Genetic Diversity of Ethiopian Groundnut (*Arachis hypogaea* L.) Accessions

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Abstract

Background: Groundnut (*Arachis hypogaea* L.) is the world's most important source of edible oil and vegetable protein. Understanding its genetic diversity is important for the sustainable use and conservation of the crop. To our knowledge, little is known about the genetic variability of cultivated Ethiopian *A. hypogaea*.

Objective: To determine the genetic variability and diversity of 43 accessions of *A. hypogaea* collected from different regions of Ethiopia using ISSR markers.

Materials and Methods: Seeds of 43 *A. hypogea* accessions collected from different regions of Ethiopia by Ethiopian Biodiversity Institute were planted in plastic pots in a greenhouse of Melkasa Agricultural Research Center. Young fresh leaves from four weeks old plants were used to extract genomic DNA using CTAB (Cetyl trimethyl ammonium Bromide) method. Four reproducible ISSR primers were used for amplification and the amplified products were separated on 1.67% agarose gel. Percentage of polymorphic bands, polymorphic information content (PIC), the mean Nei's gene diversity and Shannon's information index were determined. NTSYS-pc version 2.02 software was used to calculate the Jaccard's similarity coefficient for all possible pairs of samples.

Results: Out of 56 reproducible bands generated, 29 (51.8%) were polymorphic. The band size ranged from 120 bp to 1100 bp. The polymorphic information content (PIC) value ranged from 0.29 to 0.76 with the average value 0.49. The mean Nei's gene diversity and Shannon's information index were 0.25 and 0.33, respectively. Genetic relationship between *A. hypogaea* accessions based on Jaccard's pair wise similarity coefficients varied from 44% to 83% with an average value of 63.5%. The UPGMA analysis grouped *A. hypogaea* accessions into five distinct clusters at 63.5% similarity coefficient, and the principal coordinate analysis revealed similar grouping.

Conclusions: ISSR marker-based analysis showed the presence of genetic variability among the accessions. UPGMA and PCoA clustered most of the accessions irrespective of their geographic origins. In addition, the current study demonstrated the informativeness of ISSR markers in estimating the extent of genetic variation among *A. hypogaea* accessions. This study is a milestone for future conservation and breeding program of the crop. We recommend further investigation with more geographic range and additional molecular markers to elucidate a clear genetic diversity of groundnut in Ethiopia.

Keywords: Jaccard's similarity coefficient; Nei's gene diversity; Polymorphism; Polymorphism information content; Shannon's Weaver index

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1. Introduction

Groundnut or peanut (*Arachis hypogaea* L.) belongs to the family Leguminoseae and genus *Arachis*. Cultivated groundnut is a highly self-pollinated, allotetraploid annual legume with 2n = 4x = 40 with a basic chromosome number of x =10 (Stalker, 1997). Groundnut is cultivated and grown throughout the tropics and sub-tropics between 40° South and 40° North of the equator where the annual rainfall ranges between 500 to 1200 mm and the average daily temperature is higher than 20 °C (Mastewal Alehegn *et al.*, 2017). Major groundnut growing countries include China, India, the United States and Nigeria (Taru *et al.*, 2010). Globally, groundnut is grown in 26.4 million ha worldwide with a total production of 37.1 million metric tons and an average productivity of 1.4 metric tons ha^{-1} (Hamakareem *et al.*, 2016).

Groundnut is the world's 4th most important source of edible oil and 3rd most important source of vegetable protein (Hamakareem *et al.*, 2016). Groundnut seeds contain 42–50% oil, 26% protein, 18% carbohydrates, and are rich source of riboflavin, thiamine, nicotinic acid and vitamin E (Kathirvelan and Kalaiselvan, 2007). *Arachis hypogaea* is one of the four economically important oilseed crops along with noug, flax and sesame in Ethiopia (Mastewal Alehegn *et al.*, 2017). Besides, this crop helps small-scale producers in getting significant revenue and helps Ethiopia in getting foreign currency earnings through export. Being a legume, this plant improves soil by fixing nitrogen biologically without consuming non-renewable energies and without disturbing agro-ecological balance (Jiaramraja and Fantahun Woldesenbet, 2014).

In Ethiopia, Groundnut is grown and covers nearly 87,925.23 hectares (CSA, 2020) of arable land per annum and the major producing zones are eastern Hararghe Zone in Oromia as well as Metekel in Benishangul-Gumuz regional state (Fredu Nega *et al.*, 2015; Addisu Getahun and Ermias Tefera, 2017). Despite its importance, the national average yield produced by the farmers in Ethiopia is considerably low, 1.3 tons ha^{-1}, indicating the need of maximum effort to improve productivity (Gebreselassie Redae *et al.*, 2014). The gap observed between actual and potential yields is due to several factors, including non-availability of seeds of improved varieties, poor soil fertility, inappropriate crop management practices, insect pests and fungal diseases (Geleta Tarekegn *et al.*, 2007; Geremew Terefe and Asfaw Tulu, 1992).

Genomic research can provide new tools and resources to revolutionize crop genetic improvement and production. It also provides accurate knowledge at molecular level, which is not possible with phenotypic markers (Johan *et al.*, 2011). Assessment of genetic diversity is an important step in any crop improvement program (Bhandari *et al.*, 2017). Understanding the molecular basis of the essential biological phenomena in plants is crucial for the effective conservation, management, and efficient utilization of plant genetic resources (PGR) (Linda *et al.*, 2009). Collecting DNA marker data to determine whether phenotypically similar cultivars are genetically similar would therefore be of great interest in crop breeding programs (Duzyaman, 2005). Evaluation of genetic diversity could be based on morphological or molecular markers. Morphological features may not be efficient as they are highly influenced by environments. Molecular marker technique is an efficient tool for genetic variation evaluation in plants (Soares *et al.*, 2016). Consequently, the development of marker protocols such as RFLP, AFLP, ISSR, SSR and SNP has revolutionized the genetic analysis by detecting level of polymorphism (Raina *et al.*, 2001).

Inter Simple Sequence Repeats (ISSR) marker has been reported as a rapid, reproducible, and cheap fingerprinting technique based on the variation found in the regions between microsatellites (Zhang *et al.*, 2006; Golkar *et al.*, 2011). The ISSR method has several benefits over other techniques: first, it is known to be able to discriminate between closely related genotypes and second, it can detect polymorphisms without any previous knowledge of the crop's DNA sequence (Zeitkiewicz *et al.*, 1994; Mishra *et al.*, 2014). It is a fast, inexpensive genotyping technique based on variation in the regions between microsatellites (Zeitkiewicz *et al.*, 1994). Inter Simple Sequence Repeats analyses offer breeders and geneticists with competent means to link phenotypic and genotypic variations in various fields of plant improvement (Shimekt Tadele *et al.*, 2014).

