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# Genetic diversity analysis in *Plectranthus edulis* (Vatke) Agnew populations collected from diverse geographic regions in Ethiopia using inter-simple sequence repeats (ISSRs) DNA marker system

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## Abstract

**Background:** *Plectranthus edulis* (Vatke) Agnew (Lamiaceae), locally known as Ethiopian potato *syno*. Ethiopian dinich, is one of the native Ethiopian edible tuber crops that has been significantly contributing to household food security for millions of subsistence farmers. However, its current production is declining to the extent of total extinction from several administrative regions where it used to be widely cultivated. It is one of the less researched crops regardless of being indigenous and its contribution to food security during time of scarcity. Therefore, we intended to assess the level of genetic diversity in 67 accessions, representing nine populations that were collected from diverse agro-ecologies in the country, using ISSR markers and hence, to generate a baseline information that assists marker assisted breeding, conservation and germplasm management efforts.

**Results:** In the present study, ten polymorphic ISSR markers were screened and optimized, that generated an average of 7.4 scorable bands per marker and revealed high overall percent polymorphism (95%), Nei's gene diversity ( $h = 0.40$ ) and Shannon index ( $I = 0.62$ ) suggesting ISSR's effectiveness in detecting high levels of genetic diversity. A considerably high overall populations gene diversity (Nei's) ( $h = 0.32$ ) and Shannon index ( $I = 0.47$ ) were observed, revealing high potential of the populations for further breeding and conservation efforts particularly for population from Gurage administrative zone, which showed the highest values. Similarly, estimation of pairwise genetic distance revealed the importance of cross breeding population from Awi administrative zone to the rest populations. Analysis of hierarchical molecular variance (AMOVA) showed higher levels of genetic differentiation within populations (92%), and collection regions (94%) suggesting that either clonal mode of propagation in the crop or farmers selection pressure for important agronomic traits or both maintained the original heterozygosity in the crop. UPGMA phylogenetic analysis did not strictly group the populations based on their geographic region of origin, which could be attributed to the widely practiced tuber exchange and hence continuous human mediated exchange of genetic material and sharing of the same genetic base among the geographic regions.

**Conclusions:** The ISSR markers used in the present study were effective in revealing extent and patterns of genetic diversity in *P. edulis* populations. However, it is important to couple them with agro-morphological traits or codominant molecular markers to get more reliable information for use in breeding and conservation. Several of the potential administrative zones we covered are useful for *P. edulis* diversification and conservation. However, the crop is currently

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highly marginalized and this led to rapid decline in population size and loss of valuable agronomic traits. To address this challenge, there is an urgent need to take counteractive measures.

**Keywords:** Ethiopian potato, Genetic diversity, ISSR, Molecular marker, *Plectranthus edulis*

## Background

*Plectranthus edulis* (Vatke) Agnew is locally known by several vernacular names among which, Ethiopian potato *syno*. Ethiopian dinich, is frequently used by the scientific community. It is an ancient tuber crop and native to Ethiopia. The crop belongs to family Lamiaceae, sub-family Nepetoideae, tribe Ocimeae, and genus *Plectranthus* [1, 2]. It has a wide range of adaptations and used to be widely cultivated in the Central, Southern, Western, Northwestern and South-Western parts of Ethiopia. It is also reported to be found in warmer African countries such as Kenya, the Democratic Republic of Kongo, and Uganda, mainly in a wild form [3, 4]. The crop is one of the four economically important tuber crops of the genus *Plectranthus*, such as *P. esculentus* (Livingstone potato), *P. parviflorus* (Sudan potato) and *P. rotundifolius* (Madagascar potato) [5–7].

In Ethiopia, *P. edulis* is primarily cultivated for food and as a source of income for millions of subsistence farmers, particularly in the country's densely populated highlands and semi-highlands. The crop is also widely used as a folk medicine and a source of nectar for honeybee [8, 9]. However, production of the crop is currently declining to the extent of total extinction from several areas where it used to be widely cultivated. The decline in cultivation could be attributed to the current restricted distribution of the crop, low attention from the local scientific community, lack of awareness among younger farmers about the conservation and cultivation of the crop, research focus of the country that mainly targets cereals and commercial crops, poor shelf life of the crop, poor market opportunities, and introduction of exotic crops such as Irish potato to the area where it is cultivated [10]. Furthermore, the cultivation of the crop is currently restricted to elderly farmers mainly using marginal and degraded plots of land that are thought to be less important for other crops [11].

Genetic diversity, the total genetic variation in a species, could be assessed using a particular method or a combination of methods such as agro-morphological traits, biochemical and/or DNA marker(s) methods. Because of their ability to generate more reliable information, DNA molecular markers have been used for more than 30 years in estimating genetic diversity values [12–14]. ISSR is one of the DNA based marker systems that involves the amplification of DNA segment oriented in opposite direction between two identical microsatellites

repeat regions. It is widely applicable in genetic diversity study of crop plants and is therefore an important tool for characterizing and conserving germplasm, particularly in the breeding and management of endangered, rare and non-market-oriented endemic species [15, 16].

