Genetic diversity and population structure of Ethiopian faba bean (*Vicia faba* L.) germplasm revealed by ISSR markers

Bullo Neda^{a*}, Tileye Feyissa^{b,c}, Kifle Dagne^c, Ermias Assefa^d

^aDepartment of Plant Science, College of Agriculture and Natural Resource, Salale University, P.O. Box 245, Fitcha, Ethiopia

^bInstitute of Biotechnology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

^cDepartment of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia

^dEthiopian Biotechnology Institute, Addis Ababa, Ethiopia

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Abstract. Molecular characterization of faba bean accessions is important for future collection, conservation, and crop improvement programs. However, molecular genetic diversity of the Ethiopian faba bean accessions has not been comprehensively characterized. The present study was conducted to determine the genetic diversity and population structure of 90 faba bean landraces and six released varieties using nine inter-simple sequence repeat (ISSR) markers. The polymorphic information content (PIC), marker index (MI), resolving power (RP) and effective multiplex ratio (EMR) showed average values of 0.32, 5.87, 7.14, and 18.34 per primer, indicating high polymorphism values. The marker revealed average gene diversity of 0.26, ranging from 0.15 to 0.36. The degree of polymorphism among the landrace accessions ranged from 44.91% for the Sidama population to 72.46% for the East Hararghe population, with a mean of 57.27%. Analysis of molecular variance (AMOVA) indicated 99 % variation within and 1% variation among population, respectively. The present study revealed little to moderate population differentiation accompanied by high gene flow. Both distance-based and model-based cluster analysis distinguished seven distinct groups illustrating the conformity of results obtained from the study. Accessions from different administrative zones (AZs) of the country were distributed over most of the clusters; exhibiting high genetic diversity in collection sites. Therefore, further collection, conservation, and utilization programs would be recommended from AZs with high genetic diversity.

Keywords: Analysis of molecular variance, genetic differentiation, gene flow, Neighbor-joining (NJ), Principal component analysis (PCoA)

INTRODUCTION

The most important cool season food legume in the world are chickpeas, garden pea, lentil, and faba bean (Kaur *et al.*, 2014). The center of origin and primary diversity of faba bean is widely believed somewhere in the Middle East (Lawes *et al.*, 1983). In addition, Harlan (1969) discovered that Ethiopia and Afghanistan are the secondary centers of diversity. Recently, an archaeological

study strongly suggests that Neolithic people in Israel first domesticated faba bean 10,000 years ago; and they were eating as a staple food before grain began to be cultivated in the area (Caracuta *et al.*, 2015). The faba bean introduction to Ethiopia was supposed to be from the Middle East via Egypt around 5,000 BC soon after its domestication (Duc *et al.*, 2010). The world-

*Author for correspondence: Bullo Neda Tulu, Department of Plant Science, College of Agriculture and Natural Resource, Salale University, P.O. Box 245, Fitcha, Ethiopia. Email – tulu0913479057@gmail.com

leading producing countries for faba beans are China, Ethiopia, Egypt, and the United Kingdom (FAOSTAT, 2014). Faba bean is grown in mid and highland agro-ecologies of Ethiopia, ranging from 1,800 to 3,000 m.a.s.l.; where the mean rainfall ranges from 650 to 1,500 mm. It serves as a multi-purpose crop leading the pulse category in areas and production (CSA, 2018). Faba bean provides food for humans, maintaining soil fertility through atmospheric nitrogen fixation; it is also used as fodder; a break crop to reduce cereal diseases, insect pests, and weeds (Chintalapati, 2001). The beautiful flowers of faba bean also attract pollinators' insects (Marzinzig *et al.*, 2018).

Faba bean area coverage and production volumes are 443,966 ha and 848,655 tons, respectively of pulses grown in Ethiopia (CSA, 2014). The average yield of faba bean in the world is 5.00 t/ha (CSA, 2014), however, the average national yield of faba bean in Ethiopia is 2.10 t/ha (CSA, 2018). Bekele *et al.* (2006) reported the low yield of faba bean in Ethiopia is due to biological limitation of the crop; particularly the susceptibility to biotic (diseases, insects, and weeds) and abiotic stresses (moisture deficit, high soil acidity, waterlogging, and frost), and also the inherently low yielding potential of indigenous landrace accessions.

One way of mitigating these constraints is by developing sound crop improvement programs that increase the productivity of the crop through the development of varieties resilient to biotic and abiotic constraints. As a result, to increase production and productivity of faba bean; identifying the genetic diversity available in the landrace materials is the pre-requisite for a successful improvement of the crop. To achieve this, studying the molecular characterization of the crop is imperative. However, the molecular genetic diversity of Ethiopian faba bean landrace accessions has not been comprehensively characterized. Tores and Avilla (2011) also reported that more than 80% of faba bean cultivation is in developing countries where the applications of novel molecular breeding approaches are limited. Hence, it is crucial to further investigate the potential of molecular genetic diversity of the landraces accessions of faba beans in Ethiopia as a source for further breeding work. According to Awan *et al.* (2014),

carrying out genetic diversity assessment on landrace accessions is essential for broadening the genetic base of cultivars, identifying accessions with higher potential for yield, disease resistance, and nutritional qualities to sustain improvement. Inter-simple sequence markers (ISSR) has advantages over other markers that are low-cost, easy to use, and its methodology is easy as compared to other dominant markers; it is also an ideal genetic marker for organisms whose genetic information is lacking, though there are no complete markers in every aspect. Mishra *et al.* (2003) stated that ISSRs markers are evenly distributed throughout the genome of the organisms, for PCR requires small quantities of template DNA, and it does not need sequence data as that of other markers for primer construction. Therefore, the study aims to determine the molecular genetic diversity and population structure of Ethiopian faba bean landrace accessions collected from different administrative zones (AZs) of Ethiopia using ISSR markers. Furthermore, PIC, MI, RP, and EMR were analyzed to determine the potential of ISSR markers to detect polymorphism of faba bean landrace accessions in Ethiopia.

MATERIALS AND METHODS

Plant materials

Seeds of 90 faba bean landrace accessions collected from 14 AZs of Ethiopia were obtained from the Ethiopian Biodiversity Institute (EBI). Furthermore, six released varieties obtained from Holeta Agricultural Research Center (HARC) and Debrabirhan Agricultural Research Center (DbARC) were also included for comparison purposes (Table 1). The potential faba bean growing AZs of Ethiopia were selected and accessions from each AZ were appropriately represented according to their proportions. Accessions from each AZ were collected from altitudes ranging from 1562-3130 m.a.s.l. and each AZ is also considered as a pre-defined population. Fifteen seeds per accession were grown in a greenhouse at the College of Natural Science, Addis Ababa University (AAU). The AZs from which collections were done for the landrace accessions are shown in Figure 1.

Table 1. List of accessions, collection regions, and sites for the 90 faba bean (*Vicia faba* L.) landrace accessions used in the study including 6 released varieties.

