Coffea arabica* landraces collected from Southern Ethiopia. Moreover, the marker was able to show relationship among populations of arabica coffee in southern Ethiopia.

Ethiopia is endowed with immense potential of diverse coffee genetic resouces (Olika et al., 2011). Many previous morphological research results showed there is a narrow gene pool in *Coffea arabica* but recent researches based on molecular markers on wild coffee assured there is higher genetic diversity in Ethiopian coffee germplasm. This study on landraces of coffee from Southern Ethiopia showed that there is high polymorphism and genetic diversity in *Coffea arabica*. However, this diversity analysis should be repeated with co-dominant markers such as microsatellites to determine the levels of inbreeding and also calculate gene flow among coffee producing zones in the south.

The result of the present study has demonstrated that the ISSR technique could be applied for measuring the degree of variability within, and between cultivated coffee populations and cultivars. The marker used in this study also showed the presence of higher diversity within and among populations of cultivated coffee in south, which deserves conservation attention. The present promotion of specialty coffee which is focused on few enhanced landrace materials in south could result in extinction of other landraces with their economically important traits. Hence, the IBC and other stakeholders in federal and regional government should work jointly to conserve the landrace population in southern Ethiopia.

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