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Genetic diversity study of Ethiopian Faba bean (*Vicia faba* L.) varieties based on phenotypic traits and inter simple sequence repeat (ISSR) markers

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Faba bean (*Vicia faba* L.) is one of the earliest domesticated food legumes of the world. This study was designed to reveal the genetic diversity existing among 32 Ethiopian faba bean varieties grown at three locations (Sinana, Agarfa and Selka) using 23 phenotypic traits and 11 inter simple sequence repeat (ISSR) primers. The combined analysis of variance across the three locations showed highly significant (*p<0.05*) variations among the varieties for many of the traits. The un-weighted pair group method with arithmetic mean of phenotypic traits revealed five major clusters. Eleven ISSR primers amplified 120 bands, of which 107 loci were polymorphic. Primer 860 recorded the highest gene diversity (0.38) and Shannon index (0.56), while primer 848 and 857 exhibited the least gene diversity (0.18). Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) of ISSR primers data grouped the cultivars into three major clusters based on Jaccard's similarity coefficient ranging from 0.41 to 0.77. The principal coordinated analysis also categorized the varieties into three different groups similar to that of cluster analysis. The genetic variation in these cultivars suggests their potential use in faba bean breeding programs via introgression with other germplasm resources for faba bean improvement.

Key words: *Vicia faba*, genetic diversity, phenotypic traits, inter simple sequence repeat (ISSR).

INTRODUCTION

Faba bean (*Vicia faba* L., 2n = 12) is one of the oldest domesticated food legumes with controversial origin. Vavilov (1936) proposed that faba bean originated and was domesticated in the Central Asia and then expanded westward along the mountains to Iran, Turkey, the Mediterranean and Spain, while Cubero (1974) suggested

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that Near East, Iraq and Iran are the primary centers of origin and Afghanistan and Ethiopia are the secondary centers of origin. However, recent evidence from archeological study strongly suggests that Neolithic people in Israel (the lower Galilee: Ahihud, Nahal Zippori and Yiftah'el) first domesticated faba beans at least 10,200 years ago, and they were eating it as a staple food before grain was cultivated in the other areas (Caracuta et al., 2015).

Faba bean is one of major grain legume crops grown in Ethiopia and ranks first in terms of area in production with 574,060.45 ha and 943,964.2 tons produced (CSA, 2014). It is used both as food and feed due to its high protein content, and to enhance soil fertility (Caracuta et al., 2015). It is also used as a break crop to interrupt disease cycles and weed control for cereal-based crop rotations (Erik et al., 2012). Despite its ecological and economic contributions and the value of faba bean in Ethiopia, the productivity is still far below its potential of 1.9 ton ha^{-1}, due to chocolate spot, aschocyta blight, rust, drought, soil acidity and water logging (Asfaw et al., 1994; El-Fouly, 1982). To address these challenges, designing a sound crop breeding programs that would improve productivity of the crop through development of superior cultivars with stable performance across agro-ecologies plays an important role.

For such reason, documenting genetic information of this important crop is crucial. Characterization of genetic diversity study plays a paramount role in revealing genetic variability among and within species, developing selection criteria, selecting heterotic parents for hybridization, choosing effective breeding procedures and in determining conservation strategies (Singh, 1990). Estimation of genetic variation among faba bean varieties in Ethiopia is, therefore, the main concern of breeders as the effectiveness of selection depends on the proportion of the heritable variation.

Different marker systems such as morphological, biochemical and molecular markers are typically used to assess the genetic diversity of crops species. Morphological markers reveal genetic diversity of crops based on phenotypic appearance though it is highly influenced by environmental factors and can be more subjective than other markers (Vos et al., 1995; Hedrick, 2005; Li et al., 2009). Similarly, biochemical markers are low in abundance and polymorphisms, and are sensitive to environment. So, the limitations of both morphological and biochemical markers are addressed by the DNA based molecular markers. Several authors have reported the genetic diversity of faba beans using different molecular markers, including: SDS PAGE (Hou et al., 2014), random amplified polymorphic DNA (RAPD) (Basheer-Salimia et al., 2013; Aziz and Oman, 2015), amplified fragment length polymorphism (AFLP) (Zeid et al., 2001; Ammar et al., 2015), SRAP (Ammar et al., 2015) and inter simple sequence repeat (ISSR) (Terzopoulos and Bebeli, 2008; Abdel-razzak et al., 2012; Mejri et al., 2012; Wang et al., 2012; Salazar-Laureles et al., 2015). These markers have been instrumental in revealing genetic diversity within and among populations of faba bean genotypes.

Combining molecular markers with phenotypic markers is an important approach for varietal characterization and fingerprinting to reveal the relationship and level variability among faba bean varieties cultivated in Ethiopia. Genetic diversity of released Ethiopian faba bean varieties has not been studied yet, so, this research aimed to investigate the diversity and relationships between Ethiopian faba bean cultivars using both phenotypic traits and ISSR markers.

MATERIALS AND METHODS

Descriptions of the study area

The field experiment was conducted at three locations in southeastern Ethiopia highland, Sinana (07°07'N, 40°10'E), Agarfa (07°15'44''N, 039°50'38''E) and Selka (07°04'28''N, 040°12'18''E) during 2014/2015 for the analysis of agro-morphological performance, while ISSR analysis was carried out at Plant Genetics Research Laboratory, Addis Ababa University, Addis Ababa, Ethiopia. All the three experimental sites are characterized by pellic vertisol with a slightly acidic soil with altitude range of 2400 to 2509 m a.s.l that represent the potential production area.

Plant materials

A total of 32 faba bean varieties released from different research centers in the country were used for variability assessment using morphological traits and ISSR markers (Table 1). The field experiment was laid out using Alpha lattice design with two replications at three locations (Sinana, Agarfa and Selka). Each plot consisted of two rows 4 m long with a row-to-row spacing of 40 cm and plant-to-plant spacing of 10 cm. The genotypes were assigned to plots randomly within each replication. Fertilizer was applied at the rate of 100 kg ha^{-1} at planting. For ISSR genotyping, leaf samples were collected and dried with silica gel for DNA extraction. Silicagel dried leaf samples were ground with Mixer Mill (Retsch GmbH, Germany).

Genomic DNA extraction

Genomic DNA was extracted with a minor modification using cetyl trimethyl ammonium bromide (CTAB) method employing triple extraction to yield optimal quantities of high-quality DNA from tissues (Borsch et al., 2003). DNA quantity and quality were tested using gel electrophoresis and Nano drop spectrophotometry (Nano-drop 2000/2000c). Genomic DNAs were then diluted to approximate amount of 70 ng/µl for polymerase chain reaction (PCR) to screen primers and optimize the PCR reaction condition.