No.	Region	Administrative zone	Accession	No.	Region	Administrative zone	Accession
1	Amhara	North Shewa A	212580	49	Oromia	East Hararghe	29535
2	Amhara	North Shewa A	212572	50	Oromia	East Hararghe	29533
3	Amhara	North Shewa A	212567	51	Oromia	East Hararghe	29536
4	Amhara	North Shewa A	229299	52	Oromia	West Shewa	25270
5	Amhara	North Shewa A	212578	53	Oromia	West Shewa	25264
6	Amhara	North Shewa A	208114	54	Oromia	West Shewa	25260
7	Amhara	North Shewa A	208085	55	Oromia	West Shewa	25259
8	Amhara	North Shewa A	229310	56	Oromia	West Shewa	25274
9	Amhara	North Shewa A	212565	57	SNNPR	Sidama	28109
10	Amhara	East Gojam	25017	58	SNNPR	Sidama	28110
11	Amhara	East Gojam	27279	59	SNNPR	Sidama	28112
12	Amhara	East Gojam	229871	60	SNNPR	Sidama	28113
13	Amhara	East Gojam	26428	61	SNNPR	Gedeo	28104
14	Amhara	East Gojam	25006	62	SNNPR	Gedeo	26861
15	Amhara	East Gojam	229869	63	SNNPR	Gedeo	26883
16	Amhara	East Gojam	26400	64	SNNPR	Gedeo	26859
17	Oromia	West Arsi	30011	65	SNNPR	Gedeo	26882
18	Oromia	West Arsi	30008	66	SNNPR	Gedeo	26862
19	Oromia	West Arsi	30014	67	SNNPR	Gedeo	26885
20	Oromia	West Arsi	30016	68	SNNPR	Gedeo	26887
21	Oromia	West Arsi	30015	69	SNNPR	Gedeo	26886
22	Oromia	West Arsi	30017	70	SNNPR	Gedeo	28103
23	Oromia	Arsi	25009	71	SNNPR	Gedeo	26860
24	Oromia	Arsi	27052	72	SNNPR	Segen	26855
25	Oromia	Arsi	231293	73	SNNPR	Segen	26888
26	Oromia	Arsi	219089	74	SNNPR	Segen	26854
27	Oromia	Arsi	219355	75	SNNPR	Segen	26858
28	Oromia	Bale	30023	76	SNNPR	Segen	26889
29	Oromia	Bale	28777	77	SNNPR	Segen	26853
30	Oromia	Bale	30022	78	SNNPR	Kembata	25310
31	Oromia	Bale	28774	79	SNNPR	Kembata	25303
32	Oromia	Bale	28771	80	SNNPR	Kembata	25299
33	Oromia	Bale	28776	81	SNNPR	Kembata	25298
34	Oromia	Bale	28772	82	SNNPR	Kembata	25290
35	Oromia	Bale	30021	83	SNNPR	Kembata	25292
36	Oromia	Bale	28770	84	SNNPR	Bench Maji	27931
37	Oromia	Bale	28773	85	SNNPR	Bench Maji	235709
38	Oromia	North Shewa O	212566	86	SNNPR	Bench Maji	27929
39	Oromia	North Shewa O	25022	87	Tigray	Central Tigray	220079
40	Oromia	North Shewa O	25010	88	Tigray	Central Tigray	220076
41	Oromia	North Shewa O	25003	89	Tigray	Central Tigray	16599
42	Oromia	North Shewa O	25018	90	Tigray	Central Tigray	220086
43	Oromia	North Shewa O	229303	91	Released	Released	Bulga-70
44	Oromia	East Hararghe	29537	92	Released	Released	Lalo
45	Oromia	East Hararghe	29531	93	Released	Released	Dagim
46	Oromia	East Hararghe	29526	94	Released	Released	Walki
47	Oromia	East Hararghe	29527	95	Released	Released	Moti
48	Oromia	East Hararghe	29532	96	Released	Released	Hachalu

North Shewa A = North Shewa Amhara, North Shewa O = North Shewa Oromia, SNNPR = South Nations Nationalities and Peoples Region

Figure 1. Map of Ethiopia showing the collection AZs of faba bean landrace accessions.

DNA extraction

Young leaves of ten plants per accession from three-week-old seedlings were randomly selected and dried in silica gel. A method developed by Borsch *et al.* (2003) was used for total genomic DNA extraction using 50 mg silica gel dried leaf tissue samples of ten plants of each accession. To test optimum quality and quantity of genomic DNA, a modified CTAB method employing triple extraction was executed using 1% ethidium bromide-stained agarose gel electrophoresis under UV light and NanoDrop spectrophotometer. Based on the quality and quantity of genomic DNA, among the triple

extractions, the first extraction was selected for further PCR amplification. So, for PCR amplification, the first extraction of genomic DNA was diluted to 80 ng/μl with sterile distilled water.

PCR and electrophoresis

The experimental results on faba bean (Abdul-Razzak *et al.*, 2012) and other related crops like chickpea (Bhagyawant and Srivastava, 2008) and lentil (Meenakshi *et al.*, 2013) are the basis of the selection of 17 ISSR markers for further screening. Using five representative accessions from populations with high genetic diversity

based on morphological data, nine ISSR primers that revealed amplification of unambiguously visible and reproducible bands were selected for further analyses (Table 2). The total PCR reaction components volume per sample was 25 μ l; consisting of 17.20 μ l sterile distilled water, 2.50 μ l MgCl₂ (25 mM), 2.50 μ l 10x PCR buffer, 1.00 μ l dNTP (100 mM equimolar solution of each dATP, dCTP, dGTP and dTTP), 0.40 μ l primer (20 pmol/ μ l), 0.40 μ l Taq polymerase (5 U/ μ l) and 1 μ l template DNA (80 ng/ μ l). PCR amplification was carried out using BiometraT3 thermocycler. The initial denaturation was set at 94°C for 4 min, denaturation for 40 cycles at 94°C for 15 s, annealing temperature for 1 min ranging from 42°C to 48°C, extension at 72°C for 1:30

min, and a final extension at 72°C for 7 min. Until the time of gel electrophoresis, the PCR products were stored at 4°C (Joseph & David, 2001). Ten μ l amplification products of each sample were mixed with 2 μ l of six times concentrated loading dye and loaded on the gel. One hundred base-pair DNA marker was loaded on the first lane of the gel to estimate the sizes of the bands. Electrophoresis was conducted at 100 V for 2:00 h in a 1x TBE buffer. Ethidium bromide (10 mg/ml) mixed with 450 ml distilled water was used to be stained for 30 min and destained with 450 ml of distilled water for 30 min. The bands were visualized by Camera- fitted Gel Doc. System.

Table 2. Lists of primers, their sequence, repeat motif, annealing temperature and nucleotide.