Genetic diversity study in groundnut, based on morphological, biochemical and some molecular markers has been reported (Patel and Galakiya, 2014; Roomi *et al.*, 2014; Peng *et al.*, 2016; Dhwani *et al.*, 2017; Zekeria Yusuf *et al.*, 2017). However, more molecular marker-based genetic diversity study of *A. hypogaea* accessions in Ethiopia is demanding. Therefore, the objective of this study initiated to determine the level and pattern of genetic variability in 43 accessions of groundnut grown in different regions of Ethiopia using ISSR markers.

Moreover, we addressed the potential in formativeness of ISSR markers for identifying groundnut accessions.

2. Materials and Methods

2.1. Plant Materials

Seeds of 43 groundnut accessions collected from different regions of Ethiopia were obtained from Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia. The accessions were previously originated from different geographical locations of Ethiopia (Figure 1). The seeds of all the 43 accessions were planted in plastic pots containing sandy loamy (composted) soil and maintained in a greenhouse under controlled temperature (30 °C) for about four weeks at Awash Melkasa Agricultural Research Center. Watering was done once a day regularly. Fresh young leaves from four weeks old plants were collected from each accession in tubes containing silica gel for genomic DNA extraction.

Gene bank number, code, collection region, locality and geographical location (latitude and longitude) of accessions are listed in Table 1.

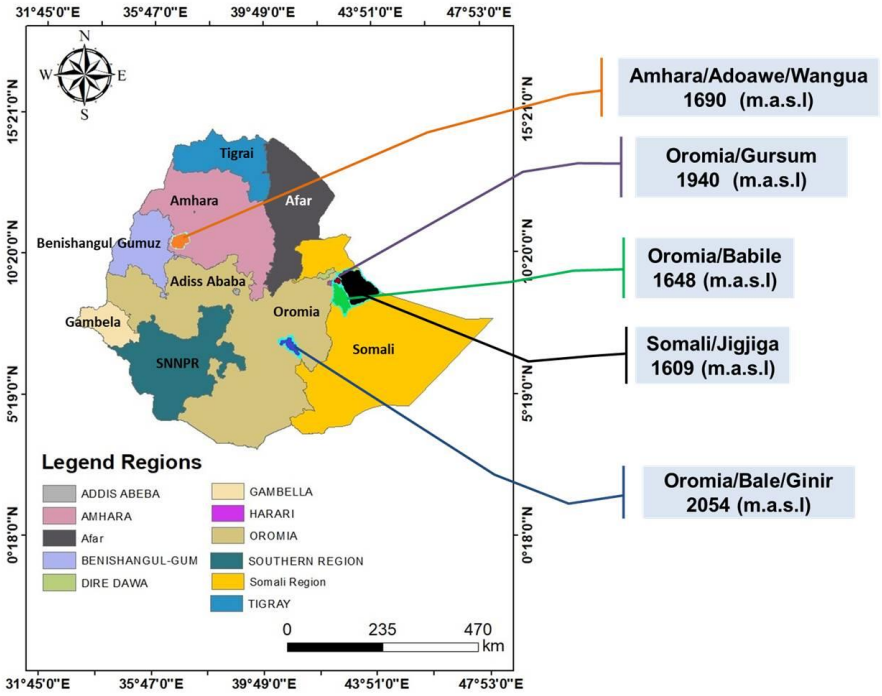

Figure 1. Map of Ethiopia showing collection sites of *A. hypogaea* accessions.

Table 1. Description of 43 *A. hypogaea* accessions used in the present study.