So far, very limited research activities have been conducted on *P. edulis* focusing only on agro-morphological diversity, ontogeny, micro-propagation, phytochemistry and nutritional analysis. As far as we know, very limited work is available using molecular DNA data such as EST-SSR [17] and no report is available on evaluation of its genetic diversity using ISSR markers. This study was therefore conducted in view of assessing the extent of genetic diversity within and among *P. edulis* populations collected from diverse agro-ecological regions in Ethiopia using ISSR markers. The generated information could be used as baseline in the future to support marker assisted breeding, conservation and germplasm management of this crop.

## Methods

### Plant material

Seeds of 67 *P. edulis* accessions, representing nine populations, were collected from the four main growing regions in the country (Table 1; Fig. 1). The seed samples for each accession were planted on separate pots filled with soil in a glasshouse at College of Natural Sciences, Addis Ababa University, Ethiopia. After planting, 5-week old young leave tissues were collected from five plants per accession and dried in a silica-gel filled zip-lockbag.

### Genomic DNA extraction, primer screening and optimization

Genomic DNA extraction and PCR amplification were performed at the Plant Genetics Research Laboratory, College of Natural Sciences, Addis Ababa University, Ethiopia. Following the CTAB (2% Cetyl Trimethyl Ammonium Bromide) protocol [18], approximately 0.5 g of silica gel dried and Restech mixer mill fine ground leaf powder was used for genomic DNA extraction with minor modifications such that the chloroform extraction step was repeated three times to yield high-quality DNA. The quality and quantity of DNA was checked using 1% w/v agarose gel and Thermo Scientific nanodrop spectrophotometer (NanoDrop 2000/2000c, Thermo Fisher Scientific, Wilmington, USA), respectively.

**Table 1 List of *P. edulis* populations and the administrative regions, zones and woredas of collection used in the present study, along with their altitude, latitude and longitude**

Population	Admin. Region	Admin. Zone	Admin. Woreda	Coll. Code	Altitude (m)	Latitude (dd)	Longitude (dd)	
Awi	Amhara	Awi	Banja	PE001	2554	10.937	36.912	
				PE002	2557	10.938	36.914	
				PE003	2643	10.973	36.948	
			Fagta Lekoma	PE004	2555	11.058	36.893	
				PE005	2590	11.044	36.891	
				Ankesha Goagsa	PE006	2380	10.873	36.896
					PE007	2369	10.874	36.893
Met	Benshangul Gumuz	Metekel	Wenbera	PE008	2505	10.635	35.892	
				PE009	2427	10.623	35.346	
				PE010	2517	10.583	35.400	
				PE011	2483	10.614	35.646	
				PE012	2501	10.613	35.745	
CHL	Central Highland, Oromia	Southwest Shewa	Darian	PE013	2599	8.694	37.896	
				PE014	1828	8.412	37.872	
				PE015	1972	8.506	37.965	
			West Shewa	Cheliya	PE016	2924	9.109	37.399
				PE017	2333	8.958	37.538	
		Dandi	PE018	2469	8.973	38.007		
			PE019	2441	8.979	38.016		
			PE020	2446	8.981	38.020		
			PE021	2445	8.978	38.024		
			IAB	Oromia	Ilu Aba Bora	Metu	PE022	1694
PE023	1714	8.285					36.585	
PE024	1696	8.285					36.587	
Alle	PE025	1794					8.134	36.553
	Didessa	PE026					1915	8.485
Bedele	PE027	1879				8.072	36.451	
	PE028	2096				8.135	36.452	
	PE029	1947				8.393	36.124	
	PE030	1901				8.483	36.370	
	Jim					Jimma	Goma	PE031
PE032			1621	7.874	36.599			
Seka Che			PE033	1806	7.605		36.690	
			Dedo	PE034	2181		7.505	36.892
Jimma			PE035	2228	7.502		36.890	
			PE036	1835	7.636		36.763	
Sokoru			PE037	1719	7.655		36.845	
			PE038	1987	7.911		37.435	
	PE039	1927	7.919	37.431				
Ewo		East Wollega	Gida Ayana	PE040	1991	9.887	36.613	
				PE041	2126	9.920	36.594	
				PE042	2087	9.895	36.628	
			Limmu	PE043	2166	9.927	36.473	
				PE044	2149	9.846	36.459	
			Kirammu	PE045	2151	9.846	36.458	
				PE046	2163	9.983	36.870	
PE047	2141	9.990	36.870					

**Table 1 (continued)**

Population	Admin. Region	Admin. Zone	Admin. Woreda	Coll. Code	Altitude (m)	Latitude (dd)	Longitude (dd)
GGo	SNNPs	Gamo Gofa	Chencha	PE048	2678	6.261	37.582
				PE049	2705	6.262	37.579
				PE050	2696	6.246	37.564
			Dita	PE051	2625	6.302	37.496
				PE052	2602	6.301	37.488
				PE053	2623	6.299	37.485
				PE054	2513	6.309	37.487
Gur	Gurage	Endegagn	PE055	2261	7.844	37.837	
			PE056	2445	7.848	37.848	
			PE057	2382	7.857	37.856	
		Enemorna Ener	PE058	2131	8.017	37.847	
			PE059	2155	8.011	37.849	
			Gumer	PE060	2878	7.964	38.063
				PE061	2929	7.961	38.067
Wso	Wolaita Sodo	Damot Gale	PE062	2906	7.958	38.071	
			PE063	2012	6.961	37.842	
		SodoZuriya	PE064	2159	6.917	37.818	
			PE065	2215	6.880	37.792	
			PE066	2188	6.901	37.821	
		Damot Sore	PE067	2099	6.905	37.640	