No.	Primer	Sequence from 5'- 3'	Repeat Motif	Annealing temp (°C)	Nucleotide types
1	UBC812	5'GAGAGAGAGAGAGAGAG3'	(GA)8G	45	Di-nucleotide
2	UBC815	5'CTCTCTCTCTCTCTG3'	(CT)8G	42	Di-nucleotide
3	UBC824	5'TCTCTCTCTCTCTCG3'	(TC)8G	48	Di-nucleotide
4	UBC835	5'AGAGAGAGAGAGAGAGYC3'	(AG)8YC	48	Di-nucleotide
5	UBC840	5'GAGAGAGAGAGAGAGAYT3'	(GA)8YT	42	Di-nucleotide
6	UBC841	5'GAGAGAGAGAGAGAGAYC3'	(GA)8YC	43	Di-nucleotide
7	UBC873	5'GACAGACAGACAGACA3'	(GACA)4	45	Tetra-nucleotide
8	UBC880	5'GGAGAGGAGAGGAGA3'	(GGAGA)3	45	Penta-nucleotide
9	UBC881	5'GGGTGGGGTGGGGTG3'	(GGGTG)3	47	Penta-nucleotide

Data analyses

Using the binary matrix representing the column as the ISSR bands and rows as the accessions, the bands were scored as present (1), absent (0), or ambiguous (?). The marker informativeness was measured through the counting of bands to determine the potential of the markers for the genetic diversity study of faba bean landrace accessions. Hence, the number of scored bands (NSB), number of polymorphic bands (NPB), number of private bands (NPvB), number of rare bands (NRB), number of common bands (NCB), and number of abundant bands (NAB) were recorded to detect marker informativeness prior to diversity analysis. Besides, PIC, MI, RP, and EMR parameters of the ISSR markers were calculated using GenAIEx 6.5 software (Peakall and Smouse, 2006) to detect the suitability of the markers for the genetic diversity study of faba bean landrace accessions. The population indices such as polymorphic loci (P), observed alleles

(Na), effective alleles (Ne), genetic diversity (GD), Shannon information index (I), expected heterozygosity (h), unexpected heterozygosity (uh), genetic distance (Gd), genetic identity (GI), genetic differentiation (PhiPT), principal component analysis (PCoA), and analysis of molecular variance (AMOVA) were also measured by using GenAIEx 6.5 software (Peakall and Smouse, 2006). The Neighbor-joining dendrogram method of Saitou and Nei (1987) has constructed clustering using the R package POPPR software (Kamvar *et al.*, 2014). Discriminant analysis of principal components (DAPC) was also measured by the Adegenet R package software (Jombart *et al.*, 2010). STRUCTURE 2.3.4 software was used to calculate population structure (Pritchard *et al.*, 2000). According to Evanno *et al.* (2005), to assess the exact number of clusters through STRUCTURE HARVESTER (Earl, 2012); a burn-in period of 50,000 with 100,000 MARKOV

Chain Monte Carlo (MCMC) iterations was run 5 times for each K value, in our case K = 1 to 10.

RESULTS

ISSR primers banding patterns and diversity

The genetic diversity of 90 Ethiopian faba bean landrace accessions collected from 14 AZs including six released varieties were evaluated using nine ISSR markers. Prior to diversity assessment, the suitability of the ISSR markers was evaluated through their efficiency, reliability, and discriminatory power to reveal markers' potential.

Nine ISSR markers revealed 167 total bands, with band size ranging from 133 bp to 3000 bp with all 100% polymorphism. The largest variation in fragment size was detected for primer UBC815 (333 bp - 3000 bp) and the lowest for UBC873 (133 bp - 800 bp). The ISSR markers showed a mean of 18.60 band types per primer, ranging from 14 to 25. The maximum and the

minimum number of bands were amplified by the primer UBC815 (25) and UBC824 (14), respectively. The mean of the NPvB is 1.40, ranging from 0 to 4. Rare bands (NRB) are polymorphic bands that were reported in less than 5% of the accessions under study. The mean of the NRB is 3.20 per primer, with its total of 29 across accession. Both the largest NPvB and the largest NRB of 4 and 7, respectively were generated by the primer UBC873. Common bands (NCB) are polymorphic bands that were reported in 5-50% of the accessions. The mean of the NCB is 13.90 per primer, with its total of 125 across accession. Both primer UBC812 and UBC841 amplified the maximum NCB with the value of 21. On the other hand, primer UBC880 and UBC881 amplified the minimum NCB with a value of 9. Abundant bands (NAB) are polymorphic bands that were reported in more than 50% of the accessions. The mean of NAB is 1.40 per primer, with its total of 13 across accession. The primer UBC881 showed the highest NAB of 6 (Table 3).

Table 3. Number of scored bands, polymorphic bands, percentage of polymorphic bands, private bands, rare bands, common bands, abundant bands and fragment size amplified by nine ISSR primers in faba bean accessions.

Primer	Repeat Motif	T (°C)	NSB	NPB	% P	NPvB	NRB	NCB	NAB	FS
UBC812	(GA)8A	45	23	23	100	2	2	21	0	200-2000
UBC815	(CT)8G	42	25	25	100	2	3	20	2	333-3000
UBC824	(TC)8G	48	14	14	100	0	0	13	1	400-1500
UBC835	(AG)8YC	48	17	17	100	2	6	10	1	150-1000
UBC840	(GA)8YT	42	15	15	100	2	1	12	2	300-1500
UBC841	(GA)8YC	43	24	24	100	0	2	21	1	350-1500
UBC873	(GACA)4	45	17	17	100	4	7	10	0	133-800
UBC880	(GGAGA)3	45	15	15	100	2	6	9	0	200-1500
UBC881	(GGGTG)3	47	17	17	100	0	2	9	6	350-1400
Total			167	167	100	13	29	125	13	-
Average					100	1.40	3.20	13.90	1.40	-

T (°C) = annealing temperature, NSB = Number of the scored bands; NPB = Number of polymorphic band; %P = percentage of polymorphism; NPvB = number of private bands; NRB = number of rare bands; NCB = number of common bands; NAB = number of abundant bands, FS = fragment size amplified.

Each accession genetic profile was evaluated through the ISSR marker performances of PIC, MI, RP, and EMR parameters. The highest and the lowest PIC values of 0.44 and 0.19 were exhibited by the primer UBC881 and UBC873, respectively, with the mean value of PIC 0.32

(Table 4). MI indicated the general usefulness of the markers. The highest and the lowest MI values of 7.46 and 3.18 were exhibited by the primer UBC815 and UBC873, respectively, with the mean value of MI 5.87. RP is the discriminatory potential of the markers. Similar to PIC, the

highest (12.13) and the lowest (2.96) RP values were exhibited by the primer UBC881 and UBC873, respectively with the mean of 7.14 (Table 4).