S/N	Gene bank accession no.	Code *	Collection region	Locality	Latitude (N)	Longitude (E)
1	19739	GOB 1	Oromia/Misrak/Babile	Berkele/s 1	9°12'25.33"	42°21'26.31"
2	19740	GOB 2	Oromia/Misrak/Babile	Berkele/s 2	9°12'25.35"	42°21'27.34"
3	19741	GOB 3	Oromia/Misrak/Babile	Babile/Shek A.	9°11'54.45"	42°21'41.22"
4	19742	GOB 4	Oromia/Misrak/Babile	Awsherit 1	9°09'21.23"	42°22'21.34"
5	19743	GOB 5	Oromia/Misrak/Babile	Awsherit 2	9°09'21.29"	42°22'21.28"
6	19744	GOB 6	Oromia/Misrak/Babile	Lecole	9°07'36.32"	42°21'02.33"
7	19745	GOB 7	Oromia/Misrak/Babile	Ifa Gende 1	9°13'42.11"	42°18'22.18"
8	19746	GOB 8	Oromia/Misrak/Babile	Ifa Gende 2	9°13'41.11"	42°18'22.28"
9	19747	GOB 9	Oromia/Misrak/Babile	Ifa Gende 3	9°15'30.21"	42°18'21.12"
10	19748	GOB 10	Oromia/Misrak/Babile	Medigana 1	9°16'06.13"	42°18'12.43"
11	19749	GOB 11	Oromia/Misrak/Babile	Medigana 2	9°16'06.21"	42°18'24.28"
12	19750	GOB 12	Oromia/Misrak/Babile	Dendaro	9°17'25.21"	42°17'25.14"
13	19751	GOB 13	Oromia/Misrak/Babile	Tofic 1	9°16'06.36"	42°17'36.25"
14	19752	GOB 14	Oromia/Misrak/Babile	Tofic 2	9°16'06.41"	42°17'36.32"
15	19753	GOB 15	Oromia/Misrak/Babile	Berkele	9°10'48.27"	42°18'23.12"
16	19754	GOB 16	Oromia/Misrak/Babile	Gende	9°09'37.42"	42°18'50.10"
17	19755	GOB 17	Oromia/Misrak/Babile	Gemechu	9°07'25.34"	42°18'54.03"
18	19756	GOB 18	Oromia/Misrak/Babile	Tula	9°13'05.11"	42°19'28.32"
19	19757	GOB 19	Oromia/Misrak/Babile	Tula About	9°12'17.23"	42°19'38.42"
20	19758	GOB 20	Oromia/Misrak/Babile	Abdul 1	9°11'49.22"	42°19'43.37"
21	19759	GOB 21	Oromia/Misrak/Babile	Abdul 2	9°11'49.28"	42°19'58.23"
22	19760	GOG 1	Oromia/Misrak/Gursum	Llalemi 1	9°19'33.14"	42°26'05.26"
23	19761	GOG 2	Oromia/Misrak/Gursum	Llalemi 2	9°19'33.38"	42°26'05.38"
24	19762	GOG 3	Oromia/Misrak/Gursum	Awdal	9°18'19.23"	42°26'11.36"
25	19763	GOG 4	Oromia/Misrak/Gursum	Oda 1	9°19'14.11"	42°27'06.18"
26	19764	GOG 5	Oromia/Misrak/Gursum	Oda 2	9°18'37.42"	42°28'38.21"
27	19765	GOG 6	Oromia/Misrak/Gursum	Oda 3	9°18'40.13"	42°28'38.16"
28	19766	GOG 7	Oromia/Misrak/Gursum	Oda 4	9°18'30.32"	42°29'41.38"
29	19767	GOG 8	Oromia/Misrak/Gursum	Oda 5	9°18'30.28"	42°29'41.25"
30	19768	GOG 9	Oromia/Misrak/Gursum	Nur Selam 1	9°19'30.26	42°28'38.32"
31	19769	GOG 10	Oromia/Misrak/Gursum	Nur Selam 2	9°19'30.53"	42°28'38.45"
32	19770	GOG 11	Oromia/Misrak/Gursum	Odaa 1	9°21'54.13"	42°29'50.34"
33	19771	GOG 12	Oromia/Misrak/Gursum	Odaa 2	9°21'54.24"	42°29'50.46"
34	19772	GOG 13	Oromia/Misrak/Gursum	Abader	9°17'51.28"	42°24'14.33"
35	19773	GOG 14	Oromia/Misrak/Gursum	Harobata 1	9°17'12.43"	42°23'43.24"
36	19774	GOG 15	Oromia/Misrak/Gursum	Harobata 2	9°17'12.38"	42°23'43.28"
37	19775	GOG 16	Oromia/Misrak/Gursum	Harobata 3	9°16'12.18"	42°23'18.21"
38	19776	GOG 17	Oromia/Misrak/Gursum	Harobata 4	9°16'22.20"	42°23'35.12"
39	19777	GOG 18	Oromia/Misrak/Gursum	Awdal 1	9°17'20.56"	42°26'26.65"
40	19778	GOG 19	Oromia/Misrak/Gursum	Awdal 2	9°17'20.48"	42°26'26.51"
41	19779	GSJ -1	Somalia/Jigjiga	Beledka	9°17'51.23"	42°39'08.34"
42	24208	GAW -1	Amhara/Adoawe	Wangua	10°48'45.43"	36°25'35.12"
43	28662	GOBG1	Oromia/Bale	Ginir	7°11'86.21"	40°37'46.21"

Note: * *GOG = Oromia/Gursum; GOB = Oromia/Babile; GOBG = Oromia/Bale-Ginir; GSJ = Somalia/Jigjiga; and GAW = Amhara/Wangua.*

2.2. DNA Extraction

DNA extraction and analysis were carried out at Plant Molecular Genetics Research Laboratory, Department of Microbial, Cellular and Molecular Biology Department, Addis Ababa University. About 50 mg of silica gel-dried leaves for each accession were ground with mix and miller machine. Genomic DNA extraction was done based on the CTAB method (Wang *et al.,* 1994) with minor modification in the amount of CTAB solution used (1000 µl), as well as incubation and centrifugation time, to get optimal amounts of DNA. The yield of DNA isolated was measured/quantified using a Nano Drop ND-8000 UV

spectrophotometer. Moreover, the purity of DNA was visually determined by agarose gel electrophoresis by running the samples on 1% agarose gel (Figure 2). The samples were stored at 4 °C until subsequent analysis is carried out.

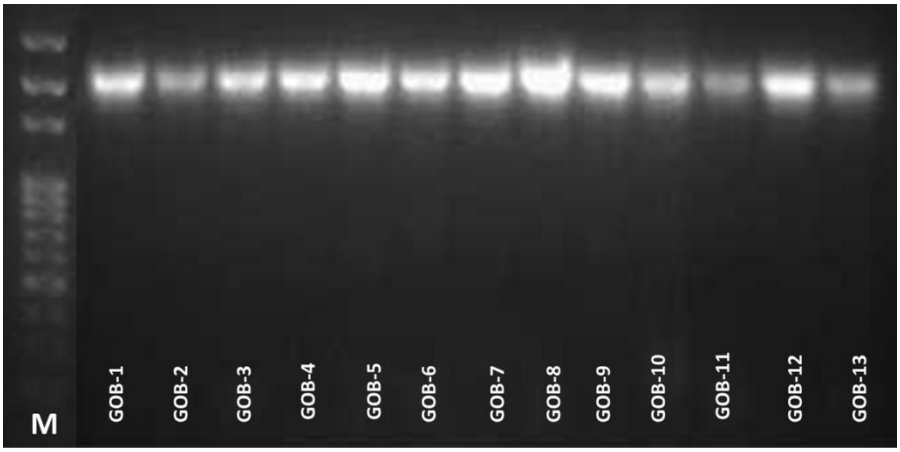

Figure 2. Quality of the DNA in 1% (w/v) agarose gels in 0.5% TBE buffer (M = molecular ladder).

2.3. Primer Selection and Optimization

For PCR optimization and screening of primers, the concentration of extracted DNA from each accession were adjusted to 50 ng μl^{-1}. A total of nine ISSR primers obtained from the Genetic Research Laboratory (Primer kit UBC), originally bought from University of British Columbia, were used for the initial testing of polymorphism and reproducibility. All the 9 primers were screened for reproducibility and polymorphism. Finally, three di-nucleotide primers (UBC810, UBC841 and UBC857), and one penta-nucleotide primer (UBC881) which showed polymorphic and reproducible bands were selected for ISSR amplification (Table 2).

Table 2. ISSR primers screened for polymorphism and reproducibility of the amplified bands.