Primer selection and PCR amplification

Initially, a total of 25 ISSR primers (Primer kit of UBC 900) were selected based on published research report on faba bean (Abdul-Razzak et al., 2012; Mejri et al., 2012; Wang et al., 2012; Salazar-Laureles et al., 2015) and other related crops: chick pea (Bhagyawant and Srivastava, 2008), lentil (Edossa et al., 2007;

Table 1. Combined mean performance analysis of 23 traits of 32 Ethiopian faba bean varieties tested at Sinanaa, Agarfa and Selka during main growing season of 2014.

Variety name	LL	LW	LA	LAI	PL	PW	IL	PHFP	PH	NBPPL	NPPL	NSPL	NSPPod
Mosisa	9.12	4.45	28.92	189.64	7.27	1.33	5.77	40.83	149.77	1.40	32.13	80.20	2.50
Tumsa	9.28	4.58	30.50	238.97	7.34	1.37	5.96	57.17	153.73	1.40	25.00	61.87	2.45
Hachalu	9.33	4.73	31.26	227.51	8.44	1.52	5.98	48.70	155.80	1.60	25.17	70.73	2.78
Dosha	9.48	4.65	31.28	281.89	7.87	1.40	5.38	40.80	142.30	2.23	33.20	87.83	2.63
Gachena	9.58	4.74	32.33	228.49	8.88	1.73	5.82	48.03	153.33	1.47	24.27	63.83	2.64
Walki	9.53	4.97	33.58	247.96	6.90	1.28	5.88	40.00	151.57	1.47	36.63	90.37	2.52
Obse	8.97	4.31	27.77	204.34	9.51	1.54	6.67	45.87	155.13	1.60	19.63	56.97	2.88
Moti	8.80	4.25	26.71	220.49	8.13	1.35	5.66	44.77	148.13	1.93	26.33	75.60	2.91
Gabelcho	9.48	4.35	29.25	249.65	7.77	1.54	6.14	47.17	150.93	1.87	26.80	68.47	2.57
Adet Hanna	9.80	4.74	32.95	212.38	7.19	1.25	6.41	44.10	150.47	1.13	36.03	90.90	2.53
NC-58	9.18	4.33	28.34	187.58	6.25	1.17	6.41	39.20	147.77	1.40	41.07	107.57	2.62
Wayu	8.94	4.07	25.95	181.65	5.72	1.10	6.39	46.40	142.87	1.43	37.53	94.13	2.50
Degaga	9.28	4.63	30.35	233.97	7.01	1.34	5.92	42.73	156.27	1.77	44.90	120.80	2.67
Dagm	8.69	3.87	23.98	203.88	5.98	1.04	5.65	45.23	138.77	1.97	40.63	109.73	2.71
Holetta-2	9.53	4.64	31.39	226.88	7.33	1.38	5.50	46.83	152.20	1.73	37.03	99.37	2.67
Shalo	9.60	4.75	32.24	254.45	7.38	1.47	5.83	51.97	154.20	1.60	31.73	76.57	2.42
Tesfa	9.39	4.81	31.80	213.00	6.93	1.28	6.11	41.57	148.73	1.30	34.57	93.23	2.73
Mesay	9.24	4.35	28.54	194.48	6.92	1.32	6.21	37.13	154.07	1.17	32.90	93.37	2.89
Bulga 70	9.08	4.44	28.49	217.94	6.33	1.14	5.99	38.83	147.33	1.83	38.67	108.93	2.83
Kassa	9.57	4.54	30.97	204.04	6.45	1.20	6.39	42.80	149.10	1.67	43.47	104.97	2.39
Gora	10.24	4.92	35.37	283.35	9.87	1.58	5.40	44.03	151.73	1.53	24.53	67.67	2.71
CS-20-DK	9.00	4.10	26.25	228.62	6.84	1.21	5.60	44.43	147.50	1.90	30.93	87.10	2.81
Kuse	9.00	4.22	27.06	198.39	6.61	1.28	5.59	40.30	145.10	1.53	35.37	95.80	2.71
Dida'a	10.03	4.71	33.17	242.11	8.86	1.58	5.53	44.30	155.57	1.53	26.17	69.33	2.64
Lalo	9.34	4.04	26.81	181.38	6.22	1.17	5.34	46.63	142.33	1.53	34.17	90.50	2.65
Bako local	8.63	4.11	25.48	233.18	6.52	1.13	6.35	42.97	141.30	1.83	37.20	99.00	2.64
Debrebirhan local	8.73	3.70	23.24	213.76	5.57	1.05	6.01	38.40	135.60	2.23	47.60	123.20	2.59
Sinana local	9.18	4.58	29.86	212.57	7.04	1.32	6.09	39.93	150.53	1.27	35.03	89.07	2.54
Agarfa local	9.07	4.89	31.52	226.31	7.08	1.45	5.59	37.13	149.43	1.33	35.73	92.33	2.56
EKCSR-02006	9.03	4.44	28.38	197.81	7.32	1.39	5.88	38.70	143.83	1.53	34.83	81.13	2.33
Adet local	9.26	4.68	30.67	251.01	7.63	1.40	6.23	48.20	151.83	1.80	34.97	89.87	2.58
Kulumsa local	10.09	5.17	36.74	254.46	7.81	1.47	6.11	46.10	151.17	1.67	38.03	91.43	2.44
Mean	9.30	4.49	29.72	223.19	7.28	1.34	5.93	43.79	149.01	1.61	33.82	88.50	2.63
CV	6.10	9.13	13.38	23.11	6.75	12.35	11.42	14.70	5.40	25.54	20.30	21.04	8.24
LSD (5%)	0.35	0.34	4.01	42.45	0.59	0.20	0.64	5.68	4.65	1.31	8.34	20.40	0.15

** = Highly significant($p<0.01$); * = significant ($p<0.05$); CV = coefficient of variation; LSD = least significant difference; LL = leaf length (cm); LW = leaf width (cm); LA = leaf area (cm^2); LAI = leaf area index; Pl = pod length (cm); PW = pod width (cm); IL = internodes length (cm); HFPN = height to first poding node (cm); PH = plant height (cm); NBPPL = number of branches per plant; NPPL= number of pods per plant; NSPL= number of seed per plant; NSPPod = number of seed per pod.

Table 1. Contd.

