Genetic Diversity of Groundnut (*Arachis hypogaea* L.) Accessions using Inter Simple Sequence Repeats (ISSRs) Marker

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Abstract

Background: Groundnut (*Arachis hypogaea* L.) belongs to the family Leguminosae. It is the world's most important source of edible oil and vegetable protein. To our knowledge, little is known about the genetic variability of cultivated Ethiopian *A. hypogaea* at a molecular level. It is important to understand the genetic diversity of the crop to collect, conserve and use the germplasm for variety development. In the present study, ISSR markers were used to determine the genetic variability and diversity of 43 accessions of *A. hypogaea* collected from different regions of Ethiopia.

Results: Four ISSR primers were used to generate 56 reproducible bands of which 29 (51.8%) were polymorphic. The band size ranges from 120 bp to 1100 bp. The number of amplified bands varied from 12 in primer UBC841 to 18 in primer UBC 881. The polymorphic bands percentage ranges from 27.8 % of Primer UBC 881 to 84.6 % of primer UBC 857. The polymorphic information content (PIC) value ranges 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 varies from 44% to 83% with an average value of 63.5%. The UPGMA dendrogram based on cluster analysis grouped *A. hypogaea* accessions into five distinct clusters at 63.5% similarity coefficient, and the principal coordinate analysis revealed similar grouping.

Conclusions: Even though in both UPGMA and PCoA most of the accessions were grouped in separate clusters irrespective of their geographic origins, the ISSR marker-based analysis shows the presence of genetic variability among the accessions. Moreover, the current study demonstrated the potential informativeness of ISSR markers in estimating the extent of genetic variation in *A. hypogaea* accessions.

Introduction

Groundnut or peanut (*Arachis hypogaea* L.) belongs to the family Leguminosae and genus *Arachis*. Cultivated groundnut (*Arachis hypogaea* L.) is a highly self-pollinated, allotetraploid annual legume with $2n = 4x = 40$ with a basic chromosome number of $x = 10$ [1]. *A. hypogaea* 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 with average daily temperature of higher than 20 °C [2]. Major *A. hypogaea* growing countries include China, India, the United States and Nigeria [3].

A. hypogaea is the world's 4th most important source of edible oil and 3rd most important source of vegetable protein [4]. *A. hypogaea* seeds contain 42–50% oil, 26% protein, 18% carbohydrates, and are also rich source of riboflavin, thiamine, nicotinic acid and vitamin E [5]. *A. hypogaea* is one of the four economically important oilseed crops along with noug, flax and sesame in Ethiopia [2]. Besides, this crop helps small scale producers in getting significant revenue and also 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 [6].

In Ethiopia, *A. hypogaea* is grown and covered nearly 80,000 hectares [7] of arable land per annum and the major producing regions are Eastern Hararghe in Oromia and Metekel in Benishangul-Gumuz regional state

[7, 8]. Despite its importance, the national average yield produced by the farmers in Ethiopia is considerably low, 1.3 tons/ha, indicating the need of maximum effort to improve productivity [9].

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 [10]. Assessment of genetic diversity is an important step in any crop improvement program [11]. 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) [12]. Collecting DNA marker data to determine whether phenotypically similar cultivars are genetically similar would therefore be of great interest in crop breeding programs [13]. 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 [14]. Consequently, the development of marker protocols such as RFLP, AFLP, ISSR, SNP and SSR have revolutionized the genetic analysis by detecting level of polymorphism [15].

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 [21, 22]. 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 [23]. In addition, it does not require genome sequence information; it leads to multilocus, highly polymorphous patterns and produces dominant markers [24]. ISSR PCR is a fast, inexpensive genotyping technique based on variation in the regions between microsatellites [23]. ISSRs segregate mostly as dominant markers following simple Mendelian inheritance. ISSR analyses offer breeders and geneticists with competent means to link phenotypic and genotypic variations in various fields of plant improvement [25].