Primer	Annealing T° (°C)	Sequence of nucleotides (5'–3')	Amplification pattern
UBC810	45	GAGAGAGAGAGAGAGAT	Polymorphic, Reproducible
UBC812	45	GAGAGAGAGAGAGAGAA	Not amplified
UBC824	48	TCTCTCTCTCTCTCTCG	Not reproducible
UBC840	45	GAGAGAGAGAGAGAGAYT	Not amplified
UBC841	48	GAGAGAGAGAGAGAGAYC	Polymorphic, Reproducible
UBC842	45	GAGAGAGAGAGAGAGAYG	Not amplified
UBC848	45	CACACACACACACACARC	Not reproducible
UBC857	48	ACACACACACACACACYG	Polymorphic, Reproducible
UBC881	48	GGGGTGGGGTGGGGTG	Polymorphic, Reproducible

Note: *Y = Pyrimidines (C or T) and* R = *Purines (A or G). The source was Primer kit 900 (UBC 900).*

2.4. ISSR Amplification

The ISSR amplification was done using Biometra 2000 T3 Thermo-cycler. The PCR amplification was carried out in a 25 µl total reaction mixture containing 50 ng μl^{-1} template DNA, 17.5 µl ddH20, 0.5 µl dNTP (1.25 mM), 2.5 µl PCR buffer (10xThermopol reaction buffer), 2.5 µl $MgCl_2$ (2 mM), 0.5 µl primer (20 pmol μl^{-1}) and 0.5 µl Taq Polymerase (5 U μl^{-1}). The amplification program was 4 min preheating and initial denaturation at 94 °C, then 39 cycles at 94 °C for 30 sec, 1 min primer annealing at 45/48 °C based on primers used, 90 sec extension at 72 °C with a final extension of 7 min at 72 °C. The PCR products were also stored at 4 °C until loaded on gel for electrophoresis.

2.5. Electrophoresis

Agarose gel (1.67%) was prepared using 300 ml TBE mixed with 5.01 g agarose using 500 ml Erlenmeyer flask and then boiled in micro-oven for 3 minutes. After it was cooled for about 20 min at room temperature, 12 µl Ethidium Bromide (10 mg ml^{-1}) was added and the gel was poured on gel casting tray to solidify. The amplified products were run on to ISSR gel using 1.67% agarose, with 1 X TBE using gel electrophoresis chamber. Eight micro litter ISSR amplification products and 2µl (6X) loading dye (0.12% bromo-phenol blue and 30% glycerol) were mixed thoroughly and loaded on the gel. A 1200 bp ladder (molecular marker) was used to estimate the molecular size of the DNA fragments. The gel was run on electrophoresis machine for 2 h at constant voltage of 100 V. The ISSR band patterns were visualized and photographed under UV light using Biometra Biodoc analyzer**.**

2.6. Data Scoring and Statistical Analysis

The ISSR bands were scored as present (1) and absent (0) representing the ISSR profile of each sample. For each ISSR marker, total amplified bands, number of polymorphic bands, and percentage of polymorphic bands (PPB) were determined. The 0/1 matrix data was analyzed using Free Tree 0.9.1.50 (Pavlicek *et al.*, 1999) and NTSYS-pc version 2.02 (Rohlf, 2000) software to calculate the Jaccard's similarity coefficient for all possible pairs of samples. Jaccard's similarity coefficient was calculated as:

$$s_{ij} = \frac{a}{a+b+c}$$

Where, S_{ij} is Jaccard's similarity coefficient, a is the total number of bands shared between individuals i and j, b is the total number of bands present in individual i but not in individual j, and c is the total numbers of bands present in individual j but not in individual i. Jaccard's similarity coefficient classified as low when its value is less than or equal to 40%, medium when it is between 40% and 60% and high when greater than or equal to 60% (Stephanie, 2016).

The resulting similarity matrices were employed to construct UPGMA-based dendrogram. The unweighted pair group method with arithmetic mean (UPGMA) was used in order to determine the genetic relationship among accessions using NTSYS- pc version 2.02 (Rohlf, 2000). The matrix of genetic similarity was also used in a principal coordinate analysis (PCoA) to resolve the patterns of clustering among the accessions based on Jaccard's coefficient.

Percent of polymorphism, Nie's pairwise gene diversity (Nei, 1987) and Shannon's Weaver pairwise diversity index (I) were determined with POPGENE software 1.32 (Yeh *et al.*, 1997). The binary data generated were used to determine levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The Shannon index varies from 0 to 1, and values closer to zero represents lower genetic diversity (Silva *et al.* 2015). In the case of di allelic loci (binary data) the maximum value of the gene diversity (H) indices equals 0.5, revealing maximum genetic diversity (Nei, 1978). According to Nei (1978), Gst is classified as low when its value is less than or equal to 0.05, medium when it is between 0.05 and 0.15, and high when it is greater than or equal to 0.15. In flowering plant, the level of Nm is divided into three grades: high, Nm greater than or equal to 1.00; moderate, Nm ranging from 0.250 to 0.99; and low, Nm ranging from 0.00 to 0.249 (Slatkin, 1985 1987).

To measure the informativeness of the ISSR markers to differentiate among accessions, polymorphism information content (PIC), effective multiplex ratio (EMR), marker Index (MI) and resolving power (RP) were calculated. The value of polymorphism information content (PIC) was calculated using software Power Marker version 3.2 (Liu and Muse, 2005). The PIC was calculated by the formula: PIC = 2Pi (1-Pi), where, Pi is the frequency of occurrence of polymorphic bands in different priers. A PIC value of less than or equal to 0.25 indicates low diversity, between 0.25 and 0.5 intermediate and value greater than or equal to 0.5 indicates high diversity (Botstein *et al.,* 1980). The EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands (Najaphy *et al.*, 2011). The MI was determined according to Powell *et al.* (1996) as the product of PIC and EMR. The RP was calculated using the formula RP = ∑Ib, where Ib is band in formativeness and Ib = 1-[2×(0.5-p)]; where, p is the proportion of genotypes containing the band (Altıntas *et al.*, 2008).

2.7. Resolving Power (RP)

The resolving power (RP) is a parameter that specifies the discriminatory potential of the primers (the ability of a primer to generate optimally informative bands). Many studies have indicated RP index as an important feature of a good marker system (Grativol *et al.*, 2001; Mondal *et al.*, 2008; Kayis *et al.*, 2010; Sadeghi and Cheghamirza, 2012).

3. Results

3.1. ISSR Polymorphism

Four of the nine ISSR primers only (Table 3) produced reproducible bands ranging from 120 to 1100 bp (Figure 2). Fifty-six bands were generated across the four ISSR primers, of which 29(51.8%) were polymorphic (Table 3). The amplified bands by the primers ranged from 12 (UBC841) to 18 (UBC881) across the accessions. The number of polymorphic bands of the primers ranged from 5 in primer UBC841 and UBC881 to 11 in primer UBC857. The percentage of polymorphism for primers ranged from 27.8% in primer UBC881 to 84.6% in primer UBC 857, with an average polymorphism percent of 51.8% (Table 3).