Variety name	BMPP	SYPP	HI	TSW	DF	DM	SFP	SPE	EGR	CP
Mosisa	2966.67	4573.92	50.91	518.85	54.00	141.67	87.67	2891.92	53.98	22.90
Tumsa	3716.67	4260.07	36.57	620.47	55.67	147.83	92.17	2903.59	39.75	22.51
Hachalu	3583.33	4311.36	39.68	575.79	55.33	147.83	92.50	2836.00	37.14	20.98
Dosha	2683.33	4093.57	48.90	556.88	55.50	148.67	93.17	2639.31	26.54	22.07
Gachena	3850.00	4867.15	40.99	562.13	53.67	146.33	92.67	3242.05	43.05	21.36
Walki	3816.67	5631.46	43.81	558.09	53.00	143.67	90.67	3464.31	42.01	22.24
Obse	3350.00	4396.33	42.02	617.15	53.17	144.33	91.17	3020.01	36.25	21.29
Moti	3416.67	5256.44	41.79	583.93	51.83	147.33	95.50	3150.76	32.82	21.44
Gabelcho	3583.33	5113.17	38.34	524.65	56.00	144.17	88.17	2580.27	36.98	22.05
Adet Hanna	3116.67	4253.14	43.52	421.82	54.17	139.83	85.67	2611.61	46.30	22.38
NC-58	3000.00	4859.89	52.54	401.00	53.50	138.83	85.33	3090.95	47.99	22.29
Wayu	3300.00	4141.30	40.40	444.10	57.33	145.33	88.00	2461.21	45.97	21.95
Degaga	3400.00	5081.81	49.37	454.36	54.17	137.50	83.33	3040.16	52.69	22.56
Dagm	3166.67	3551.45	36.24	362.51	56.33	144.17	87.83	2137.32	31.53	22.92
Holetta-2	3575.00	4629.20	43.00	488.32	55.17	146.00	90.83	2938.92	37.93	20.59
Shalo	3750.00	4913.17	42.26	506.99	54.50	141.17	86.67	3003.48	51.81	22.65
Tesfa	3183.33	4339.87	44.09	411.39	55.33	138.33	83.00	2559.77	46.61	22.92
Mesay	3283.33	4393.18	42.24	406.32	54.67	140.83	86.17	2677.26	49.93	22.51
Bulga 70	2900.00	4283.07	47.79	412.81	54.50	141.83	87.33	2701.40	36.85	22.34
Kassa	3350.00	4678.05	44.85	412.42	53.00	142.50	89.50	3085.26	44.26	22.48
Gora	3500.00	4112.37	38.62	690.06	54.83	146.67	91.83	2660.75	37.28	22.87
CS-20-DK	3391.67	4469.20	42.15	504.47	55.50	145.33	89.83	2787.27	36.36	21.95
Kuse	3366.67	4905.53	47.27	470.99	53.67	142.33	88.67	3179.12	56.01	21.80
Dida'a	3616.67	4855.91	43.86	596.38	53.83	146.83	93.00	3351.94	40.14	22.63
Lalo	3216.67	3847.39	37.99	380.43	55.00	146.50	91.50	2430.73	32.72	22.12
Bako local	2616.67	2955.59	36.06	333.56	56.50	140.33	83.83	1716.27	26.67	22.33
Debrebirhan local	2583.33	3791.04	49.16	358.80	54.50	140.83	86.33	2357.21	39.68	22.79
Sinana local	3050.00	4737.69	50.21	406.84	53.33	140.17	86.83	2993.91	53.26	22.51
Agarfa local	3783.33	4286.42	44.34	519.28	52.33	142.33	90.00	3546.15	53.69	22.14
EKCSR-02006	3766.67	5305.15	45.53	509.26	54.00	147.00	93.00	3585.34	42.24	21.99
Adet local	3733.33	4608.15	39.97	520.38	54.00	147.00	93.00	3139.85	34.52	21.68
Kulumsa local	4200.00	4320.91	44.09	545.65	53.67	147.00	93.33	3862.48	42.98	20.95
Mean	3369.27	4494.44	43.39	489.88	54.44	143.77	89.33	2895.21	41.75	22.13
CV	17.45	7.20	15.84	14.09	2.18	1.45	2.50	9.16	28.02	3.29
LSD (5 %)	360.95	123.94	8.19	80.19	1.33	2.32	2.40	302.35	8.5	0.88

** = Highly significant($p<0.01$); * = significant ($p<0.05$); CV = coefficient of variation; LSD = least significant difference; SYPP = seed yield per plot(gm/m^2); HI = harvest Index; TSW =thousand seed weight (gram); DF = days to flowering; DM = days to maturity; SFP =seed filling period; SPE = seed production efficiency; SFR = seed filling rate; SYPD = seed yield per day; BMPR = biomass production rate; EGR = economic growth rate; CP = crude protein content.

Table 2. Combined analysis of variance for 23 traits of thirty-two Ethiopian faba bean varieties tested at Sinana, Agarfa and Selka during cropping season of 2014.

Traits	Loc (df=2)	Rep(loc) (df=3)	Variety (df=31)	Variety xloc (df=62)	Pooled error (df=93)	Mean	CV (%)	R^2
Leaf length (cm)	22.37**	0.22	0.91**	0.26**	0.09	9.32	3.26	0.91
Leaf width W (cm)	9.52**	0.40**	0.70**	0.14**	0.08	4.49	6.40	0.87
Leaf area (cm^2)	1108.08**	51.06**	59.38**	12.341**	10.21	29.72	10.75	0.84
Leaf area index	235538.98**	1329.23	4385.03**	3065.35**	1326.59	223.19	16.32	0.87
Pod length (cm)	0.96*	0.46	7.28**	0.3ns	0.24	7.28	6.78	0.91
Pod width (cm)	0.24**	0.07	0.17**	0.03ns	0.03	1.34	12.65	0.75
Internod length (cm)	27.54**	1.32	0.73**	0.51**	0.3	5.93	9.22	0.80
Plant height to first pod (cm)	4396.84**	24.60	118.54**	35.81*	22.78	43.79	10.90	0.87
Plant height (cm)	1380.68**	23.14	163.21**	42.12**	15.56	149.07	2.64	0.88
Numbers of branch per plant	2.48ns	1.08	1.57ns	1.44ns	1.20	1.69	25.68	0.74
Numbers of pods per plant	3081.89**	131.75	265.52**	53.14ns	51.61.00	33.82	21.24	0.79
Numbers of seeds per plant	23249.83**	42.41	1547.71**	328.94ns	284.56	88.49	19.08	0.81
Numbers of seeds per pod	0.13**	0.01	0.12**	0.05**	0.02	2.63	5.10	0.81
Biomass per plot (g/plot)	4189960.94**	65377.60	858732.78**	233697.5**	90807.71	3361.72	8.96	0.85
Seed yield per plot (g/plot)	3441447.89**	23325.12	179331.08**	888862.18**	10620.27	1438.22	7.17	0.95
Harvest index (%)	1590.82**	171.10	117.53**	62.55ns	44.90	43.39	15.45	0.73
Thusound seed weight (g)	288830.59**	547.00	46671.63**	17542.47**	4873.54	489.88	14.25	0.85
Days to flowering	649.83**	3.24	9.10**	5.05**	1.40	54.44	2.17	0.94
Days to flowering	482.92**	9.53	60.92**	33.95**	4.21	143.77	1.40	0.93
Seed filling period	188.26**	10.11	65.44**	32.92**	4.84	89.32	2.46	0.91
Seed production efficiency	310466638.86**	38423.81	1161147.27**	419447.58**	69442.9	2895.21	9.10	0.95
Economic growth rate	1926.35**	7.92	38227**	144.09**	53.89	41.75	17.58	0.83
Crude protein content (%)	23.81**	0.37	2.23**	1.98**	0.53	22.13	3.30	0.83

*** = Significant at p<0.001; ** = significant at p<0.01; * = significant at p<0.05; ns = no significance; CV = coefficient of variation.