The highest and the lowest EMR values of 25.00 and 14.00 were exhibited by the primer UBC815 and UBC824, respectively, with a mean of 18.34 per primer (Table 4). The number of Na was 2. The mean value of the number of Ne is 1.40, ranging from 1.99 to 1.60. The highest I of

0.53 and GD of 0.36 were observed from primer UBC881 followed by UBC824 with I of 0.50 and GD of 0.33. The least I of 0.25 and GD of 0.15 were obtained from primer UBC873 followed by UBC835 with I of 0.32 and GD of 0.20. Allelic evenness is ranging from 0.52 to 0.79, with a mean of 0.66. The data obtained from each primer allowed the differentiation of a mean of 72.67 accessions per primer, ranging from 62 to 83 accessions identified (Table 4).

Table 4. Marker parameters and statistics calculated across nine ISSR primers in 96 faba bean accessions.

Locus	EMR	PIC	MI	RP	Na	Ne	I	GD	Evenness	NG
UBC812	21.04	0.41	6.35	6.73	2	1.36	0.39	0.24	0.64	66
UBC815	25.00	0.36	8.96	11.50	2	1.47	0.45	0.30	0.71	78
UBC824	14.00	0.42	5.85	6.79	2	1.54	0.50	0.33	0.75	66
UBC835	17.00	0.25	4.22	4.65	2	1.30	0.32	0.20	0.59	67
UBC840	15.00	0.33	4.97	6.38	2	1.44	0.43	0.28	0.69	79
UBC841	24.00	0.32	7.60	8.85	2	1.38	0.41	0.26	0.66	74
UBC873	17.00	0.19	3.18	2.96	2	1.20	0.25	0.15	0.52	62
UBC880	15.00	0.28	4.22	4.27	2	1.33	0.34	0.21	0.61	79
UBC881	17.00	0.44	7.46	12.13	2	1.60	0.53	0.36	0.79	83
Total					-	-	-	-	-	654
Mean	18.34	0.32	5.87	7.14	2	1.40	0.40	0.26	0.66	72.67

EMR = effective multiplex ratio; PIC = Polymorphism information content; MI = marker index; RP = resolving power, Na = Number of observed alleles; Ne = Number of effective alleles; I = Shannon's index; GD = Nei's gene diversity and NG = number of genotypes identified per primer.

Table 5. Genetic parameter estimates based on ISSR Marker among faba bean subpopulations.

Based on Geographical Origin							
Pop	N	% P	Na	Ne	I	h	uh
North Shewa A	9.00	68.86	1.38	1.31	0.31	0.19	0.21
East Gojam	7.00	57.49	1.15	1.21	0.23	0.14	0.15
West Arsi	6.00	57.49	1.15	1.23	0.24	0.15	0.17
Arsi	5.00	50.90	1.04	1.25	0.24	0.16	0.17
Bale	10.00	55.69	1.11	1.15	0.19	0.11	0.11
North Shewa O	6.00	54.49	1.09	1.23	0.24	0.15	0.17
East Hararghe	8.00	72.46	1.45	1.29	0.30	0.19	0.20
West Shewa	5.00	64.07	1.29	1.33	0.31	0.20	0.23
Sidama	4.00	44.91	0.90	1.20	0.21	0.13	0.15
Gedeo	11.00	65.27	1.31	1.20	0.23	0.14	0.15
Segen	6.00	62.87	1.28	1.27	0.28	0.18	0.19
Kembata	6.00	47.31	0.96	1.20	0.20	0.13	0.14
Bench Maji	3.00	46.11	0.92	1.24	0.24	0.15	0.19
Central Tigray	4.00	53.89	1.09	1.27	0.26	0.17	0.20
Total	6.43	57.27 %	1.15	1.24	0.25	0.16	0.17
Based on Breeding Status							
Landraces	90.00	98.20	1.96	1.24	0.29	0.17	0.17
Released	6.00	65.27	1.33	1.31	0.30	0.20	0.21
Total	48.00	81.74	1.65	1.27	0.29	0.18	0.19

N = number of sample, % P = Percentage of Polymorphic Loci; Na = Number of observed Alleles; Ne = Number of Effective Alleles; I = Shannon's Information Index; h = expected heterozygosity, uh = Unbiased expected heterozygosity

Table 6. Important allelic patterns for each pre-defined population.

Pop	No. alleles	No. alleles freq. $\geq 5\%$	No. of private alleles	No. LComm alleles freq. $\leq 25\%$	No. LComm alleles freq. $\leq 50\%$	h $\pm$ SD	uh $\pm$ SD
North Shewa A	115	115	1	2	30	0.25 $\pm$ 0.02	0.28 $\pm$ 0.02
East Gojam	96	96	0	2	15	0.20 $\pm$ 0.02	0.24 $\pm$ 0.02
West Arsi	96	96	1	2	16	0.21 $\pm$ 0.02	0.25 $\pm$ 0.02
Arsi	89	89	0	0	15	0.20 $\pm$ 0.02	0.25 $\pm$ 0.02
Bale	93	93	0	1	17	0.17 $\pm$ 0.01	0.19 $\pm$ 0.02
North Shewa O	91	91	0	1	15	0.20 $\pm$ 0.02	0.24 $\pm$ 0.02
East Hararghe	121	121	5	4	30	0.26 $\pm$ 0.01	0.29 $\pm$ 0.02
West Shewa	108	108	2	3	22	0.25 $\pm$ 0.02	0.32 $\pm$ 0.02
Sidama	75	75	0	1	13	0.19 $\pm$ 0.02	0.25 $\pm$ 0.02
Gedeo	109	109	1	4	26	0.21 $\pm$ 0.01	0.23 $\pm$ 0.02
Segen	109	109	1	2	26	0.23 $\pm$ 0.02	0.28 $\pm$ 0.02
Kembata	81	81	0	1	10	0.17 $\pm$ 0.02	0.20 $\pm$ 0.02
Bench Maji	77	77	0	2	11	0.21 $\pm$ 0.02	0.31 $\pm$ 0.03
Central Tigray	92	92	0	3	17	0.22 $\pm$ 0.02	0.30 $\pm$ 0.02
Released	113	113	2	2	27	0.26 $\pm$ 0.02	0.31 $\pm$ 0.02

Number of alleles, number of alleles with frequencies greater than or equal to 5%, number of private alleles, number of less common alleles with frequencies less than or equal to 25 % and 50 %, h: expected heterozygosity, uh = unbiased expected heterozygosity, SD = standard deviation.

Table 7. AMOVA within and among fifteen pre-defined populations.