Genetic diversity study in *A. hypogaea*, based on morphological, biochemical and some molecular markers has been reported [16–20]. However, more molecular marker-based genetic diversity study of *A. hypogaea* accessions in Ethiopia is demanding. In this paper we report the level and pattern of genetic variability in 43 accessions of *A. hypogaea* grown in different regions of Ethiopia using ISSR markers. Moreover, we addressed the potential informativeness of ISSR markers for identifying *A. hypogaea* accessions.

Material And Methods

Plant Materials

Seeds of 43 *A. hypogaea* accessions collected from different regions of Ethiopia were obtained from Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia. The accessions were collected from different geographical locations of Ethiopia (Fig. 1). The seeds of all the 43 *A. hypogaea* 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 week old plants were collected from plants of each

accession in tubes containing silica gel for genomic DNA extraction. These accessions, their gene bank number, code, collection region, locality and geographical location (latitude and longitude) are listed in Table 1.

Table 1

List of accessions included in the present study: Gene bank accession number, the corresponding code, collection region and sites along with their latitude and longitude

S. No.	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"

Key: GOG – Oromia/Gursum, GOB – Oromia/Babile, GOBG – Oromia/Bale-Ginir, GSJ – Somalia/Jigjiga, GAW – Amhara/Wangua

S. No.	Gene bank accession no	Code	Collection region	locality	Latitude (N)	Longitude (E)
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"

Key: GOG – Oromia/Gursum, GOB – Oromia/Babile, GOBG – Oromia/Bale-Ginir, GSJ – Somalia/Jigjiga, GAW – Amhara/Wangua

S. No.	Gene bank accession no	Code	Collection region	locality	Latitude (N)	Longitude (E)
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"
Key: GOG – Oromia/Gursum, GOB – Oromia/Babile, GOBG – Oromia/Bale-Ginir, GSJ – Somalia/Jigjiga, GAW – Amhara/Wangua						

DNA Extraction

DNA extraction and analysis were carried 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 [26] 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 (Fig. 2). The samples were stored at 4 °C until subsequent analysis is carried out.

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. 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 shows polymorphic and reproducible bands were selected for ISSR amplification (Table 2).

Table 2
ISSR primers screened for polymorphism and the 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	TCTCTCTCTCTCTCG	Not reproducible
UBC840	45	GAGAGAGAGAGAGAGAYT	Not amplified
UBC841	48	GAGAGAGAGAGAGAGAYC	Polymorphic, Reproducible
UBC842	45	GAGAGAGAGAGAGAGAYG	Not amplified
UBC848	45	CACACACACACACARC	Not reproducible
UBC857	48	ACACACACACACACYG	Polymorphic, Reproducible
UBC881	48	GGGGTGGGGTGGGGTG	Polymorphic, Reproducible
Source: Primer kit 900 (UBC 900); Y = Pyrimidines (C or T), R = Purines (A or G).			

ISSR Amplification

The ISSR amplification was done using Biometra 2000 T3 Thermo-cycler. PCR amplification was carried out in a 25 µl total reaction mixture containing 50 ng/µl template DNA, 17.5 µl ddH₂O, 0.5 µl dNTP (1.25 mM), 2.5 µl PCR buffer (10xThermopol reaction buffer), 2.5 µl MgCl₂ (2 mM), 0.5 µl primer (20 pmol/µl) and 0.5 µl Taq Polymerase (5 U/µl). 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.

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) 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 2 µl (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.

Data scoring and statistical analysis

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 [27] 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 . 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 [28]. 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, Nei's pairwise gene diversity and Shannon's Weaver pairwise diversity index (I) were determined with POPGENE software 1.32 [29]. The binary data generated were used to determine levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Genetic diversity measures were tested using Nei's gene diversity statistics. Shannon's diversity index (I) was also used to examine partitioning of genetic diversity within and among populations.