Meenakshi et al., 2013) and mung bean (Singh et al., 2013). The primers were tested for their variability and reproducibility using four representative varieties selected based on their genetic background. Finally, 11 primers with good banding pattern, polymorphism and reproducibility for genotyping were used (Table 6).

ISSR-PCR amplification was carried out in a total reaction volume of 25 µl: 16.7 µl double-distilled water, 3.0 µl of $MgCl_2$ (25 mM), 2.5 µl of 10x PCR buffer, 1.0 µl of dNTP (100 mM equimolar solutions of each dATP, dCTP, dGTP and dTTP), 0.4 µl primer (20 mM), 0.4 µl (5 U/ µl) of Taq polymerase, and 1 µl of template DNA (70 ng/µl). PCR amplification were carried out in BiometraTpersonal (Applied Biosystems, USA). Amplification condition was set as: an initial denaturing at 94°C for 4 min followed by 40 cycles denaturation at 94°C for 15 s, primer-specific annealing temperature for 1 min and extension at 72°C for one and half min and completed with extension at 72°C for 7 min and the PCR products were stored at 4°C. The amplified products were separated on 1.67% agarose gel (w/v) in 1xTBE (Tris base, boric acid and EDTA) buffer and then post stained with ethidium bromide. The fragment size and molecular weight were estimated using 100 bp DNA ladder.

Data collection and analysis

Morphological data analysis

Field data were collected on either an individual plant basis for some characters (from five random plants) or on a plot basis according to descriptors of IBPGR, ICRISAT and ICARDA (1985) (Table 2). The protein analysis was measured based on Micro-Kjeldahl technique (AOAC, 2000). The SAS software packages (SAS Institute, 2003), was used to test the normality of the experimental error and detect the presence of outliers. Analysis of variance

(ANOVA) was performed using the generalized linear regression model to compare variation between the genotypes and means were separated by least significant difference (LSD) test at p<0.05. Genotypic and phenotypic variance with their coefficients of variation was estimated as described by Sharma (1998). Data for all traits were pre-standardized to means of zero and variance of unit before clustering and principal component analysis to avoid bias due to differences in measurement scales (Sneath and Sokal, 1973). Cluster analysis based on phenotypic traits using UPGMA clustering was done to group the faba bean genotypes into genetically distinct classes. Appropriate number of clusters was determined by using points where local peaks of pseudo F-statistics join with small values of the Pseudo t^2 statistics followed by a larger Pseudo t^2 for the next cluster fusion (SAS Institute, 2003). Genetic distance between pair of clusters as standardized Mahalanobis's D^2 statistics was calculated based on the recommendation of Singh and Chaudhary (1996). The principal component analysis (PCA) was done using MINTAB version 14.00 (MINTAB, 2003).

Molecular data analysis

Clearly distinguishable and reproducible fragments generated on gel from ISSR- PCR product was photographed using UV (Bioscens SC750) and scored based on a binary matrix "0" coded for absence, and "1" for presence of a band. Genetic diversity parameters: number of polymorphic loci, percent polymorphism, means of Nei's genetic diversity and Shannon diversity index were analyzed using POPGENE version1.32 (Yeh et al., 1999). Jaccard's similarity coefficients were computed using NTSYS- pc version 2.02 (Rohlf, 2000) set on SIMQUAL module. A dendrogram was constructed based on the similarity matrix using un-weighted pair group method with arithmetic mean procedure of the Sequential, Agglomerative, Hierarchical and Nested (SAHN) clustering methods (Sneath and Sokal, 1973). Principal coordinate analysis (PCoA) based on Jaccard's coefficient were done to examine the patterns of variation among individual genotypes using PAST version 1.18 (Hammer et al., 2001) software.

RESULTS AND DISCUSSION

Morphological diversity

Performance of varieties

The highest pooled mean yield was recorded for the variety, Walki (5391.84 kg ha^{-1}), whereas the lowest mean was obtained from Bako local variety (2955.59 kg ha^{-1}) (Table 1). The mean crude protein content over combined locations ranged from 20.59 to 22.92% for varieties Holetta-2 and Dagm, respectively. The report by Griffiths and Lawes (1978) revealed a wide range of crude protein contents variation among faba bean genotypes ranging from 20 to 40%; though, they observed low protein percentage from Ethiopian faba beans. Chavan et al. (1989) also reported variation among faba bean genotypes ranging from 20 to 41%, which agrees, at least in part, with the present study. Kelly (1973) and Bond et al. (1985) reported differences in the crude protein contents of legumes for environmental conditions, genotypes, and agricultural practices and reported inheritance of this trait is additive with some partial dominance.

Pooled analysis of variance across the three locations showed highly significant differences between varieties (*p<0.01*) for all measured traits (Table 2) consisted with observed genetic variability among the varieties. Genotype by environment interaction was highly significant (p< 0.01) for the traits considered except for plant height to first pod (p< 0.05). Sharifi (2015), Ammar et al. (2015) and Alghamdi (2007) reported highly significant differences in days to flowering and maturity, pod length, plant height, number of seeds per plant and thousand seeds weight. Sharifi (2014) found highly significant differences between faba bean genotypes from Iran in seed yield per plot, numbers of pods per plant, number of seeds per pod, pod length and thousand seeds weight. Gemechu et al. (2005) also reported highly significant differences between landraces across combined locations in days to maturity, thousand seeds weight and seed yield per plot. This indicates that the performance of faba bean varieties could be affected significantly by environmental condition. Ammar et al. (2015) reported similar result. This suggests that the performance of faba bean varieties is significantly and perhaps differentially affected by environmental conditions.

Estimation of variance components

The amount of genotypic and phenotypic variability existing in a species is the most important point for crop improvement selection criteria. High GCV (>10%) were observed for pod length, pod width, number of pods per plant, number of seeds per plant, biomass weight per plant, seed yield per plant, thousand seeds weight and seed production efficiency (Table 5). In contrast, the lowest GCV (<5%) were observed for leaf length, internode length, plant height, days to flowering, days to maturity and crude protein content. Higher genotypic coefficient of variation for thousand seeds weight and moderate for number of seeds per plant were observed for faba bean from Sudan (Aziz and Oman, 2015). They also reported low genotypic coefficient of variation for number of pods per plant, number of seeds per pod and seed yield per plant (Aziz and Oman, 2015). Tafere et al. (2013) also found high GCV for biomass and number of pods per plant in their study of genetic variability, heritability and correlation in some faba bean genotypes grown in Northwestern Ethiopia. Alghamdi (2007) also reported high genotypic coefficient of variation for days to flowering, number of pods plant and thousand seeds weight.