Based on Geographical Origin						
Source of variation	df	SS	MS	EV	PV	P-value
Among region	3	47.51	15.84	0.00	0 %	0.93
Among populations	10	225.31	22.53	0.25	1 %	0.25
Within populations	76	1593.80	20.97	20.97	99 %	0.58
Total variations	89	1866.62		21.22	100 %	
Based on Breeding Status						
Among populations	1	27.67	27.67	0.57	3 %	0.14
Within populations	94	1994.62	21.22	21.22	97 %	0.17
Total variations	95	2022.29		21.79	100 %	

df = degrees of freedom; SS = sum of squares; MS = mean squares; EV = estimated variance, PV = percentage variance

Population genetic diversity

The most polymorphic loci was obtained for accessions from East Hararghe (% P = 72.46) followed by accessions from North Shewa Amhara (% P = 68.86); while accessions from Sidama and Bench Maji showed the least percentage polymorphism (% P = 44.91) and (% P = 46.11), respectively (Table 5). Accessions from East Hararghe, North Shewa Amhara and Gedeo populations revealed the highest number of Na. On the other hand, accessions from West Shewa, North Shewa Amhara and East Hararghe populations revealed the highest number of Ne. Similarly, accessions from West Shewa are the most diverse (I = 0.31, h = 0.20, and uh = 0.23)

followed by accessions from North Shewa Amhara (I = 0.31, h = 0.19, and uh = 0.21) and East Hararghe (I = 0.30, h = 0.19, and uh = 0.20) (Table 5). Among the landrace populations evaluated, accessions from Bale (I = 0.19, h = 0.11, and uh = 0.11), Kembata (I = 0.20, h = 0.13, and uh = 0.14) and Sidama (I = 0.21, h = 0.13, and uh = 0.15) are the least diverse (Table 5). The highest number of alleles and the number of alleles with frequencies greater than or equal to 5 % were exhibited by accessions from both East Hararghe and North Shewa Amhara populations (Table 6).

Private bands (NPvB) were found in seven populations with East Hararghe possessing five

NPvB, which is the highest among all the populations. The highest number of less common alleles with frequencies less than or equal to 25% was revealed by East Hararghe populations (4) and Gedeo populations (4). Both accessions from North Shewa Amhara and East Hararghe populations also revealed the less common alleles with frequencies less than or equal to 50%. Accessions from East Hararghe had the highest *h* (0.26) followed by released variety (0.26) and West Shewa (0.25). The highest *uh* was exhibited by West Shewa (0.32) followed by released varieties (0.31) and Central Tigray (0.30). So, having the highest number of alleles, the number of alleles with frequencies greater than or equal to 5%, and the number of less common alleles with frequencies less than or equal to 50%; East Hararghe and North Shewa Amhara could be the most important populations. The two populations also had the highest % *P* (72.46%, 68.86%), *I* (0.30, 0.31), and *h* (0.19, 0.19), respectively (Table 5).

Analysis of molecular variance (AMOVA)

Based on 14 AZs that are the geographic origin of 90 landrace accessions, AMOVA revealed that the within-population variation is higher than among

population variation with values of 99% and 1%, respectively. Based on the breeding status that is landraces and released varieties, similarly, AMOVA revealed that the within-population variation is higher than among population variation with values of 97% and 3%, respectively. Hence, in both cases, higher genetic diversity was exhibited within the population (Table 7).

Cluster analysis

Neighbor-joining (NJ) clustering analysis separated the 96 faba bean accessions into seven groups (Figure 2). The maximum and the minimum number of accessions across the seven clusters are 24 and 5, respectively. The first cluster consisted of 12 landraces and two released varieties. Among the seven clusters; the second cluster comprised of the minimum accessions of four landraces and one released variety. While the third cluster contains the maximum accessions with 23 landraces and one released variety. The fourth and fifth clusters consisted of 13 and 14 landraces, respectively. The sixth cluster consisted of 11 landraces and one released variety. The last cluster consisted of nine landraces and one released variety (Table 8).

Table 8. Clustering 96 faba bean accessions into seven clusters using nine ISSR primers.

Cluster	Number of accessions	Accessions
C1	14	30021, 26858, 25006, 30014, 26428, 220076, Lalo, 29535, 208114, 27052, 28770, Bulga-70, 25270, 25310
C2	5	29536, Moti, 26887, 229303, 29533
C3	24	25292, 26400, 28104, 28113, 25290, 29537, 26853, 25003, 219089, 30015, 219355, 25017, 25018, 27929, 26860, 25274, 212565, 229310, Hachalu, 231293, 25298, 25010, 26889, 30017
C4	12	Walki, 29527, 29532, 212572, 212566, 212567, 229299, 26854, 26888, 212578, 28772, 25009
C5	18	26886, 30016, 30022, 229871, 26883, 229869, 26885, 28110, 28773, 28776, 208085, 28774, 25022, 235709, 25303, 25299, 28103, 16599
C6	13	26861, 29531, 220079, 28777, 3008, 28109, 30011, 30023, 25264, 26859, 29526, 26882, 27279
C7	10	28771, Dagim, 26862, 27931, 220086, 26855, 28112, 25260, 212580, 25259

Principal Coordinate Analysis (PCoA)

PCoA in the two-dimensional coordinates revealed 39.04% of the total variation, with 20.80% and 18.15% explained by PC1 and PC2, respectively (Figure 3). PCoA in the three-dimensional coordinates revealed 51.23% of the total variation, with 20.80 %, 18.15 %, and 12.28% explained by PC1, PC2, and PC3, respectively (Figure 4). This indicates that accessions from different AZs were clustered together in both coordinates. The clusters identified through PCoA analysis were similar to those identified in the NJ cluster analysis and also from STRUCTURE analysis thereby illustrating the conformity of results obtained from the study.

Figure 4. PCoA analysis of 96 Ethiopian faba bean accessions of fifteen pre-defined populations using ISSR data based on three-dimensional coordinates.

Figure 5. ΔK values for each number of subpopulations (K) for faba bean population.

Population differentiation and structure

Genetic distance and identity

Genetic distance (Gd) among the fifteen pre-defined populations is ranging from 0.01 to 0.04 (Table 9). Accessions from Bale were distantly related to accessions of Arsi (0.04) followed by accessions from Segen that were distantly related to accessions of Gedeo (0.04) and Bench Maji (0.04). The least Gd was revealed between accessions from Gedeo and Sidama populations (0.01) followed by accessions from Bale and East Gojam populations (0.01). Genetic identity (GI) coefficients using the ISSR data were used to assess the extent of genetic similarity among the 96 faba bean accessions. Genetic identity (GI) coefficients are ranging from 0.96 to 0.99. Based on the estimated GI coefficient; the highest GI value was observed between Sidama and Gedeo (0.99) followed by East Gojam and Bale (0.99). The least GI value was observed between accessions of Arsi and Bale (0.96) followed by Gedeo and Segen (0.96).