Table 3. Percent of polymorphism, polymorphism information content (PIC), marker index (MI) and effective multiplex ratio (EMR) and resolving power (RP) of the four ISSR primers in the studied groundnut accessions.

Primer	Sequence 5'–3'	Annealing T^{o} (^{o}C)	Total number of bands	No. of polymorphic bands	Polymorphism (%)	PIC	MI	EMR	RP
UBC810	(GA)8T	45	13	8	61.5	0.49	3.9	4.9	12.51
UBC841	(GA)8YC	48	12	5	41.7	0.42	2.1	2.1	10.23
UBC857	(AC)8YG	48	13	11	84.6	0.76	8.4	9.3	18.34
UBC881	(GGGGT)3G	48	18	5	27.8	0.29	1.5	1.4	2.65
Total			56	29	51.8	0.49	3.98	4.43	10.46

Note: Y = Pyrimidines (C or T) and R = Purines (A or G). The source was Primer kit 900 (University of British Colombia).

In the present study, the di-nucleotide primers, namely UBC810, UBC841 and UBC857 were observed to have 61.5%, 41.7% and 84.6% of polymorphism, respectively. The penta-nucleotides primer UBC881 showed 27.8% polymorphism. A representation of the ISSR band profile obtained with primer UBC857 is shown (Figure 3). The di-nucleotide ISSR primers UBC857 with AC repeats and UBC810 with GA repeats, detected higher polymorphism among accessions compared with penta-nucleotide primer.

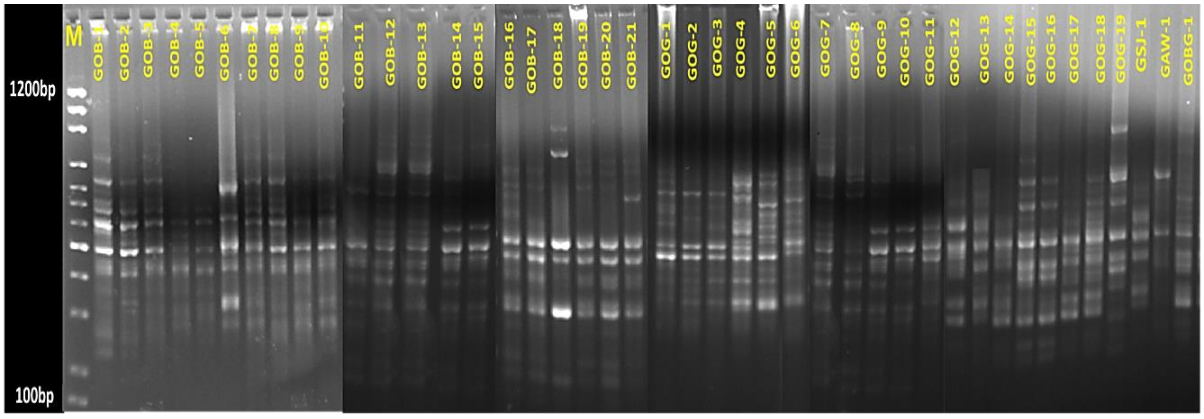

Figure 3. ISSR banding patterns generated from 43 groundnut accessions using primers UBC857.

3.2. Polymorphism Information Content (PIC)

In the present study, the PIC value varied from 0.29 (primer UBC881) to 0.76 (primer UBC857) with an average value of 0.49 (Table 3).

3.3. Marker Index (MI) and Effective Multiplex Ratio (EMR)

The calculated Marker Index (MI) value for all primers ranged between 1.5 (UBC881) and 8.4 (UBC857). The effective multiplex ratio (EMR), the number of polymorphic fragments detected per assay, varied from 1.4 (UBC881) to 9.31 (UBC857) with a mean value of 4.43 (Table 3).

3.4. Resolving Power (RP)

In the present study, the estimated RP for primers varied from 2.65 (UBC881) to 18.34 (UBC857) with an average value of 10.46 (Table 3). The highest RP values suggesting the capacity of the primers used to distinguish among different accessions. The RP was positively correlated with total amplified bands, number of polymorphic bands, MI and EMR at P<0.01. Both MI and EMR were positively correlated with RP (r = 0.924 and r = 0.738, respectively, P < 0.01) and PIC.

3.5. Genetic Diversity

The lowest Nei's gene diversity (H) value (0.11) was obtained between GOG-6 (Gursum/Oda-3) and GOB-10 (Babile/Medigana-1) and the highest value (0.38) was between GOBG-1 (Bale/Ginir) and GOB-14 (Babile/ Tofic-2) with a mean value of 0.245. The lowest Shannon's indices (I) value (0.24) was recorded between GOG-6 (Gursum/Oda-3) and GOB-10 (Babile/Medigana-1) and the highest value was 0.41 between GOBG-1 (Bale/Ginir) and GOB-14 (Babile/ Tofic-2) with a mean value of 0.325. The highest genetic distance values belonged to GOBG-1 (Bale/Ginir) and GOB-14 (Babile/Tofic-2) accessions, which were genetically the most distant accessions.

The lowest observed number of alleles (Na) (1.347 ± 0.433) was recorded in 'GOB14 (Babile/ Tofic2)' and 'GOG6 (Gursum/Oda3)' accessions and the highest observed number of alleles (1.574± 0.485) was recorded in 'GOBG1 (Bale/Ginir)' accession with a mean value of 1.461 ± 0.459. The lowest values of the effective number of alleles (Ne) (1.163 ± 0.316) were observed in 'GOB14 (Babile/Tofic2)' and the highest values (1.328 ± 0.393) was observed in 'GOBG1 (Bale/Ginir)' with a mean value of 1.245 ± 0.355. Total gene diversity (Ht) and gene diversity among accessions (Hs) were 0.3166 ± 0.042 and 0.141 ± 0.065, respectively. The coefficient of gene differentiation (Gst) among accessions was 0.294. Based on the Gst value, the mean estimated number of gene flow (Nm) between accessions was found to be 0.827 (Table 4).

Table 4. Overall genetic variability of the studied *groundnut* accessions.