The highest PCV (>10%) were observed for traits such as leaf area, leaf number per plant, leaf area index, pod length, pod width, number of branches per plant, internode length, plant height to first pod, numbers of pods per plant, numbers of seeds per plant, biomass weight per plant, seed yield per plant, harvest index per plant, biomass weight per plot, seed yield per plot,

Table 3. Mean, genotypic variance, phenotypic variance, environmental variance, genotypic coefficient of variation, phenotypic coefficient of variation, heritability in broad-sense and genetic advance as percent in mean of Ethiopian faba bean.

Traits	Mean	σ^2e	σ^2g	σ^2gl	σ^2p	GCV (%)	PCV (%)	Hb (%)	GA	GA (%)
Leaf length (cm)	9.32	0.09	0.11	0.08	0.28	3.54	5.69	71.88	0.79	8.43
Leaf width W (cm)	4.49	0.08	0.09	0.03	0.20	6.59	9.99	78.30	0.72	16.11
Leaf area (cm^2)	29.72	10.21	7.83	1.10	19.14	9.41	14.72	79.09	7.13	23.99
Leaf area index	223.20	1326.59	219.63	870.33	2416.55	6.64	22.02	30.05	30.43	13.63
Pod length (cm)	7.28	0.25	1.00	0.03	1.27	13.71	15.49	95-.20	2.21	30.37
Pod width (cm)	1.34	0.03	0.02	0.00	0.05	11.28	17.22	81.03	0.38	28.75
Internod length (cm)	5.93	0.30	0.04	0.11	0.44	3.21	11.20	29.81	0.41	6.88
Plant height to first pod (cm)	43.79	22.78	13.79	6.52	43.09	8.48	14.99	69.78	9.44	21.55
Plant height (cm)	149.07	15.56	20.18	13.28	49.02	3.01	4.70	74.19	10.70	7.18
Numbers of branch per plant	1.69	1.20	0.02	0.12	1.34	8.81	68.57	8.45	0.20	11.94
Number of pod per plant	33.82	51.61	33.62	0.77	86.00	17.14	27.42	79.15	15.12	44.71
Number of seed per plant	88.43	284.56	203.13	22.19	509.88	16.12	25.54	78.75	36.63	41.42
Number of seed per pod	2.63	0.02	0.01	0.02	0.05	4.34	8.25	60.86	0.27	10.34
Biomass per plot (g/plot)	3361.72	90807.71	104172.55	71444.90	266425.15	9.60	15.35	72.79	773.93	23.02
Seed yield per plot (g/plot)	1438.22	10620.27	15078.15	39120.96	64819.38	8.54	17.70	50.45	264.58	18.40
Harvest index (%)	43.39	44.90	9.16	8.83	62.89	6.98	18.28	46.78	7.64	17.61
Thousand seed weight (g)	489.88	4873.54	4854.86	6334.47	16062.86	14.22	25.87	62.41	162.95	33.26
Days to flowering	54.44	1.40	0.68	1.83	3.90	1.51	3.63	44.49	1.81	3.33
Days to flowering	143.77	4.21	4.49	14.87	23.58	1.47	3.38	44.26	4.43	3.08
Seed filling period	89.33	4.84	5.42	14.04	24.30	2.61	5.52	49.70	5.05	5.65
Seed production efficiency	2895.21	69442.90	123619.45	174993.84	368056.19	12.14	20.95	63.88	798.32	27.57
Economic growth rate	41.75	53.89	39.70	45.10	138.68	15.09	28.20	62.31	15.12	36.20
Crude protein content (%)	22.13	0.53	0.04	0.73	1.30	0.92	5.15	11.23	0.26	1.19

σ^2e = Environmental variance; σ^2g = genotypic variance; σ^2gl = genotype by environment interaction variance; σ^2p = phenotypic variance; GCV = genotypic coefficient of variation; PCV = phenotypic coefficient of variation; Hb = heritability; GA = genetic advance.

harvest index, thousand seed weight, seed production efficiency and economic growth rate (Table 3). In this study, the PCV values were greater than GCV values across the environment which is consistent with other scientist report (Alghamdi, 2007; Tafere et al., 2013; Aziz and Oman, 2015).

Heritability and genetic advance as a percent of mean

In this study, heritability value ranged from 8.45 to 95.2% for number of branches per plant and pod length, respectively (Table 3). Heritability values were sufficiently high for traits like pod length (95.2%) and pod width (81.03%) suggesting that these traits could be selected for in tradition breeding program. This show that environmental factors exerted minimal influence on detectable heritability, that is, environmental responses were separate heritable component (Sharifi, 2015). It is concluded that selection based on means would be useful for the selection for these traits in the faba bean varietal development.

Moderately high heritability values were observed for characters such as leaf length, leaf width, leaf area, plant height to first pods, plant height, number of pod per plant, number of seed per plant, number of seed per pod, biomass weight per plot, thousand seed weight, and seed production efficiency which indicates the possibility of improvement via selection for these traits. Consistent with the results presented here, other workers have reported moderately high heritability for these traits. Hence, high heritability values for most of the characters could be

Table 4. Lists of genotypes grouped under different clusters.

Clusters	Number of genotypes	Name of varieties
1	10	Mosisa, Adet Hanna, Tesfa, Mesay, NC-58, Degaga, Kassa, Kuse, Bulga 70, Sinana Local
2	14	Tumsa, Gebelcho, Holetta-2, Hachalu, Obse, Gachena, Didae, Shallo, Walki, Moti, EKCSR-02006, Agarfa local, Adet local, Kulumsa local
3	6	Dagm, Wayu, CS-20-DK, Lalo, Bako local, Eniwari local
4	1	Dosha
5	1	Gora

attributed to the relatively favorable environment at combined locations (Alghamdi, 2007; Mellion et al., 2012; Teferen et al., 2013; Aziz and Oman, 2015). Intermediate heritability values were recorded for harvest index, grain yield, grain filling period, days to flowering, days to maturity, biomass weight per plant and seed yield per plant. Alghamdi (2007) obtained the highest heritability for days to flowering and maturity. Conversely, low heritability values were recorded for leaf number per plant, leaf area index, internode length, number of branch per plant, harvest index per plant and crude protein content.

Genetic advance as a percent mean ranged from 1.19% for crude protein content to 44.71% for number of pods per plant (Table 3). Johnson et al. (1955) concluded that broad sense heritability, together with genetic advances are usually more useful than heritability alone in predicting the resultant effect of selection. In the present study, high genetic advances as percent of mean with high heritability was observed on traits: pod length, pod width, leaf area, number of pods per plant, number of seed per plant, biomass weight per plot, thousand seed weight and seed production efficiency. The report by Kalia and Sood (2004) showed high heritability and high genetic advance for number of pod per plant which indicated high additive gene action and possibility of trait improvement through selection.