Genetic differentiation and gene flow

Genetic differentiation (Φ_{iPT}) on faba bean landraces collected from AZs of Ethiopia revealed that Φ_{iPT} among pairs of the population are ranging from 0.00 to 0.27, with an overall mean of 0.07. Between Central Tigray and Bench Maji ($\Phi_{iPT}=0.27$) population, very great Φ_{iPT} was observed. Among pair-wise populations of Bench Maji and Sidama ($\Phi_{iPT}=0.21$), Bench Maji and West Shewa ($\Phi_{iPT}=0.20$), Central Tigray, and West Shewa ($\Phi_{iPT}=0.18$), Sidama and West Arsi ($\Phi_{iPT}=0.17$), and Bale and Arsi ($\Phi_{iPT}=0.17$) large degree of Φ_{iPT} were observed. Among the Central Tigray and Sidama populations ($\Phi_{iPT} = 0.14$) moderate Φ_{iPT} was revealed. Among 105 pair-wise of Φ_{iPT} values; 52 of them ranged from 0.00 - 0.05, 46 of them ranged from 0.05 - 0.15, 6 of them ranged from 0.15 - 0.25, and 1 pair above 0.25; showing little, moderate, large, and very large Φ_{iPT} , respectively (Table 10). The gene flow of 90 faba bean landrace accessions collected from fourteen AZs including six released varieties are ranging from 0.68 to 24.75. The highest level of gene flow (Nm) was observed between populations of West Shewa and North Shewa Amhara; released variety and East Gojam, West Arsi, Arsi, East Hararghe; Central Tigray

and Kembata; Segen and East Hararghe, West Shewa, Sidama, while the lowest level of Nm was observed between Central Tigray and Bench Maji (Table 10).

Population structure

In the present study, the number of subpopulations was determined as ($K = 7$) due to the relatively high ΔK value at $K = 7$, according to Evanno *et al.* (2005) (Figure 5). The results of $K = 7$ populations indicated accessions from 15 pre-defined populations distributed between the seven populations as demonstrated by the bar plot (Figure 6). A vertical line broken into K colored segments and proportional to each of the K inferred clusters represent the accessions in the study. Different colors represents different subpopulations. In the figure, the accessions in the populations and the percentage identity to the cluster to which it belongs were indicated by X-axis and Y-axis, respectively. If a membership coefficient of the genotype is ≥ 0.80 , it belongs to a cluster but if a membership coefficient is < 0.80 , it regarded as an admixed individual. (Table 11). Among the 15 pre-defined populations; ten of them had admixed individuals with the proportion ranged from 9.09 for the Gedeo population to 40.00 for the Bale population; while the other populations do not have admixed individuals. The average proportions of admixed individuals are 13.90. Genetic divergence among clusters, expected heterozygosity within a cluster, and the proportion of the membership of each

pre-defined population in each of the clusters are presented in Table 12. The maximum net nucleotide distance among the seven clusters was detected between C1 and C4 followed by C4 and C6, with values of 0.24 and 0.21, respectively. While, a minimum distance was detected between C2 and C3 followed by C2 and C5, with the value of 0.10 and 0.11, respectively. The maximum and minimum expected heterozygosity within a cluster were observed within C2 and C5, with values of 0.19 and 0.02, respectively. Among the seven clusters; C3 and C1 exhibited the first and second-largest proportions of 21.99 and 15.59, respectively. The minimum proportion of the membership of samples was observed in C2 and C6, with values of 3.66 and 8.33, respectively.

Discriminant analysis of principal components (DAPC)

The results of the scatterplot of faba bean accessions using DAPC are exhibited in Figure 7. In the figure, the accessions and the clusters are represented by the dots and the inertia ellipses, respectively. So, based on DAPC also, the accessions were separated into seven clusters. The DAPC results in Table 12 indicated that the largest and smallest proportion of DAPC was exhibited in C6 and C2, with 0.23 and 0.05, respectively. Both the NJ and the DAPC analysis classified the 96 faba bean accessions into seven clusters; even though the number of accessions in each cluster is not similar since DAPC is based on the retained discriminant functions.

Figure 6. Classification of the 96 faba bean accessions using STRUCTURE 2.3.4 software into seven distinct clusters by the model-based method at $k = 7$.

Table 9. Genetic distance (below diagonal) and Nei's genetic identity (above diagonal) among fifteen pre-defined populations.

	NSA	EG	WA	A	B	NSO	EH	WS	SD	G	S	K	BM	CT	RV
NSA	****	0.98	0.98	0.98	0.98	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
EG	0.02	****	0.99	0.97	0.99	0.99	0.98	0.97	0.99	0.99	0.97	0.99	0.98	0.98	0.97
WA	0.02	0.01	****	0.97	0.99	0.99	0.99	0.98	0.99	0.99	0.97	0.98	0.98	0.98	0.97
A	0.02	0.03	0.03	****	0.96	0.98	0.97	0.97	0.96	0.96	0.98	0.98	0.97	0.97	0.97
B	0.02	0.01	0.01	0.04	****	0.98	0.98	0.97	0.99	0.99	0.96	0.98	0.98	0.98	0.96
NSO	0.01	0.01	0.02	0.02	0.02	****	0.99	0.97	0.98	0.99	0.98	0.99	0.98	0.97	0.97
EH	0.02	0.02	0.01	0.03	0.02	0.02	****	0.97	0.98	0.99	0.97	0.98	0.97	0.98	0.97
WS	0.02	0.03	0.02	0.03	0.03	0.03	0.03	****	0.98	0.97	0.96	0.97	0.98	0.98	0.97
SD	0.03	0.01	0.01	0.04	0.01	0.02	0.02	0.02	****	0.99	0.96	0.98	0.98	0.98	0.96
G	0.02	0.01	0.01	0.04	0.01	0.01	0.01	0.03	0.01	****	0.96	0.98	0.99	0.98	0.96
S	0.02	0.04	0.03	0.03	0.04	0.02	0.03	0.04	0.04	0.04	****	0.97	0.96	0.97	0.98
K	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.03	0.02	0.02	0.03	****	0.98	0.98	0.97
BM	0.02	0.02	0.02	0.04	0.02	0.02	0.03	0.03	0.02	0.02	0.04	0.02	****	0.98	0.97
CT	0.02	0.02	0.02	0.04	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.02	****	0.98
RV	0.02	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.04	0.04	0.02	0.03	0.03	0.02	****

NSA=North Shewa Amhara, EG=East Gojam, WA=West Arsi, A=Arsi, B=Bale, NSO=North Shewa Oromia, EH=East Hararghe, WS=West Shewa, SD=Sidama, G=Gedeo, S=Segen, K=Kembata, BM=Bench Maji, CT=Central Tigray, and RV=Released Varieties

Table 10. PhiPT values (above diagonal) and Gene flow (Nm, below diagonal).