To measure the informativeness of the ISSR markers to differentiate between the *A. hypogaea* 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 [30]. The PIC was calculated by the formula: $PIC = 2P_i(1-P_i)$, where, P_i is the frequency of occurrence of polymorphic bands in different priers. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands [31]. MI was determined according to Powell et al. [32] as the product of PIC and EMR. RP was calculated using the formula $RP = \sum I_b$, where I_b is band informativeness and $I_b = 1 - [2 \times (0.5-p)]$, where p is the proportion of genotypes containing the band [33].

Results

ISSR polymorphism

Four of nine ISSR primers used (Table 3) could produce reproducible bands ranging in size from 120 to 1100 bp (Fig. 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 range 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 and polymorphism information content (PIC) generated in *A. hypogaea* using four ISSR Primers

Primer	Sequence 5'-3'	Annealing T (°C)	Total number of bands	No. of polymorphic bands	% of polymorphism	PIC
UBC810	(GA)8T	45	13	8	61.5%	0.49
UBC841	(GA)8YC	48	12	5	41.7%	0.42
UBC857	(AC)8YG	48	13	11	84.6%	0.76
UBC881	(GGGGT)3G	48	18	5	27.8%	0.29
Total			56	29	51.8%	0.49
Source: Primer kit 900 (University of British Colombia); Y = Pyrimidines (C or T), R = purines (A or G).						

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 was observed to have 27.8% polymorphism. A representation of the ISSR band profile obtained with primer UBC857 is shown (Fig. 3). In the present study, the di-nucleotide ISSR primers UBC857 with AC repeats and UBC810 with GA repeats, detected higher polymorphism among accessions compared with penta-nucleotide primer.

Polymorphism Information Content (PIC)

Polymorphism information content (PIC) is the probability of detection of polymorphism by a primer/primer combination between two randomly drawn genotypes and depends on the number of detectable alleles and the distribution of their frequency. 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).

Marker Index (MI) and Effective multiplex ratio (EMR)

The calculated Marker Index (MI) value for all primers ranged between 1.49 (UBC881) and 11.01 (UBC857). The effective multiplex ratio (EMR), the number of polymorphic fragments detected per assay, varied from 5 to 26 with a mean value of 15.94.

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 [34, 35, 36, 37]. 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. The highest RP values suggesting the capacity of the primers used to distinguish among different accessions. RP was

positively correlated with total amplified bands, number of polymorphic bands, MI and EMR at $P < 0.01$. MI and EMR were positively correlated with RP ($r = 0.924$ and $r = 0.738$, respectively, $P < 0.01$) and also positively correlated with PIC.

Genetic Diversity

The lowest Nei's gene diversity (H) value was 0.11 between GOG-6 (Gursum/Oda-3) and GOB-10 (Babile/Medigana-1) and the highest was between GOBG-1 (Bale/Ginir) and GOB-14 (Babile/ Tofic-2) at 0.38, with a mean value of 0.245. The lowest Shannon's indices (I) value was 0.24 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 mean observed number of alleles (Na) ranged from 1.347 ± 0.433 in 'GOB-14 (Babile/ Tofic-2)' and 'GOG-6 (Gursum/Oda-3)' accessions to a maximum of 1.574 ± 0.485 in 'GOBG-1 (Bale/Ginir)' accession. Values of the effective number of alleles (Ne) were less than those for (Na) with regard to every accessions and ranged from 1.163 ± 0.316 in 'GOB-14 (Babile/ Tofic-2)' to 1.328 ± 0.393 in 'GOBG-1 (Bale/Ginir)'. Total gene diversity (Ht) and gene diversity among accessions (Hs) were 0.3166 ± 0.042 and 0.141 ± 0.065 , respectively. The heterozygosity among the accessions ranged from 0.098 in 'GOB-14 (Babile/ Tofic-2)' to 0.174 in 'GOBG-1 (Bale/Ginir)'. 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 across all studied *A. hypogaea* L. 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
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 accessssions. Gst = Coefficient of genetic differentiation among accessions. 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) was 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 in primer UBC841 with 0.25 ± 0.22 and (0.364 ± 0.326) value respectively.