Extent and pattern of diversity based morphological characteristics

Cluster analysis based on morpho-agronomic traits distinguished five distinct groups of faba bean genotypes. The number of individuals in each of the five clusters ranged from one to fourteen in the smallest and largest clusters, respectively. Cluster I consisted of 9 genotypes, cluster II of 14 genotypes, cluster III of 6 genotypes, cluster IV and V, one genotype each (Table 4 and Figure 1). The first cluster contained genotypes derived from hybridization, introduced materials and selected from landraces. Even though genotypes were grouped together based on their morphological similarity, the clusters did not necessarily include all genotypes from same genetic background. The genetic diversity of faba bean genotypes using cluster analysis and relationships within and among individuals and populations has been described elsewhere (Polignano et al., 1993; Gemechu et al., 2005; Chaieb et al., 2011; Yahia et al., 2012).

The pair wise generalized squared distances (D^2) showed highly significant difference ($p<0.01$) among inter-clusters (Table 5). The maximum distance was found between C1 and C5 (D^2 =554.60), while a minimum distance (D^2 = 54.80) was observed between C1 and C3. The high values of inter cluster distances indicated divergence among the varieties and might be used in breeding programs for better genetic recombination and selection of genetically divergent parents for exploitation in crossing programs. This finding is consistent with Million and Habtamu (2012) who used twenty-five elite faba bean genotypes to study genetic variability of seed yield and yield related traits and found high D^2 value. Gemechu et al. (2007) also reported divergence between Ethiopian germplasms.

The principal components analysis (PCAs) with Eigenvalue greater than one contributed 85% of the entire diversity among the genotypes. The first three PC_s contributed 66% (PC1 = 38%, PC2 = 19% and PC3 = 9%) of total variation among Ethiopian faba bean varieties. This agrees with the results reported by Gemechu et al. (2005) and Yahia et al. (2012).

Molecular diversity

Magnitude of diversity as revealed by ISSR markers

Eleven ISSR primers amplified a total 120 bands, of which 107 loci were polymorphic ranging from 5 (ISSR 818) to 17 (ISSR 811) with an average of 90% polymorphism (Table 6). The size of all amplified bands ranged from 200 to 3000 bp (Figure 2). Average number of bands and polymorphic fragments per primer were 11 and 10, respectively. Analysis of percent polymorphisms per primer signified 100% polymorphic for primers ISSR 811, ISSR 860, ISSR 873 and ISSR 881 followed by the primer ISSR 854 with 90% polymorphisms, indicating that these primers were much better for resolving genetic

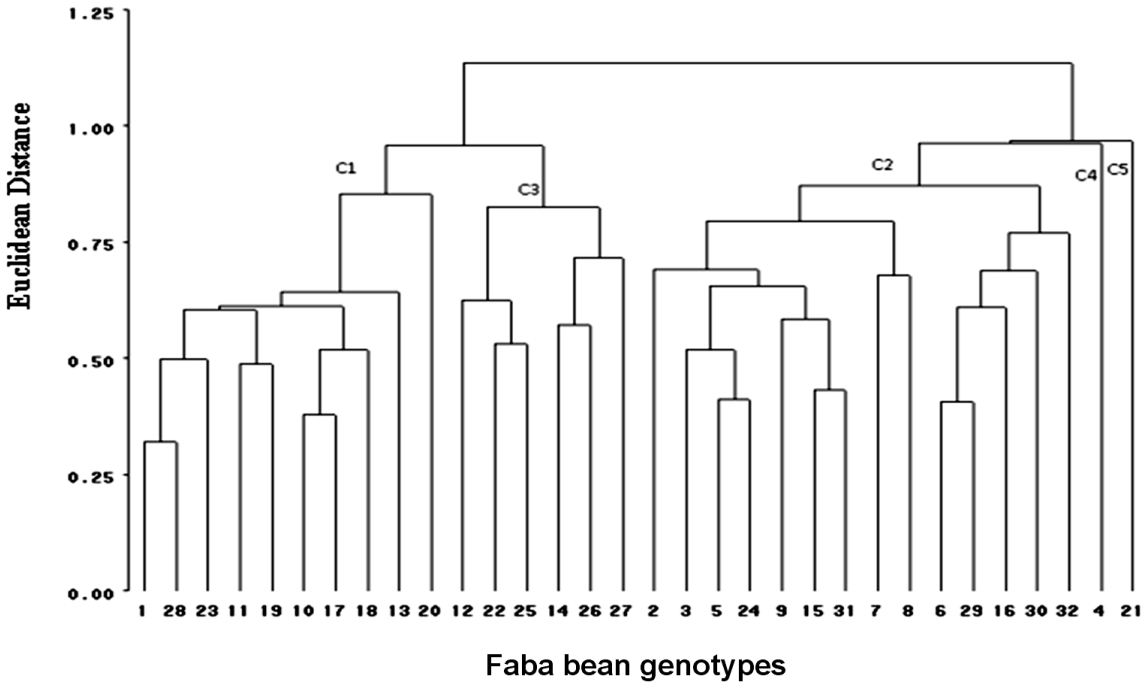

Figure 1. Dendrogram with UPGM and Euclidean distance showing relationship among 32 Ethiopian faba bean genotypes using 23 quantitative traits. Where 1 = Mosisa; 2 = Tumsa; 3 = Hachalu; 4 = Dosha; 5 = Gachena; 6 = Walki; 7 = Obse; 8 = Moti; 9 = Gabelcho; 10 = Adet Hanna; 11 = NC-58; 12 = Wayu; 13 = Degaga; 14 = Dagm;15 = Holetta-2; 16 = Shalo; 17 = Tesfa; 18 = Mesay; 19 = Bulga 70; 20 = Kassa; 21 = Gora; 22 = CS-20-DK; 23 = Kuse; 24 = Dida'a; 25 = Lalo; 26 = Bako local; 27 = Eniwari local; 28 = Sinana local; 29 = Agarfa local; 30 = EKCSR-02006; 31 = Adet local; 32 = Kulumsa local.

Table 5. Distances between nine clusters of 32 faba bean varieties grown in Ethiopia.

Clusters	C1	C2	C3	C4	C5
C1	0	80.54**	54.80**	411.55**	554.60**
C2		0	89.76**	376.91**	358.07**
C3			0	388.79**	459.37**
C4				0	306.27**
C5					0

** = Highly significant at probability level, $p<0.01$ ($\chi^2_{22} = 40.29$).

diversity of faba bean varieties

There is substantial variation in the degree of polymorphisms in reported genetic diversity of faba bean germplams using ISSR markers in the literature, while investigators have reported lower average percentages polymorphism, than found in the present study on genetic diversity of faba bean germplasms using ISSR markers (Terzopoulos and Bebeli, 2008; Abdel-razzak et al., 2012; Basheer-Salimia et al., 2013; Hou et al., 2014; Aziz and Oman, 2015).

Recently, Ammar et al. (2015) showed highest level of percent polymorphisms (100%) using 6 SRAP and 4 AFLP primers on faba bean genetic diversity assessment from Saudi Arabia. Mejri et al. (2012) also described 97.3% of polymorphism to study the effect of gamma radiation on 22 faba bean genotypes from Tunisia using15 ISSR primers. Similarly, Wang et al. *(*2012) reported percentage of polymorphisms that ranges from 91 to 100% with an average of 93% in a study of genetic diversity and relationship of global faba bean germplasms collected from across the world. Salazar-Laureles et al. (2015) also found percent polymorphisms ranging from 71.4 to 100% with an average of 91.3% in an analysis of genetic variability within Chilean faba bean accession using ISSR markers.