PE: *Plectranthus edulis*; Col.: collection; Admin.: administrative; SNNPs: South Nations Nationalities and Peoples; Seka che: Seka Chekorsa; dd: decimal degree geographic coordinate system

Twenty-five ISSR primers (Source: Primer kit 900 (UBC 900) obtained from University of British Columbia, Vancouver, Canada) were used during the initial screening for variability and reproducibility. Ten (seven di-nucleotides—five of which were anchored, two tri-nucleotides and one penta-nucleotide) polymorphic primers capable of generating reproducible bands were selected and optimized for the study (Table 2).

#### PCR amplification and gel electrophoresis

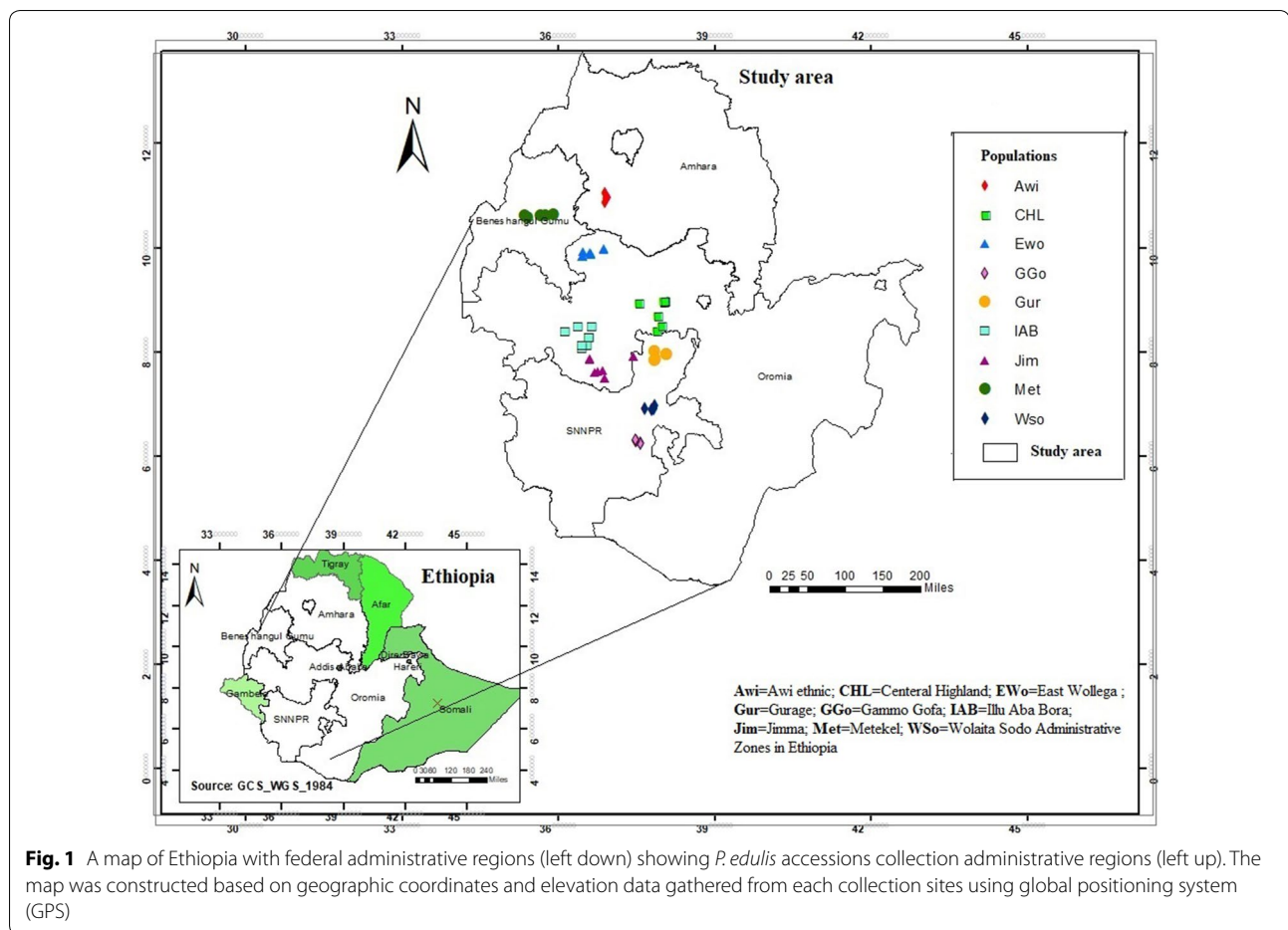
PCR amplification was conducted in a final volume of 25  $\mu$ l reaction mixture per sample containing ddH<sub>2</sub>O (15.2  $\mu$ l), MgCl<sub>2</sub> (25 mM) (3.0  $\mu$ l), Taq buffer (10 $\times$  reaction buffer S) (2.5  $\mu$ l), dNTPs (1.25 mM) (1.0  $\mu$ l), primer (20 pmol  $\mu$ l<sup>-1</sup>) (0.4  $\mu$ l), Fire Pol DNA Polymerase (5 U  $\mu$ l<sup>-1</sup>) (0.4  $\mu$ l), (FIREPOL<sup>®</sup> DNA polymerase, Solis Bio-Dyne, Estonia) and template DNA (10 ng  $\mu$ l<sup>-1</sup>) (2.5  $\mu$ l). The amplification program was set at 94 °C for 4 min pre-heating and initial denaturation, followed by 40 cycles of denaturation at 94 °C for 15 s, primer annealing at specific annealing temperature for each primer (Table 2) for 1 min, extension at 72 °C for 1 min and 30 s each cycle and final extension at 72 °C for 7 min. The PCR amplification products were electrophoresed using 1.67%