Sample size	Na ± (SD)	Ne ± (SD)	H ± (SD)	I ± (SD)	Ht ± (SD)	Hs ± (SD)	Gst	Nm
43	1.461 ± 0.459	1.245 ± 0.355	0.245 ± 0.095	0.325 ± 0.164	0.3166 ± 0.042	0.141 ± 0.065	0.294	0.827

Note: *Na = Observed number of alleles; Ne = Effective number of alleles; H = Nei's (1973) gene diversity; I = Shannon's Information index; Ht = Total genetic diversity; HS = Gene diversity among accessions; Gst = Coefficient of genetic differentiation among accessions; and Nm = Estimate of gene flow from Gst or Gcs. e.g., Nm = 0.5(1 - Gst)/Gst.*

For primers, the highest observed number of alleles (1.980 ± 0.640) and effective number of alleles (1.85 ± 0.12) were recorded by primer UBC857 and the least observed number of alleles (1.600 ± 0.520) and effective number of alleles (1.42 ± 0.41) were shown by primer UBC841. The highest gene diversity (0.46 ± 0.036) and Shannon index (0.657 ± 0.037) was shown by primer UBC857 and followed by primer UBC810, with gene diversity and Shannon index value of (0.46 ± 0.036 and 0.413 ± 0.322), respectively (Table 5) and the least value of genetic diversity and Shannon index was recorded by primer UBC841 with 0.25 ± 0.22 and (0.364 ± 0.326) value, respectively.

Table 5. Overall genetic variability of ISSR primers used.

Primer	Na ± (SD)	Ne ± (SD)	H ± (SD)	I ± (SD)
UBC810	1.643 ± 0.500	1.54 ± 0.42	0.31 ± 0.23	0.413 ± 0.322
UBC841	1.600 ± 0.520	1.42 ± 0.41	0.25 ± 0.22	0.364 ± 0.326
UBC857	1.980 ± 0.640	1.85 ± 0.12	0.46 ± 0.036	0.657 ± 0.037
UBC881	1.680 ± 0.480	1.52 ± 0.39	0.29 ± 0.21	0.419 ± 0.291
Average	1.726 ± 0.535	1.58 ± 0.32	0.32 ± 0.17	0.463 ± 0.244

Note: *Na = Observed number of alleles; Ne = Effective number of alleles; H = Nei's gene diversity; and I = Shannon's Information index.*

3.6. Genetic Relationship

The generated similarity matrix by ISSR based on the Jaccard's pairwise similarity coefficient matrices showed similarity ranged from 44% to 83%. The average similarity across the 43 accessions was found to be 63.5 %. The lowest genetic similarity value (i.e., maximum diversity) (44%) was found between accessions GOBG-1 (Bale/Ginir) and GOB-14 (Babile/Tofic-2), followed by similarity value of 46% between GOG-1 (Gursum/Llalemi-1) with GAW-1 (Amhara/Wangua) and GOB-17 (Babile/Gemechu) with GOBG-1 (Bale/Ginir). The highest similarity coefficient (i.e., minimum diversity) (83%) was found between the accessions GOG-6 (Gursum/Oda-3) with GOB-10 (Babile/Medigana-1) and GOB-7 (Babile/Ifa-gendi-1) with GOB-16 (Babile /Gende), followed by that between GOG-12 (Gursum/Odaa-2) with GOB-9 (Babile/Ifa-gendi-3) at a similarity value of 82%.

3.7. Cluster Analysis

UPGMA clustering analysis grouped accessions into five clusters at cut-off point of 63.5% similarity (Figure 4). The dendrogram did not divide the accessions into distinct groups based on geographical origin. The UPGMA analysis revealed that individuals in each accession were distributed and inter-mixed with individuals of another locality. For instance, the accessions from Gursum and/or Babile were distributed in all 5 distinct clusters (Figure 4). Moreover, accessions from distant geographical locations were clustered together with other accessions from different regions (Table 6).

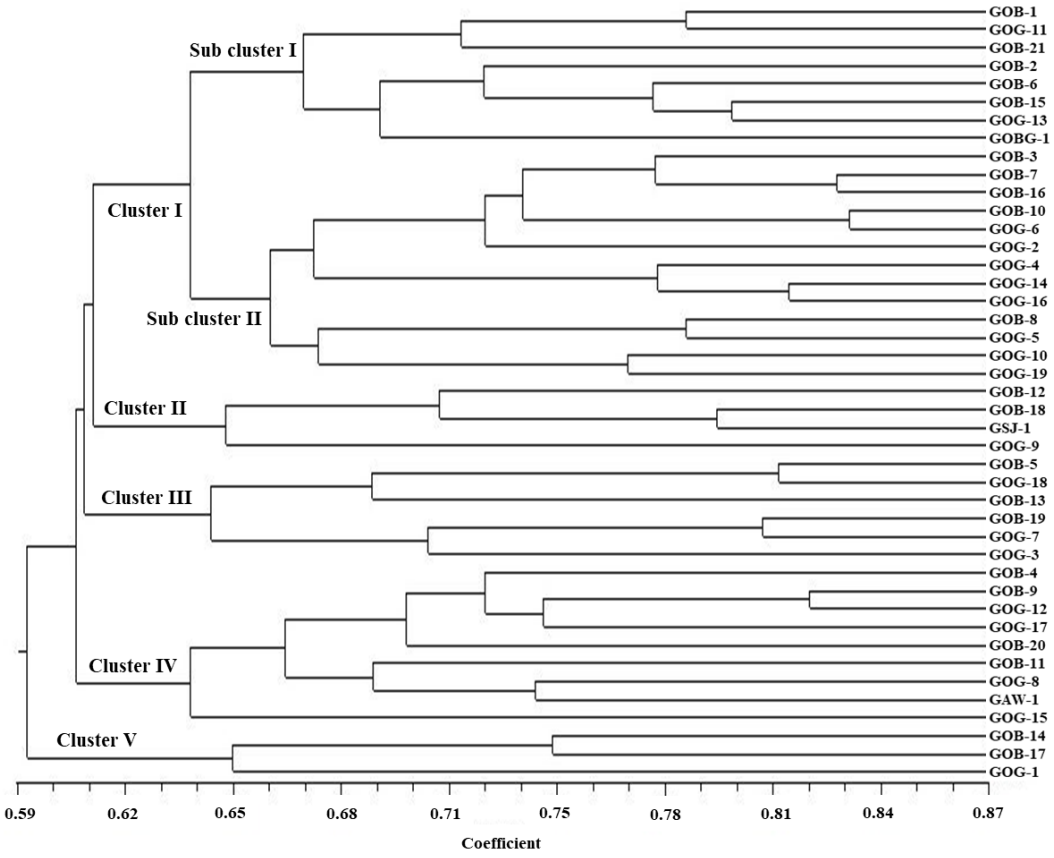

Figure 4. UPGMA cluster analysis of 43 accessions of *A. hypogaea* (GOG = Oromia/Gursum; GOB = Oromia/Babile; GOBG = Bale/Ginir; GSJ = Somalia/Jigjiga; and GAW = Amhara/Wangua).