The higher average percent polymorphism per primer

Table 6. Diversity parameters used to reveal diversity of Ethiopian faba bean varieties based on 11 ISSR primers

Primer	RM	NSB	NPL	PP	Na	ne	h	I
811	(GA)8C	17	17	100.00	2.00±0.00	1.40±0.31	0.25±0.15	0.41±0.18
812	(GA)8G	15	12	80.00	1.800±0.41	1.45±0.40	0.26±020	0.39±0.28
818	(CA)8G	7	5	71.43	1.71± 0.49	1.38± 0.40	0.23±0.20	0.35±0.28
848	(CA)8RG	9	7	77.78	1.78±0.44	1.27±0.27	0.18±0.15	0.30±0.22
854	(TC)8RG	10	9	90.00	1.90±0.32	1.32±0.2	0.22±0.12	0.37±0.18
857	(AC)8YG	10	8	80.00	1.80±0.42	1.30±0.37	0.18±0.20	0.28±0.27
860	(TG)8RA	9	9	100.00	2.00±0.00	1.66±0.29	0.38±0.11	0.56±0.13
864	(ATG)6	11	9	81.82	1.82±0.40	1.36±0.32	0.23±0.18	0.36±0.24
873	(GACA)4	13	13	100.00	2.00±0.00	1.57±0.34	0.33±0.15	0.50±0.18
880	(GGAGA)3	9	8	60.00	1.60±0.52	1.29±0.36	0.18±0.20	0.27±0.28
881	(GGGTG)3	10	10	100.00	2.00±0.00	1.52±0.31	0.32±0.13	0.49±0.16
Total		120	107	90.00	1.90±0.30	1.43±0.32	0.27±0.16	0.41±0.23

RM = repeat motif; NSB = numbers of scored bands; NPL = number of polymorphic loci; PP = percent polymorphisms; Na = number of allele; ne = effective number of allele; h = gene diversity; I = Shannon diversity index.

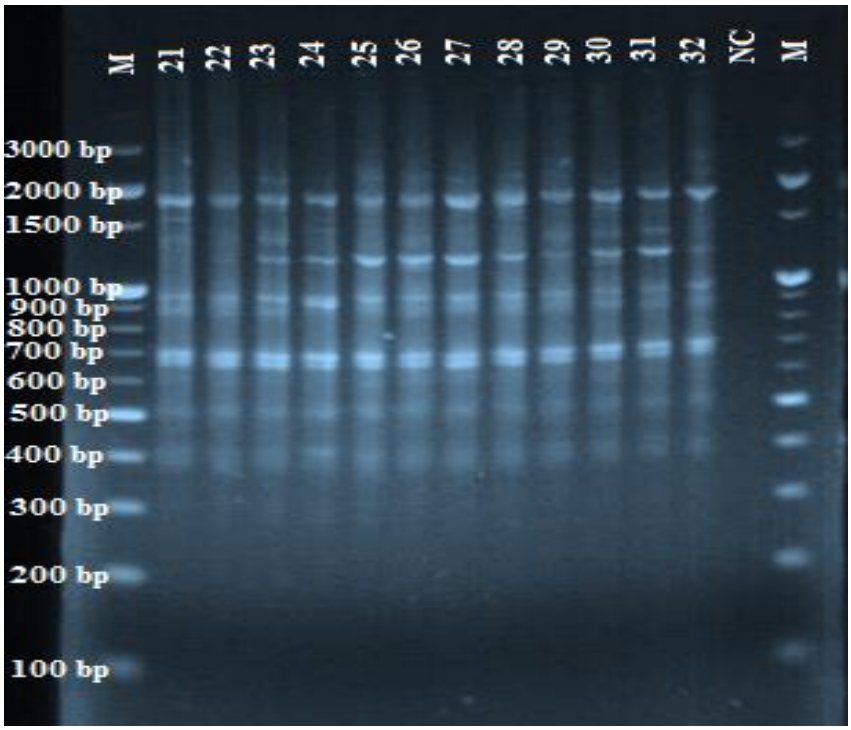

Figure 2. ISSR fingerprint generated from 12 individuals of faba bean varieties using primer 873.

(90%) observed in this study showed that the capacity of ISSR primers were capable of revealing the genetic diversity within and between groups of faba bean varieties, at least those investigated here. The ISSR primers considered in the present study could be used in further studies to identify genetic diversity of faba bean germplasms. The degree of polymorphism among the groups/category of faba bean varieties ranged from 37.5% (45 loci) for the local (farmer) varieties to 84.17% (101loci) for the varieties derived from hybridization (Table 7). The highest polymorphisms observed in the varieties derived from hybridization as compared to local varieties could also be explained by the broader spectrum initially acquired from subsequent genetic recombination during crossing program in the national breeding programs which introduced new alleles to these

Table 7. Diversity parameters indicating the variability of four categories of faba bean genotypes in the present study.

Population type	NPL	PP	na	ne	h	I
Hybridization	101	84.17	1.84+0.37	1.44+0.34	0.27+0.17	0.40+24
Local selection	92	76.67	1.77+0.42	1.39+0.34	0.24+0.18	0.37+0.25
Introduction selection	56	44.67	1.44+0.5	1.30+0.35	0.17+0.20	0.26+0.28
Local varieties	45	37.5	1.37+0.48	1.19+0.32	0.12+0.17	0.18+0.25
Over all genetic diversity	107	90.00	1.90±0.30	1.43±0.34	0.27±0.16	0.41±0.23

NPL = number of polymorphic loci; PP = percent polymorphisms; na = number of allele; ne = effective number of allele; h = gene diversity; I = Shannon diversity index.

varieties.

Using 11 ISSR primers, varieties developed via hybridization revealed the highest gene diversity (h = 0.27) and Shannon diversity index (I = 0.41), whereas farmer (local) varieties showed the least gene diversity (0.12) and Shannon diversity index (0.18). The average gene diversity and Shannon diversity index per primer ranged from 0.18 to 0.38 and 0.27 to 0.56 with mean value of 0.27 and 0.41, respectively (Table 6). The present study is comparable with Wang et al. (2012) who used 11 ISSR primers and found gene diversity and Shannon diversity indices ranging from 0.18 to 0.26 and 0.27 to 0.39, respectively using 11 ISSR primers.

The highest gene diversity (0.38) and Shannon index (0.56) were recorded by primer 860 and followed by primer 873 with high gene diversity (0.33) and Shannon index (0.50) and this indicated that primers 860 and 873 were better able to detect genetic diversity of these Ethiopian faba bean varieties. The least gene diversity (0.18) was obtained from primers 848 and 857.