	NSA	EG	WA	A	B	NSO	EH	WS	SD	G	S	K	BM	CT	RV
NSA	0	0.07	0.05	0.02	0.13	0.03	0.02	0.01	0.04	0.14	0.10	0.08	0.07	0.03	0.06
EG	3.32	0	0.11	0.04	0.04	0.04	0.00	0.03	0.11	0.02	0.03	0.07	0.12	0.08	0.01
WA	4.75	2.02	0	0.03	0.02	0.05	0.06	0.02	0.17	0.08	0.02	0.06	0.13	0.13	0.01
A	12.25	6.00	8.08	0	0.17	0.09	0.05	0.00	0.02	0.11	0.05	0.10	0.10	0.02	0.01
B	1.67	6.00	12.25	1.22	0	0.09	0.05	0.11	0.09	0.00	0.14	0.10	0.00	0.01	0.12
NSO	8.08	6.00	4.75	2.53	2.53	0	0.03	0.04	0.06	0.00	0.06	0.08	0.17	0.00	0.02
EH	12.25	****	3.92	4.75	4.75	8.08	0	0.01	0.06	0.00	0.01	0.05	0.04	0.08	0.01
WS	24.75	8.08	12.25	****	2.02	6.00	24.75	0	0.12	0.06	0.01	0.06	0.20	0.18	0.04
SD	6.00	2.02	1.22	12.25	2.53	3.92	3.92	1.83	0	0.13	0.01	0.03	0.21	0.14	0.04
G	1.54	12.25	2.88	2.02	****	****	****	3.92	1.67	0	0.12	0.03	0.10	0.02	0.12
S	2.25	8.08	12.25	4.75	1.54	3.92	24.75	24.75	24.75	1.83	0	0.02	0.09	0.08	0.06
K	2.88	3.32	3.92	2.25	2.25	2.88	4.75	3.92	8.08	8.08	12.25	0	0.09	0.01	0.05
BM	3.32	1.83	1.67	2.25	****	1.22	6.00	1.00	0.94	2.25	2.53	2.53	0	0.27	0.11
CT	8.08	2.88	1.67	12.25	24.75	****	2.88	1.14	1.54	12.25	2.88	24.75	0.68	0	0.13
RV	3.92	24.75	24.75	24.75	1.83	12.25	24.75	6.00	6.00	1.83	3.92	4.75	2.02	1.67	0

Table 11. The proportion of the membership of each fifteen pre-defined population in each of the clusters.

Population	Number of accessions	Admixed individual	The proportion of membership in each cluster (%)						
			C1	C2	C3	C4	C5	C6	C7
North Shewa A	9.00	11.11	11.11	0.00	22.22	33.33	11.11	0.00	11.11
East Gojam	7.00	0.00	28.57	0.00	28.57	0.00	42.86	14.29	0.00
West Arsi	6.00	33.33	16.67	0.00	16.67	0.00	0.00	33.33	0.00
Arsi	5.00	20.00	60.00	0.00	20.00	0.00	0.00	0.00	0.00
Bale	10.00	40.00	10.00	0.00	0.00	0.00	40.00	10.00	10.00
North Shewa O	6.00	16.67	0.00	16.67	33.33	16.67	16.67	0.00	0.00
East Hararghe	8.00	0.00	12.50	12.50	12.50	25.00	0.00	25.00	0.00
West Shewa	5.00	20.00	20.00	0.00	20.00	0.00	0.00	0.00	40.00
Sidama	4.00	0.00	0.00	0.00	25.00	0.00	25.00	25.00	25.00
Gedeo	11.00	9.09	0.00	9.09	18.18	0.00	27.27	27.27	9.09
Segen	6.00	16.67	0.00	0.00	33.33	33.33	0.00	0.00	16.67
Kembata	6.00	0.00	16.67	0.00	50.00	0.00	33.33	0.00	0.00
Bench Maji	3.00	0.00	0.00	0.00	33.33	0.00	33.33	0.00	33.33
Central Tigray	4.00	25.00	25.00	0.00	0.00	0.00	0.00	25.00	25.00
Released	6.00	16.67	33.33	16.67	16.67	16.67	0.00	0.00	0.00

Table 12. Genetic divergence among the clusters (net nucleotide distance), within the clusters (expected heterozygosity), and proportion of the accessions in each of the clusters.

Net nucleotide distance								Expected heterozygosity	Proportion of membership	DAPC
C1	C2	C3	C4	C5	C6	C7				
C1	-	0.17	0.18	0.19	0.20	0.24	0.18	0.12	15.59	0.12
C2		-	0.10	0.17	0.11	0.14	0.16	0.19	3.66	0.05
C3			-	0.20	0.14	0.19	0.17	0.14	21.99	0.14
C4				-	0.20	0.21	0.19	0.12	8.33	0.10
C5					-	0.16	0.18	0.02	15.31	0.22
C6						-	0.18	0.12	10.66	0.23
C7							-	0.16	11.35	0.15

DISCUSSION

The ISSR markers amplified a total of 167 different types of bands. The average total bands per primer were 18.56; ranging from 14 to 25 with all 100% polymorphism. In contrast to this study, Terzopoulou and Bebeli (2008) reported an average percent of polymorphisms of 67.48% by using 11 ISSR primers in studying the genetic diversity of the Greek faba bean population. Abdel-razzak *et al.* (2012) also reported an average percent of polymorphisms of 69.10% within 10 faba bean genotypes from Egypt using nine ISSR primers. Moreover, Merji *et al.* (2012) found 97.30% average polymorphism in the analysis of the effect of gamma radiation on various

characters of 22 faba bean genotypes grown in Tunisia using 15 ISSR primers. Similarly, using ISSR markers, the study on global faba bean germplasm by Wang *et al.* (2012) indicated an average percentage of polymorphism of 93%. The present study exhibited a higher average percentage of polymorphism than the finding reported by all authors, which could be explained by the diversified nature of landrace accessions and also a large number of accessions used. Therefore, the higher average percent of polymorphism resulted in current study indicates the use of ISSR markers for future genetic diversity studies.

PIC, MI, and RP measure the efficiency of ISSR markers, as described by Roldan-Ruiz *et al.* (2000), Varshney *et al.* (2007), and Prevost and

Wilkinson (1999), respectively. The highest and the lowest PIC values of 0.44 and 0.19 were revealed by the primers UBC881 and UBC873, respectively, with a mean of 0.32. Similar to the current study, Ya-ming *et al.* (2011) and Abdollah *et al.* (2011) found average PIC values of 0.29 and 0.22, respectively. The high PIC values for ISSR primers in this study indicates the suitability and efficiency of the markers in the assessment of genetic diversity of faba bean landrace accessions in Ethiopia. In detecting polymorphism, MI is also calculate the efficiency of the markers. MI in the current study is ranging from 3.18 to 7.46, with an average of 5.87 in nine ISSR primers,

which is higher than MI described by Abdollah *et al.* (2011) with an average of 1.34. In the current study, the high value of MI was derived from the high average EMR value of 18.38. In contrast to the current study, Abdolla *et al.* (2011) found an average EMR value of 5.69. The discriminatory power of ISSR markers is measured by RP. The mean value of RP is 7.14, ranging from 2.96 to 12.13. Similarly, Abdolla *et al.* (2011) reported RP values ranging from 7.20 to 16.50. Hence, the result in the current study confirmed that the suitability, efficiency, and discriminatory power of the ISSR markers, and they would be recommended for further diversity analysis..