Table 6. Clusters of accessions as shown in the dendrogram based on UPGMA.

Clusters	Sub-clusters	Accessions	Similarity (%)
1	1	GOB (1, 2, 6,15 and 21), GOBG1 and GOG (11 and 13)	67
	2	GOB (3,7, 8, 10, and 16), GOG (2,4,5,6,10,14,16 and 19)	66
2		GOB (12 and 18), GSJ1 and GOG-9	65
3		GOB (5,13 and 19) GOG (3,7 and 18)	64.8
4		GOB (4,9, 11 and 20), GOG (8,12,15 and 17) and GAW-1	63.5
5		GOB (14 and 17) and GOG1	65

Note: *GOG = Oromia/Gursum; GOB = Oromia/Babile; GOBG = Bale/Ginir; GSJ = Somalia/Jigjiga; and GAW = Amhara/Wangua.*

3.8. Principal Coordinate Analysis (PCoA)

All the data obtained using four ISSR primers were used for PCoA using Jaccard's coefficients of similarity. The first two components of the coordinates of the PCoA having eigen values of 16.3 and 8.2 with variance of 33.2% and 16.4%, respectively, and together 49.6% of the total variance. Similar to the UPGMA clustering pattern, the 43 groundnut accessions were grouped into five groups (clusters) based on the principal co-ordinate analysis (Figure 5). The PCoA plot indicated that most of the accessions did not group together with other accessions originated from the same geographical location. This result is in line with the result obtained from UPGMA. However, most of the accessions that show geographical proximity were found to form distinct groups and spread all over the plot (Figure 5). In addition, accessions from distant geographical locations tend to form similar group. As the result, low coefficient of variation was observed among groundnut accessions considered in this study.

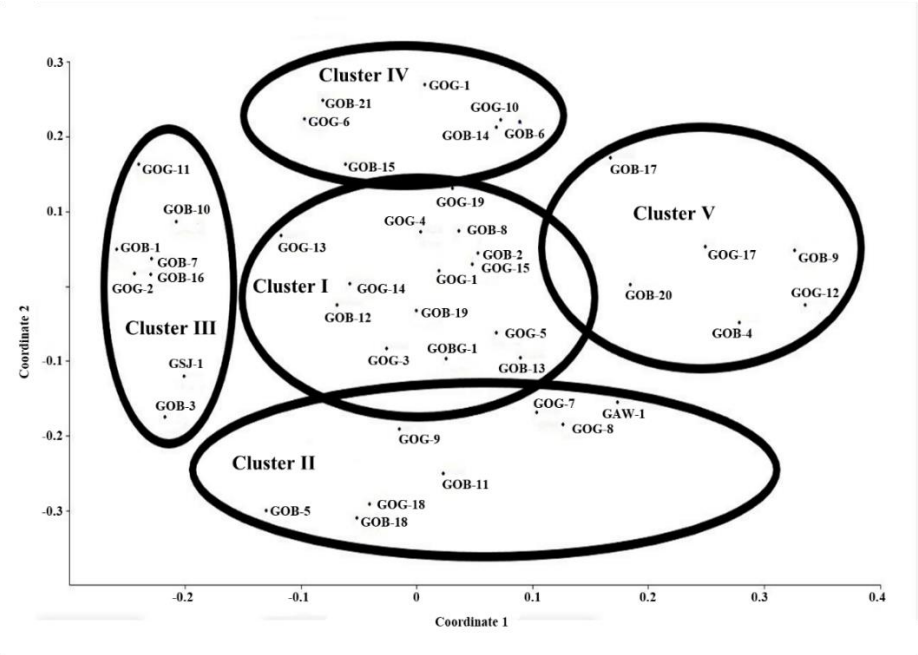

Figure 5. PCoA scatter plot diagram showing genetic relationships of *A. hypogaea* accessions.

4. Discussion

In the present study, ISSR profile was used to determine diversity and relationship among different groundnut accessions. Several studies on populations indicated the percentage of the polymorphic locus as an important measure of genetic diversity (Soares *et al.*, 2016). Groundnut showed moderate genetic diversity (p = 51.8%) as indicated by percent polymorphic loci (Table 3). In the Raina *et al.*, (2001) study, the ISSR markers revealed 54% polymorphism among 13 *A. Hypogaea*

accessions. In the Dhwani *et al.* (2017) and Suvendu *et al.* (2009) study, the ISSR markers revealed a higher level of polymorphism (87% and 74.7%, respectively) in groundnut.

The observed highest Nei's gene diversity (H) value (0.38 ± 0.174), and the highest Shannon's indices (0.41 ± 0.194) showed presence of genetic diversity among the studied accessions. The gene diversity obtained in this study (0.38), which is relatively higher than 0.11 obtained by Ren *et al.* (2014). On the other hand, the higher value gene diversity (0.74) was observed in the groundnut 'reference set' of ICRISAT (Pandey *et al.*, 2014). In the present study, the PIC value varied from 0.29 (primer UBC881), less informative to 0.76 (primer UBC857), high informative with an average value of 0.49. PIC value. PIC is a statistic that measures the usefulness of a genetic marker for linkage analysis (Hildebrand *et al.*, 1992). In this study, primer UBC857 can retrieve more information on the genetic polymorphism of groundnut accessions and could be used as a genetic marker for further study. The variations in the in formativeness of the molecular markers might be due to the difference in the genetic background of germplasm collections, length of the repeat unit and type, the sequence composition and distribution or frequency throughout the genome of groundnut (Dwivedi *et al.*, 2001). Thus, the choice of appropriate primer motives in ISSR fingerprint is critical to detect high polymorphism and reveal relationship between different groundnut accessions.

The genetic differentiation of a species reflects the interactions of various evolutionary processes such as shifts in distribution, habitat fragmentation and population isolation, mutation, genetic drift, mating system, gene flow and natural selection (Schall *et al.*, 1998). Possibly, these factors might have influenced the genetic differentiation of the groundnut accessions under study. The coefficient of gene differentiation (Gst) for the entire accessions was 0.29 (Table 4), suggesting a restricted genetic differentiation between accessions. The Gst value recorded indicating about 29% of the total genetic divergence among the accessions. This might be the result of interactions of different evolutionary processes.