w/v agarose gel and size of the fragments was estimated against 100 bp DNA ladder (Thermo Fisher Scientific, Massachusetts, USA) (Fig. 2).

#### Band scoring and data analysis

The resulting bands were considered as unit character and scored as present (1), absent (0) and ambiguous (?). After successful scoring, the data were assembled into a binary data matrix with the samples in a row and the ISSR markers (loci) in column.

The resulting data matrix was analyzed using appropriate software. POPGENE ver. 1.32 [19] was used in particular to determine the percentage of polymorphic loci (PPL), gene diversity (h), and Shannon's information index (I). Arlequin ver. 3.01 [20] was used to determine the analysis of molecular variance (AMOVA) within and among the populations. Using NTSYS-pc version 2.02 [21] and Free Tree 0.9.1.50 [22], Jaccard's similarity coefficient-based unweighted pair group method with arithmetic average (UPGMA) [23] and neighbor-joining (NJ) [24, 25] clusters were drawn to determine the genetic relationship between individual samples and populations considered in this study. To further examine the patterns of variation among individual samples,



**Fig. 1** A map of Ethiopia with federal administrative regions (left down) showing *P. edulis* accessions collection administrative regions (left up). The map was constructed based on geographic coordinates and elevation data gathered from each collection sites using global positioning system (GPS)

a principal coordinate analysis (PCoA) was performed using GenAlEx 6.5 [26].

## Results

### ISSR primers and their banding patterns

The ten screened and optimized ISSR primers produced 74 clear and scorable bands over the entire nine populations (an average of 7.4 per primer). The molecular weight of the fragments ranged between 200 and 2000 bp. UBC-834 produced the highest number of scorable bands (12), whereas UBC-835 and UBC-868 scored the lowest (5 bands each) (Table 3).

### Evaluation of the ISSR primers and their diversity indices

The screened primers revealed that at least 91% of the loci were polymorphic throughout the entire population. Eight of the ten initially tested ISSR primers showed 100% polymorphism across the entire population, while UBC-866 showed the least percentage (71%) of polymorphic loci. The highest gene diversity ( $h=0.48$ ) and Shannon's information index ( $I=0.68$ ) were recorded for UBC-812, followed by UBC-834, 844 and 854. UBC-835 showed the

least gene diversity ( $h=0.39$ ) and UBC-866 showed the lowest Shannon's information index ( $I=0.58$ ). Overall, there were 0.40 and 0.62, respectively, gene diversity and Shannon's information index over the entire population (Table 3).

Among the nine populations studied, the population from Gurage administrative zone revealed the highest polymorphism (PPL=96.08%), gene diversity ( $h=0.44$ ) and Shannon's information index ( $I=0.62$ ), followed by population from Central Highland (PPL=84.31%,  $h=0.36$  and  $I=0.51$ ). Populations from Metekel and Wolaita Sodo administrative zones showed the lowest values for PPL,  $h$  and  $I$  (Table 4).

### Population genetic differentiation and distance

Hierarchical AMOVA in both without prior grouping and grouping the populations according to their collection regions revealed a significantly higher ( $p=0.001$ ) percentage of differentiation (92% and 96%, respectively) due to variance within populations and regions than differentiation among populations and regions (8% and 4%,

**Table 2 List of ISSR primers along with their annealing temperature, respective sequences and amplification efficiency used during optimization**