Figure 7. A scattered plot of 96 faba bean accessions using DAPC based on ISSR data.

The degree of polymorphism among the breeding status of faba bean accessions ranged from 65.27 for the released varieties to 98.20 for the landrace accessions. The highest polymorphism observed in the landrace accessions as compared to the released varieties could be explained by the broader spectrum of landrace accessions as a reservoir of different alleles. The present study revealed moderate diversity indexes ($I = 0.25$, and $h = 0.16$) values for the faba bean landrace accessions as compared to gene diversity indexes ($I = 0.53$, and $h = 0.35$) reported by Abdel-Razak

et al. (2012) who studied faba bean using ISSR markers. Accessions from West Shewa followed by North Shewa Amhara and East Hararghe were found to be high diversity as opposed to those from the other AZs. Hence, further collection, conservation, and utilization programs would be carried out from AZs with high genetic diversity. The study of genetic distance between fifteen pre-defined populations would also be important for accessions improvement. The present study showed the highest inter-population genetic distance between Bale and Arsi populations as

compared to the other pair-wise distance values. As a result, hybridization between accessions from distantly related populations of Bale and Arsi is supposed to yield higher hybrids as compared to less distantly related populations.

AMOVA revealed that the variation within a population and among a population of 99 % and 1%, respectively. Similarly, Oliviera *et al.* (2006) observed 81% within a population and 19% among-population variation in genetic diversity and structure analysis using 26 nuclear SSRs. According to Behailu (2016) the variation within a population and among a population of 91 % and 9%, respectively, were reported in genetic diversity study of 32 Ethiopian faba bean varieties using ISSR markers. Hence, this study showed that variation within populations is large in faba bean. Jin *et al.* (2006) and Belaid *et al.* (2006) have also reported similar results on *Glycine max* and *Lathyrus sativus* populations, respectively. The high level of genetic variation within populations as compared to among populations may be the transfer of seeds among AZs by a farmer to farmer seed exchange, during harvesting, through the market, and exchange of seeds through relief agencies like NGOs. Faba bean growers in Ethiopia get their seed mostly by the informal seed exchange system. In this line, there has been a lot of genetic exchange among farmers' landrace for seed (Alemu *et al.*, 2010). The partially allogamous nature of faba bean could also be the other possible factor for high genetic variation within groups. Because, the pollen of faba bean can easily move by insect pollinators like bee and bee beetles causing outcrossing of the varieties (Ellwood *et al.*, 2008). Hence, high genetic variation within populations indicates the existence of high gene flow among pre-defined populations.

The current study grouped the AZs based on 14 pre-defined populations into seven clusters using ISSR primers based on NJ which had the potential to detect genetic variability in faba bean accessions. This is similar to Abdel-razzak *et al.* (2012) who studied genetic diversity in ten faba bean genotypes from Egypt using ISSR clustered the individuals depending on their genetic similarity rather than their geographical origin.

Wang *et al.* (2012) also reported the clustering of genotypes from different origins into the same cluster without showing a clear-cut category. In

this study, accessions from similar AZs were separated into different clusters and also accessions from different AZs were clustered together showing the existence of variation within a population. So, the analysis of the NJ tree showed no clear-cut pattern about the geographical origin, which could be due to higher gene flow between pre-defined populations. Similar to Keneni *et al.* (2005) report on local field pea and faba bean accessions, the current study revealed the separation of accessions from non-neighboring populations into the same clusters. The PCoA results revealed that the first two and three axes represent 39.04% and 51.23%, respectively, of the total variability. The result of PCoA in both two-dimensional and three-dimensional coordinates were similar to NJ result indicating the conformity of the results obtained from the study.

The degree of population differentiation was measured with PhiPT. According to Wright (1978), a PhiPT value greater than 0.25 is referred to as very great PhiPT, a PhiPT within 0.15 to 0.25 is referred to as a large PhiPT, a PhiPT within 0.05 to 0.15 is referred to as moderate PhiPT, and values lying within 0.00 to 0.05 considered little PhiPT. In the present study, the values of most of the pair-wise comparisons among the fifteen pre-defined populations were ranging from little to moderate PhiPT. Waples (1987) categorized Nm into three that are $Nm \geq 1$ is referred to as high, Nm value within 0.25– 0.99 is referred to as intermediate, and Nm value within 0.00 – 0.25 is referred to as low. According to Wright (1951) if $Nm > 1$, the PhiPT is little, and if $Nm < 1$, the PhiPT is high. If $Nm > 1$, PhiPT does not results from genetic drift only, but if $Nm < 1$ it results from genetic drift only (Slatkin, 1987). So, in this study, the relatively little to moderate PhiPT and high level of Nm detected strongly indicate that the exchange of genetic materials between 15 pre-defined populations is sufficient to counter the genetic composition of the population.

To confirm their complementarity, the STRUCTURE and DAPC were also used to analyze the population structure of 96 faba bean accessions. However, the variation between NJ and DAPC in grouping accessions into clusters is; NJ method is based on the agglomerative hierarchical method of clustering, and DAPC is based on the non-hierarchical clustering method

(Campoy *et al.* 2016). The STRUCTURE analysis also agreed with the dendrogram analysis in that seven clusters were found in the population. The complementarity of this result suggests the representative nature of the used markers. Wang *et al.* (2012) reported similar results with the current study that is the congruency between dendrogram and PCoA results.

CONCLUSION

Faba bean plays a significant role in both the human diet and animal feed since it has high protein and starch content in its seeds. Determining genetic diversity is an important element of crop improvement programs. Although there are more than 2,300 faba bean accessions in the Ethiopian Biodiversity Institute; its molecular genetic diversity has not been comprehensively studied. In the current study, the molecular genetic diversity of Ethiopian faba bean landrace accessions was estimated using nine ISSR primers. The present study detected a higher percent of polymorphism as compared to other studies based on faba bean using ISSR primers and also different markers on other legume crops. Furthermore, it also showed that based on different diversity indices faba bean landraces accessions possess a wide range of genetic diversity within AZs, indicating its importance for further collection and utilization programs. The current finding displays the presence of high genetic diversity in the Ethiopian faba bean landrace accessions across the geographic regions. The high genetic diversity within the population as compared to the population is due to the high gene flow between the AZs. Based on the primers' performance of the nine ISSR markers; PIC, MI, RP, and EMR parameters were able to detect a substantial level of genetic diversity in faba bean landrace accessions and it would also be recommended for use in faba bean improvement programs. Landrace accessions from 14 AZs of the country were distributed over the majority of the clusters, exhibiting high genetic diversity in most of the collection sites. As a result, further collection, conservation, and utilization programs would be recommended from AZs with high genetic diversity.

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