Based on the Gst value, the mean estimated number of gene flow (Nm) for the entire accessions was found to be 0.827 (Table 4). In flowering plant, the level of Nm is divided into three grades: high, Nm equal to or larger than 1.0; moderate, Nm ranging from 0.25 to 0.99; and low, Nm ranging from 0.00 to 0.249 (Slatkin, 1987). Gene flow is generally considered as the main factor that could homogenize the genetic structure of populations in their distribution area. According to Wright (1931), Nm¼ 1 is sufficient to overcome the effects of genetic drift. Also, species with low gene flow have higher genetic differentiation than species with high gene flow. However, our results indicated that virtually moderate gene flow occurred among groundnut accessions.

Based on Jaccard's similarity coefficient, highest levels of distance between the accessions from GOBG1 (Bale/Ginir) and GOB14 (Babile/Tofic2) could be attributed to the fact that the accessions have been cultivated in the respective regions over time giving enough time for significant genetic differentiation along these particular geographical lines. Geographically isolated accessions could accumulate genetic differences and evolve unique traits as they adapt to different environment. Differential environmental conditions with respect to soil composition, altitude and annual rainfall could drive the acquisition of local adaptations. Nybom (2004) has addressed that genetic diversity is strongly associated with life form, geographic range, breeding system, seed dispersal mechanism, and successional status.

On the other hand, the comparison of the genetic distances between accessions of GOB10 (Babile/Medigana1) with GOG6 (Gursum/Oda3), GOB7 (Babile/Ifa-gendi1) with GOB16 (Babile/Gende) and GOG12 (Gursum/Odaa2) with GOB9 (Babile/Ifa-gendi-3) revealed a closer genetic relationship. This high level of similarity could be due to close geographical distance among accessions and/or selecting groundnuts with similar traits by Gursum and Babile farmers during crop production. Possible explanation for the genetic relatedness could be high rates of gene flow due to exchange of seed materials and limited time for significant genetic differentiation along geographical lines. Based on ISSR marker, genetic relationship analysis of groundnut showed higher level of genetic similarity between groundnut genetic resources (Baloch *et al.*, 2010; Dhwani *et al.*, 2017).

Both PCoA and UPGMA cluster analysis shows the clustering of all 43 accessions into five clusters without clear geographical differentiation (Figures 4 and 5). Accessions from the same geographical location were distributed in various clusters. For instance, the accessions from Gursum and/or Babile were distributed in all 5 distinct clusters. Such pattern has been reported by (Raina *et al.*, 2001; Varshney *et al.*, 2009; Peng *et al.*,

2016), suggesting that there was no significant grouping of genotypes as per geographical region in the population structure of groundnut. Moreover, the present finding is consistent with studies, which addressed cultivated groundnut and reported limited genetic diversity within them (Jiang *et al*., 2007; Janila *et al*., 2013).

Accessions from distant geographical locations were clustered together. This is evident, for example, in cluster I (sub cluster I) GOBG-1 accession from Bale/Ginir was clustered with accessions from Babile and Gursum. Similarly, in cluster IV the accession from GAW1 (Amhara/wangua) and GSJ1 (Somalia/Jigjiga/Beledka) were clustered with accessions from Babile and Gursum which is unexpected because they are geographically far distant locations (Figure 4). This pattern is observed possibly due to admixture as the result of short and long distance marketing of groundnut seeds or exchange of germplasm across different regions (Fredu Nega *et al*., 2015; Jemal Yousuf and Nick, 2015; Addisu Getahun and Ermias Tefera, 2017).

Knowledge on the genetic diversity of the selected individuals is of ultimate importance, since it contributes to the information on the species and allows the selection of genotypes to be included in future conservation programs. Thus, the most divergent genotypes can be selected to maintenance the level of genetic diversity of a species to keep its ability to adapt to novel environmental changes. The present finding also contributes valuable information on the genetic diversity of groundnut accessions in Ethiopia.

Parameters such as MI and EMR have been used for assessing the informative potential of molecular markers in various genetic diversity studies (Mondal *et al*., 2008; Najaphy *et al*., 2011). In the present study, the primers that generated high number of bands had higher MI and EMR values. Both MI and EMR were positively correlated with RP ($r = 0.924$ and $r = 0.738$, respectively, $P < 0.01$) and PIC. The resolving power (RP) is a parameter that specifies the discriminatory potential of the primers (the ability of a primer to generate optimally informative bands. Many studies have indicated RP index as an important feature of a good marker system (Mondal *et al*., 2008; Kayis *et al*., 2010; Grativol *et al*., 2011; Sadeghi and Cheghamirza, 2012). Many studies have indicated RP index as an important feature of a good marker system (Mondal *et al*., 2008; Kayis *et al*., 2010; Grativol *et al*., 201; Sadeghi and Cheghamirza, 2012). In the present study, the highest RP value 18.34 (UBC-857) suggesting the capacity of the primer used to distinguish among different accessions. RP was positively correlated with total amplified bands, number of polymorphic bands, MI and EMR at $P<0.01$, suggesting the informativeness of the ISSR primers used in the present study.

ISSR markers have demonstrated their efficiency in the study of genetic variability for several other species. Many studies have proved the effectiveness of this marker on genetic diversity and characterization of accessions between and within populations of *Capparis spinosa* L. (Liu *et al*., 2015), *Pitcairnia flammea* (Souza-Sobreira *et al*., 2015) *Erythrina velutina* (Souza *et al*., 2016) and *Croton tetradenius* (Almeida-Pereira *et al*., 2017).

5. Conclusions and Recommendations

The study has revealed genetic polymorphism (51.8%) among the groundnut accessions. The highest genetic similarity observed among the accessions of groundnut and the observed five clusters without clear geographical differentiation suggest the existence of genetic similarity among the accessions possibly due to gene flow caused by seed exchange. The ISSR based fingerprinting of groundnut accessions demonstrated the usefulness of the marker in estimating the extent of genetic variation and genetic relationships among accessions. The present finding is an important milestone for future germplasm collection, sound conservation, improvement and breeding of the crop. Further study with more geographic range and the use of additional molecular markers would give additional picture of the genetic diversity of groundnut accessions in Ethiopia.

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