S/N	Primer	Annealing temperature (°C)	Primer sequences	Amplification efficiency
1	UBC-812	47 °C	(GA)8A	Excellent
2	UBC-817	48 °C	(CA)8A	Poor
3	UBC-818	47 °C	(CA)8G	No band
4	UBC-826	47 °C	(AC)8C	No band
5	UBC-834	47 °C	(CA)8AG	Excellent
6	UBC-835	48 °C	(AG)8YC	Very good
7	UBC-839	49 °C	(TA)8RG	Poor
8	UBC-841	48 °C	(GA)8YC	Excellent
9	UBC-844	48 °C	(AG)8YT	Excellent
10	UBC-848	48 °C	(CA)8RG	No band
11	UBC-851	49 °C	(GT)8YG	Poor
12	UBC-852	48 °C	(AC)8T	Poor
13	UBC-854	49 °C	(TC)8RG	Excellent
14	UBC-857	49 °C	(AC)8AYG	Excellent
15	UBC-860	52 °C	(TG)8RA	Poor
16	UBC-864	48 °C	(CA)8RT	Very poor
17	UBC-865	47 °C	(CCG)6	Poor
18	UBC-873	45 °C	(GACA)4	No band
19	UBC-878	48 °C	(GGAT)4	Poor
20	UBC-879	48 °C	(CTTCA)3	Poor
21	UBC-866	55 °C	(CTC)6	Good
22	UBC-868	55 °C	(GAA)6	Excellent
23	UBC-880	48 °C	(GGAGA)3	Excellent
24	UBC-881	49 °C	(GGTG)3	No band
25	UBC-888	47 °C	BDB(CA)7	Poor

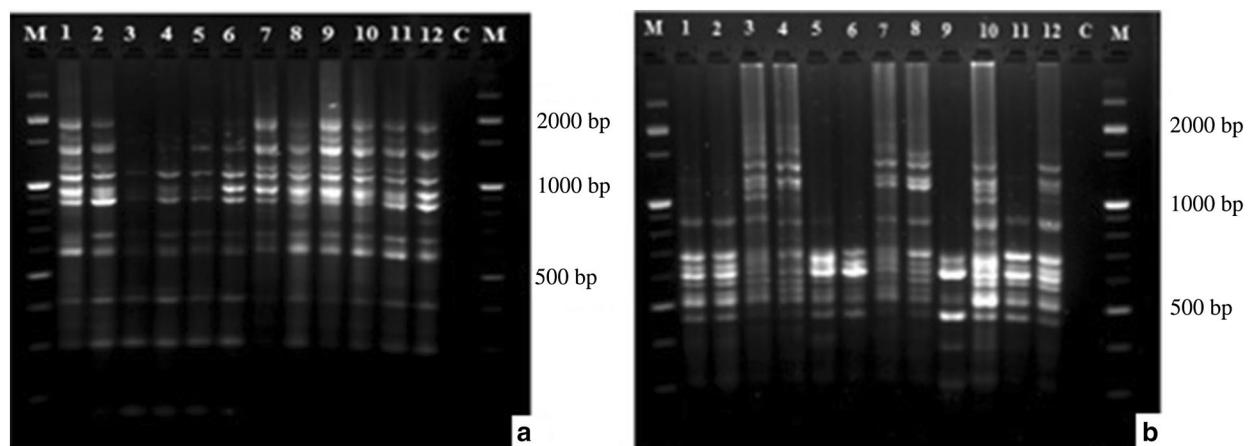
Source: Primer kit 900 (UBC 900); Single-letter abbreviations for mixed base positions: R = (A, G); Y = (C, T)

respectively) (Table 5). The lower percentage of differentiation among the populations and regions was supplemented by low  $F_{st}$  (0.08 and 0.04, respectively) value (Table 5) and a higher overall gene flow ( $N_m=1.54$ ) (Table 4).

The populations pairwise genetic distance (D) ranged from 0.11 to 0.39. In this regard, in the magnitude order, individuals from the Awi administrative zone population were relatively distantly related to the populations of Wolaita Sodo, Metekel, and Illu Aba Bora administrative zones. Similarly, population from Jimma administrative zone was relatively distant from the populations of Wolaita Sodo and Metekel administrative zones. The lowest estimate of genetic distance was observed between individuals of the Central Highland and East Wollega administrative zone populations (Table 6).

#### Population genetic relationships

Analysis of UPGMA based on Jaccard's similarity coefficients grouped the 66 accessions into five main clusters in which several accessions (31) were grouped together under cluster I followed by cluster II (24). Clusters III and IV included three accession each, while cluster V contained five accessions (Fig. 3). Population level grouping, however, formed three main clusters in which populations from Jimma, Awi, Central Highland and East Wollega administrative zones were grouped together (C3) and populations from Gamo Gofa, Metekel, Gurage and Illu Aba Bora administrative zones were grouped together (C1). Population from Wolaita Sodo administrative zone appeared as a monophyletic group (C2) (Fig. 4). In both phylogenetic trees, we observed a weak tendency to group according to their geographical origin except some collections from Northwest Ethiopia (population from Awi administrative area) and Southwest Ethiopia



**Fig. 2** ISSR profiles generated for 12 representative individual samples of *P. edulis* populations using UBC 324 (a) and UBC 812 (b). M = 100 bp DNA ladder; C = negative control; numbers from 1 to 12 represents PE001, PE004, PE008, PE013, PE016, PE022, PE025, PE034, PE042, PE050, PE056, and PE065 accessions, respectively



**Table 3** Number of reproducible scored bands (NRSB), number of polymorphic loci (NPL), percent of polymorphic loci (PPL), Nei's gene diversity (h), and Shannon's information index (I) scored over the entire nine populations studied