Table 5
Overall genetic variability across used ISSR primers

Primer	Na $\pm$ (SD)	Ne $\pm$ (SD)	H $\pm$ (SD)	I $\pm$ (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
Na = Observed number of alleles. Ne = Effective number of alleles. H = Nei's gene diversity. I = Shannon's Information index.				

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) was found between accessions GOBG-1 (Bale/Ginir) and GOB-14 (Babile/Tofic-2) (44%) followed by that between GOG-1 (Gursum/Llalemi-1) with GAW-1 (Amhara/Wangua) and GOB-17 (Babile/Gemechu) with GOBG-1 (Bale/Ginir) at a similarity value of 46%. The highest similarity coefficient (i.e. minimum diversity) 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) 83% followed by that between GOG-12 (Gursum/Odaa-2) with GOB-9 (Babile/Ifa-gendi-3) at a similarity value of 82%.

Cluster Analysis

UPGMA clustering analysis put the accessions into one cluster at 59.2% similarity. However, the accessions were clustered in to five clusters based on the cut-off point of 63.5% similarity (Fig. 4). The dendrogram for the accessions (Fig. 4) did not divide the accessions into distinct groups resembling their geographical origin. The UPGMA analysis revealed that individuals in each accession were distributed and inter-mixed with individuals of another locality. No specific/significant trend of grouping of accessions was observed. For instance, the accessions from Gursum and/or Babile were distributed in all 5 distinct clusters. Moreover, accessions from distant geographical locations were clustered together with other accessions from different regions. This fact becomes evident, for example, at the top of the cluster 'GOBG-1' accession from Bale/Ginir was clustered with accessions from Babile and Gursum. Similarly, at the bottom and middle of the clusters the accessions from GAW-1 (Amhara/wangua) and GSJ-1 (Somalia/Jigjiga/Beledka) were clustered with accessions from Babile and Gursum.

Moreover, a loose clustering of accessions as per their geographical proximity was observed. For example, accessions from Babile (62.5%) and Gursum (61.5%) were grouped in cluster I sub cluster I and II,

respectively (Fig. 4).

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% used to show the grouping of individuals using two co-ordinates. Similar to the UPGMA clustering pattern, the 43 accessions of *A. hypogaea* were grouped into five groups (clusters) based on the principal co-ordinate analysis (Fig. 5).

The PCoA plot indicated that most of the accessions did not group together with other accessions originated from the same geographical location (Fig. 5). This result is in line with the result obtained in UPGMA. However, most of the accessions that show geographical proximity were found to form distinct groups and spread all over the plot (Fig. 5). In addition, accessions from distant geographical locations tend to form similar group or cluster. As the result, low coefficient of variation was observed in *A. hypogaea* accessions considered in this study.

Discussion

In the present study ISSR profile was used for diversity analysis and relationship among different *A. hypogaea* accessions. Several studies on populations indicate the percentage of the polymorphic locus as an important measure of genetic diversity [14]. *A. hypogaea* showed moderate genetic diversity as indicated by percent polymorphic loci ($p = 51.8\%$). A study reported 54% of polymorphism among 13 *A. hypogaea* accessions using ISSR markers [15]. The observed highest Nei's gene diversity (H) value (0.38 ± 0.174), and the highest Shannon's indices (0.41 ± 0.194) shows presence of genetic diversity among the studied accessions. The Shannon index vary from 0 to 1, and lower genetic diversity is represented by values closer to zero [39].

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 of 0.70 and above is highly informative while a value of ~ 0.44 is moderately informative. PIC is a statistic that measures the usefulness of a genetic marker for linkage analysis [38].