Primers	NRSB	NPL	PPL	h ± SD	I ± SD
UBC-812	9	9	100%	0.48 ± 0.02	0.68 ± 0.02
UBC-834	12	12	100%	0.44 ± 0.03	0.63 ± 0.05
UBC-835	5	4	80%	0.39 ± 0.01	0.60 ± 0.02
UBC-841	6	6	100%	0.42 ± 0.05	0.61 ± 0.03
UBC-844	7	7	100%	0.44 ± 0.04	0.63 ± 0.04
UBC-854	7	7	100%	0.44 ± 0.04	0.63 ± 0.04
UBC-857	9	9	100%	0.41 ± 0.09	0.60 ± 0.10
UBC-866	7	5	71%	0.40 ± 0.04	0.58 ± 0.04
UBC-868	5	5	100%	0.41 ± 0.03	0.62 ± 0.03
UBC-880	7	7	100%	0.40 ± 0.10	0.59 ± 0.12
Mean	7.4	7.1	95%	0.40 ± 0.05	0.62 ± 0.05

(populations from Gurage and Jimma administrative areas) that formed their own mini-clusters (Fig. 3).

Analysis of principal components (PCoA) based on Nei's [27] genetic distance revealed 29.68% of the total variation for the first three (12.77%, 9.52%, 7.39%, respectively) principal axes. The 2D coordinates showed similar patterns of clustering with that of populations' UPGMA, except for a considerable number of accessions from the administrative zones of Awi, Jimma and Illu Aba Borathat tended to form their own distinct cluster (Fig. 5).

**Discussion**

**Population genetic diversity and implications for selection and conservation**

ISSR marker system is one of the widely used molecular markers for assessing the extent and patterns of genetic diversity and for deducing phylogenetic relationships in a variety of crop species. Similarly, it has been used in a

**Table 4** Number of individuals per population (NIPP), Number of polymorphic loci (NPL), percent polymorphism (PP), Nei's gene diversity (h), and Shannon's information index (I) for the nine populations recorded over the entire ten loci studied

Pop	NIPP	NPL	PPL	h ± SD	I ± SD	G <sub>st</sub> <sup>a</sup>	N <sub>m</sub> <sup>a</sup>
Awi	7	61	82.35	0.33 ± 0.19	0.48 ± 0.26		
CHL	9	62	83.78	0.36 ± 0.18	0.51 ± 0.25		
EWo	8	59	79.73	0.33 ± 0.19	0.48 ± 0.26		
Gur	8	71	95.94	0.44 ± 0.11	0.62 ± 0.15		
GGo	7	51	68.91	0.31 ± 0.22	0.44 ± 0.30		
IAB	9	52	70.27	0.30 ± 0.21	0.43 ± 0.29		
Jim	9	59	79.73	0.34 ± 0.19	0.49 ± 0.26		
Met	5	45	60.81	0.26 ± 0.22	0.38 ± 0.32		
WSo	5	44	59.5	0.26 ± 0.23	0.37 ± 0.32		
Mean	7.4	56	75.66	0.32 ± 0.19	0.47 ± 0.27	0.24	1.54

See Table 1 for the description

Pop populations

<sup>a</sup> N<sub>m</sub> = estimate of gene flow from G<sub>st</sub>, where N<sub>m</sub> = 0.5(1 - G<sub>st</sub>)/G<sub>st</sub>

**Table 5** Analysis of hierarchical molecular variance (AMOVA) without prior grouping and with grouping the populations into their collection regions

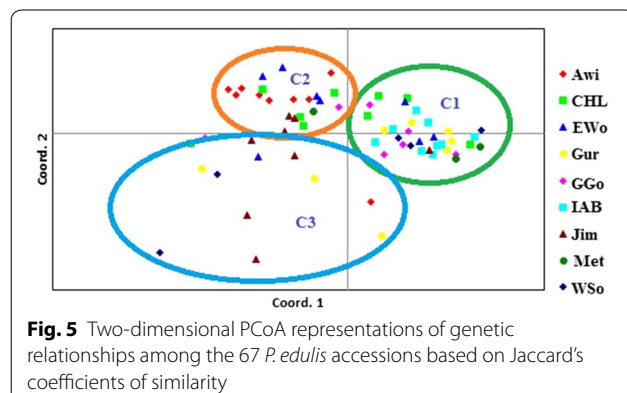
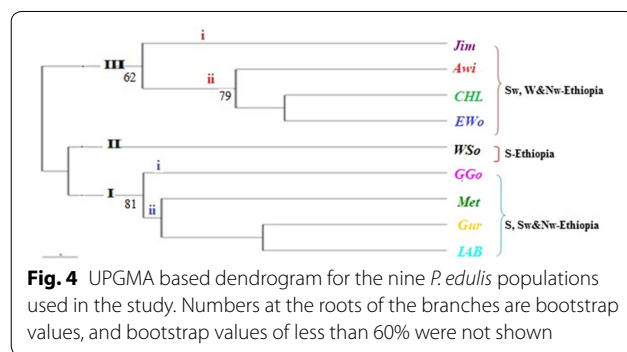
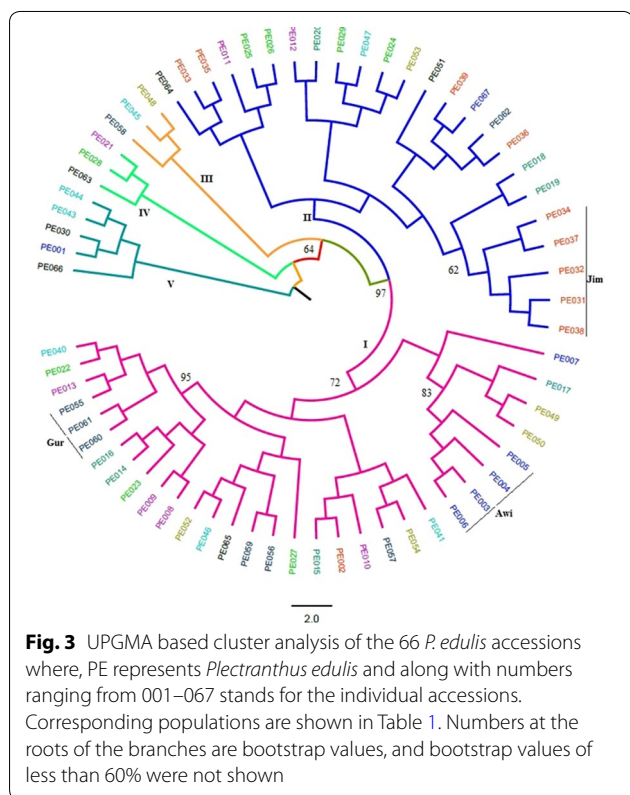
	Sources of variation	df	Sum of squares	Variance components	Percentage of variation (%)	Fixation index	p value
Without grouping	Among populations	8	140.6	0.93Va	8	0.08	< 0.001
	Within population	58	616.4	10.81Vb	92		
	Total	66	757.00	11.74	100		
With grouping	Among regions	3	53.73	0.48Va	4	0.04	< 0.001
	Within regions	62	703.27	11.34Vb	96		
	Total	65	757.00	11.82	100		

df degrees of freedom

**Table 6** Nei's original measure of pairwise genetic distance for the nine *P. edulis* populations considered in the study (the italic values stand for the higher and lower pairwise genetic distances)

Pop	<i>Awi</i>	<i>CHL</i>	<i>EWo</i>	<i>Gur</i>	<i>GGo</i>	<i>IAB</i>	<i>Jim</i>	<i>Met</i>	<i>WSo</i>
<i>Awi</i>	****								
<i>CHL</i>	0.13	****							
<i>EWo</i>	0.11	0.09	****						
<i>Gur</i>	0.21	0.12	0.16	****					
<i>GGo</i>	0.20	0.17	0.19	0.15	****				
<i>IAB</i>	0.34	0.18	0.22	0.11	0.20	****			
<i>Jim</i>	0.15	0.18	0.19	0.15	0.18	0.29	****		
<i>Met</i>	0.35	0.19	0.24	0.16	0.17	0.16	0.30	****	
<i>WSo</i>	0.39	0.20	0.22	0.17	0.24	0.17	0.32	0.27	****

Pop population  
 \*\*\*\* Not applicable



wide variety of plants worldwide to study genetic variation [12, 28].

The ten reproducible ISSR markers selected and used in the present study showed an overall high level of polymorphism (95%) which implies their great usefulness in revealing and evaluating the level of genetic diversity among and within *P. edulis* populations. There have been similar reports in other endemic root and tuber crops such as sweet potato [28], yam [29] and anchote

[30] populations where ISSR markers showed high but varying levels of polymorphism. ISSR technique was also reported to be effective and successful in assessing genetic variability in other endemic and exotic species including lentil (*Lens culinaris medikus*) [31] and coffee (*Coffea arabica* L.) [32] from Ethiopia, Chinese grown pecan (American pecan) [33], and sesame [34].

The extent of genetic diversity in plant species is the result of one or more factors such as reproductive



biology, life (evolutionary) history, geographic range of distribution, and various environmental factors that, in one way or another, affect mutation rates [35–37]. In this regard, the values in overall percent polymorphism, gene diversity and Shannon's information index observed in *P. edulis* populations could be attributed to the crop's clonal propagation nature in which only few parts of the population can preserve the original genetic diversity in the basal population. Furthermore, the efforts of local farmers, especially the elders, to maintain the original genetic diversity by preserving tubers over generations could be another reason for the observed higher genetic diversity indices, although the population size is rapidly declining from vast areas where it used to be widely cultivated. Twenty EST-SSR DNA markers detected a larger number of alleles and revealed a similar increasing trend in genetic diversity in twelve *P. edulis* populations that were collected from diverse agro-ecologies in the country [17].

High level of genetic variability is desirable because it increases fitness and thus reduces the likelihood of local extinction [38]. In this regard, *P. edulis* populations from Gurage, Central Highland, Awi, East Wollega and Jimma administrative zones, in order of magnitude, are important sources for improving the germplasm and taking immediate conservation actions.