The genetic differentiation of a species reflects the interactions of various evolutionary processes including long-term evolutionary history, such as shifts in distribution, habitat fragmentation and population isolation, mutation, genetic drift, mating system, gene flow and natural selection [42]. The coefficient of gene differentiation (G_{st}) for the entire accessions of *A. hypogaea* was 0.29, suggesting a restricted genetic differentiation between accessions. The average value of differentiation might reflect the interactions of various factors including reduced geographic range in most of the accessions, inter and intra-regional climates, their breeding system and limited genetic drift or genetic isolation of the samples.

Based on the G_{st} value, the mean estimated number of gene flow (N_m) for the entire accessions was found to be 0.827 (Table 4). In flowering plant, the level of N_m is divided into three grades: high, N_m equal to or

larger than 1.0; moderate, N_m ranging from 0.250 to 0.99; and low, N_m ranging from 0.00 to 0.249 [43]. 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) [44], $N_m \geq 1$ 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 between the accessions of *A. hypogaea*.

Based on Jaccard's similarity coefficient, highest genetic similarity observed among accessions suggests that the existence of genetic similarity among the accessions possibly due to gene flow. On the other hand, the least genetic similarity observed between some accessions is useful in broadening genetic base of *A. hypogaea* accessions in Ethiopia and these accessions should be considered as the primary/valuable sites in conservation and breeding program of the crop. Both PCoA and UPGMA cluster analysis shows the clustering of all 43 accessions into five clusters without clear geographical differentiation. This might be due to gene flow caused by the exchange of seeds by farmers in Ethiopia.

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 *A. hypogaea* accessions grown in Ethiopia.

Parameters such as MI and EMR have been used for assessing the informative potential of molecular markers in various genetic diversity studies [31, 34]. In the present study, the primers that generated high number of bands had higher MI and EMR values. 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 [34, 35, 36, 37]. 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 in articles on genetic diversity and characterization of accessions between and within populations, such as those with, *Capparis spinosa* L. [45], *Pitcairnia flammea* [46] *Erythrina velutina* [47] and *Croton tetradenius* [48].

Conclusions

The study has revealed genetic polymorphism (51.8%) among the *A. hypogaea* accessions. The highest genetic similarity observed among the accessions of *A. hypogaea* 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 *A. hypogaea* accessions demonstrated the usefulness of the marker in estimating the extent of genetic variation and genetic relationships among *A. hypogaea* accessions. To conclude, 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 *A. hypogaea* accessions in Ethiopia.

Abbreviations

EBI: Ethiopian biodiversity institute; ISSR: Inter simple sequence repeats; PCoA: Principal coordinate analysis; PCR: Polymerase Chain Reaction; PIC: Polymorphism information content; UPGMA: Unweighted pair group method with arithmetic mean

Declarations

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Authors' contributions

MA designed and preformed the experiment, analyzed data, prepared the draft manuscript; MK designed the experiment, supervised the research, revised the manuscript and correspondence of the paper; and TF designed the experiment, supervised the research and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Ethics approval and consent to participate

Not applicable

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Competing interests

The authors declare that they have no competing interests

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Figures

Figure 1

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

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Figure 2

Quality of the DNA in 1% (w/v) agarose gels in 0.5% TBE buffer

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Figure 3

ISSR banding patterns generated from 43 accessions of *A. hypogaea* using primers UBC857

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Figure 4

UPGMA cluster analysis of 43 accessions of *A. hypogaea* Key: GOG – Oromia/Gursum, GOB – Oromia/Babile, GOBG – Bale/Ginir, GSJ – Somalia/Jigjiga, GAW – Amhara/Wangua

Figure 4

UPGMA cluster analysis of 43 accessions of *A. hypogaea* Key: GOG – Oromia/Gursum, GOB – Oromia/Babile, GOBG – Bale/Ginir, GSJ – Somalia/Jigjiga, GAW – Amhara/Wangua

Figure 5

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