On the other hand, the low values of percent polymorphism, gene diversity and Shannon's information index observed in populations from Metekel and Wolaita Sodo administrative zones could be attributed to the smaller sample size used from these specific areas. In addition, the recent introduction and domestication of the crop to some areas, especially, the Metekel zone, which is a bit pocketed and relatively recently dominated by the Oromo and Awi ethnic groups, could be also the probable reason. Compared to other ethnic groups in the area, these ethnic groups are known to be more familiar with *P. edulis* domestication and cultivation. In agreement with this, Rampersad et al. [39] suggested that there are higher levels of maintained gene diversity in larger and older populations compared to a newly colonized habitat. This is the result of an older population having sufficient timeframe to allow mutational events to introduce new genetic variants and to decrease the effects of genetic drift thus increasing the frequency of the alleles.

#### Population genetic differentiation and distance

*Plectranthus edulis* populations revealed a lower extent of genetic differentiation among the populations and the regions that could result from historical or contemporary exchange of germplasms, especially tubers for immediate planting, between or among zones and regions included in this study. The higher values of the overall gene flow ( $N_m = 1.54$ ) and lower overall  $G_{st}$  (equivalent to  $F_{st}$ ) we

observed could also support this premise as suggested by Wright [40] since the higher extent of gene flow ( $N_m > 1$ ), and hence migration, is a powerful force for decreasing differentiation among populations [41].

On the other hand, the significantly higher genetic differentiation within populations and regions could be attributed to the clonal propagation nature of the crop, an important aspect in maintaining heterozygosity in the basal population over generations regardless of the current population size fluctuation. This can be explained by the fact that the crop is historically sexually reproduced through seeds, which is still rarely practiced at research sites, and this has allowed the crop to accumulate heterozygosity in the basal population to some extent. Tadele et al. [32] and Nascimento et al. [42] reported higher within population variation in clonally propagating endemic tuber crops such as anchote (*Coccinia abyssinica*) and yam, respectively. Similarly, Wodajo [43], and Seid et al. [44] reported higher levels of within population variation in safflower and *Lepidium sativum*, respectively.

The wide range of pairwise genetic distance that we detected (the highest being more than 4× of the lowest) generally indicates the high genetic variability in *P. edulis* populations that could be a valuable source for selection breeding. For this purpose, individuals from the Awi administrative zone versus Wolaita Sodo, Metekel, Illu Aba Bora and Gurage administrative zones, showing considerable pairwise genetic distance, could be used as parental sources. On the other hand, Mantel test revealed that the genetic distance did not correlate with geographic distance (data not shown) which again confirms intensive seed tuber exchange between or among farmers or the recent divergence of the crop from a common genetic base. Moulin et al. [45] reported lack of distance-related genetic variability among sweet potato landraces because of widespread practice of exchanging accessions between neighboring farmers and relatives.

#### Population genetic relationship

The genetic relationships between and among *P. edulis* populations showed poor correlation between geographic origin and the patterns of clustering. Samples or populations from distant zones or regions have been found lumped together the same cluster and those from the same or nearby geographic areas have been placed under a different cluster and such grouping weakly supports the concept of "isolation by distance" [40]. Generally speaking, the clustering patterns once again denote the widely practiced tuber exchange and consequently, continuous gene flow and extensive sharing of genetic material among regions that led to reduced differentiation among the populations. However, results from other

molecular marker systems such as short tandem repeats (STRs) is highly important in order to discriminate sufficiently and identify unique accessions before implementing the information for breeding and conservation.

## Conclusions

From the present study, we conclude that ISSR marker system is useful in estimating the extent of genetic diversity and generating valuable information for use in further breeding and conservation measures in indigenous crops. However, in order to generate a more reliable information, it is important to combine it with other marker systems such as agro-morphological traits-based genetic diversity assessment or co-dominant molecular marker systems, such as SNPs, which have a higher potential for estimating population genetic structure.

On the basis of genetic diversity indices such as gene diversity, Shannon's information index, and percent of polymorphic loci, Gurage, Central Highland, Awi, East Wollega and Jimma administrative zones are relatively better areas for *P. edulis* diversification and conservation. The higher gene flow among the different regions, coupled with the current decline in population size, may result in loss of valuable agronomic traits unless counteracting action is taken.

Representative samples from all potential growing areas need to be collected exhaustively to provide a good estimate of the crop's existing genetic diversity for use in improving it and reversing its current rapid genetic erosion.

## Abbreviations

AMOVA: analysis of molecular variance; CTAB: cetyl trimethyl ammonium bromide; ISSR: inter-simple sequence repeats; SNNPs: Southern Nations, Nationalities, and Peoples' Region; PCoA: principal coordinates analysis; UPGMA: unweighted pair group with arithmetic mean.

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## Authors' contributions

MG carried out DNA extraction and the laboratory work. MG, FG carried out sample collection, planting, leaf sample collection and data analysis. MG, TH, KT and FG participated in experimental design, data interpretation and drafting the manuscript. All authors revised the draft the final 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.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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