Cluster analysis and pattern of grouping

UPGMA cluster analysis based on data from 11 ISSR primers grouped faba bean varieties into three distinct clusters and showed relationships among Ethiopian faba bean varieties (Figure 3). However, some of the varieties spread all over the dendrogram without forming strict grouping based on their breeding information. The out-crossing habit of faba bean has its own impact on the intermixing of varieties from different genetic backgrounds into similar or the same cluster. The C1 mostly contained varieties derived from hybridization which includes: Mosisa, Hachalu, Tumsa, Gachena, Obse, Walki, Moti and Gebelcho and two varieties derived from local collection (Dosha and Adet Hanna). The other possible reasons for grouping of these varieties into same cluster could be the breeding objectives designed by breeder. The breeding objectives of faba bean were ultimately designed to improve faba bean genotypes for their seed yield, resistance to biotic and abiotic factors and recently for seed size. Therefore, these common objectives could make the materials to carry similar gene responsible for yield, seed size and resistance to biophysical stresses

The present clustering concedes with the finding of Abdel-razzak et al. (2012) who studied the genetic diversity in 10 Egyptian faba bean genotypes using ISSR grouped into individuals depending on their genetic similarity. Wang et al. (2012) also grouped 802 global faba bean accessions into four groups based on their genetic similarity in their studies of genetic diversity and relationship of global faba bean accession. Salazar-Laureles et al. (2015) grouped 39 faba bean accessions into six clusters based on their genetic similarity coefficient ranging from 0.38 to 0.83, suggesting wide genetic variability between accession at molecular level. So, these findings agree with the recent result obtained in genetic diversity studies of Ethiopian faba bean varieties.

From the cluster analysis, the estimated genetic similarity among faba bean varieties in this study ranged from 0.43 to 0.77 (Figure 3). Depending on an estimated genetic similarity matrix, the highest genetic similarity value was observed between Mesay and Bulga-70 (0.77), followed by between Sinana local and Agarfa local (0.76) and between Kuse and Lalo (0.75). The causes of high similarity between Sinana and Agarfa local varieties, could be, both varieties are found in similar geographical location and the probability of seed exchange between farmers is high. Kuse and Lalo varieties also showed high genetic similarity with each other and these varieties were released for vertisol areas and they could carry similar gene to tolerate waterlogging problem. This similarity coefficient shows that these varieties are genetically more similar and hence the hybridization between these groups may not be considered useful in getting desirable segregating materials. The least similarity value was observed between varieties Obse and Didea (0.29), followed by association between Gachena and Didea (0.32), Tumsa and Didea (0.35) and Mosisa and Lalo (0.36), which were the most genetically distant of all varieties in the present study. It is suggested that it may be useful to include such lines in hybridization programs that seek to enhance genetic variability of Ethiopian faba bean varieties. This may additionally

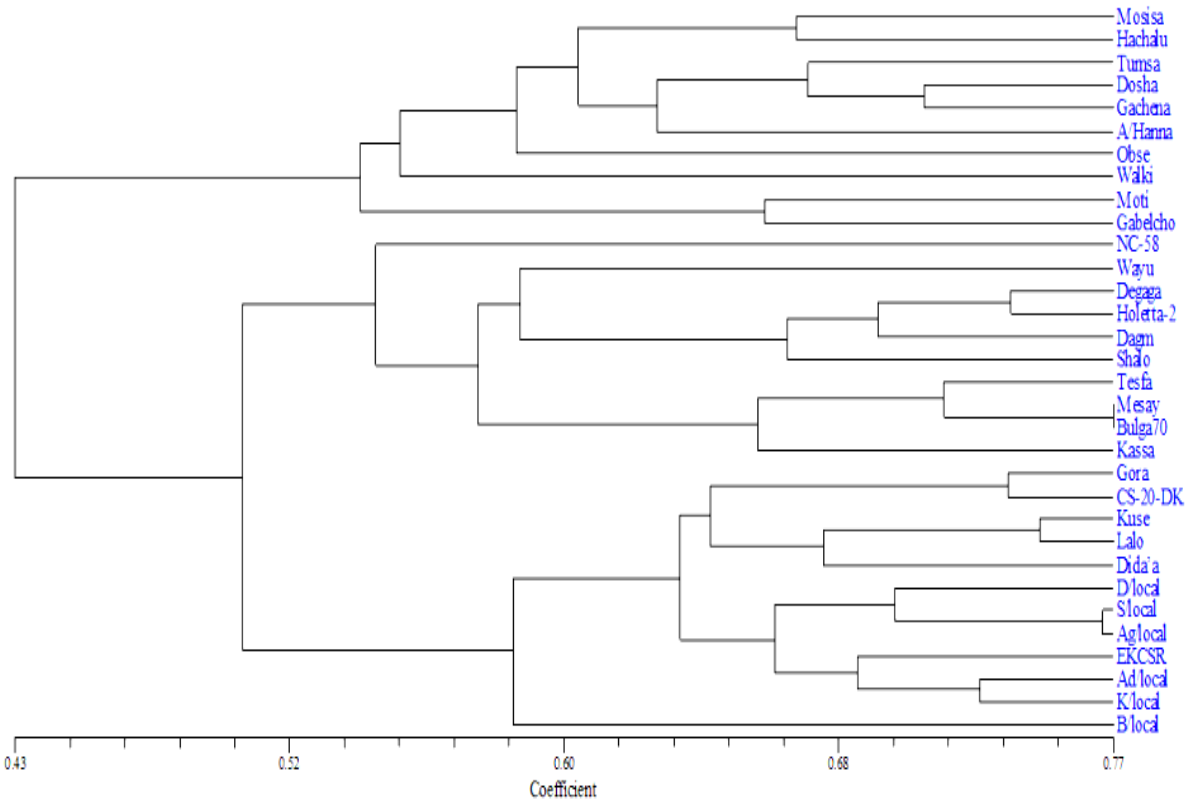

Figure 3. Dendrogram for 32 faba bean genotypes obtained using UPGMA of 120 amplified bands by 11 ISSR primers. The UPGMA algorithm is based on Jaccard's similarity coefficients obtained after pair wise comparison of the presence-absence fragments.

provide insight into the potential for trait selection from F2 and subsequent segregating generations from hybridization programs.

Principal coordinates analysis (PCO) based on ISSR data clearly differentiated Ethiopian faba bean varieties and resolved these varieties into three distinct groups. The first three groups predominantly separated faba bean varieties on the basis of their pedigree relationships and , when taken together, explained 24.02% of total variation with PC_1, PC_2 and PC_3 explaining 11.48, 7.28 and 5.26%, respectively. This result is consistent with other studies on faba bean genetic diversity study using ISSR markers (Wang et al., 2012; Salazar-Laureles et al., 2015). Local varieties were clearly separated from the cultivars by dendrogram and PCoA clustering. The "Farmer" variety from Bako, clustered separately from the groups in both dendrograms. Bako and Kulumsa local varieties separated solely from the group in PCoA and indicated genetic distinctness from the materials used in the present study.

Conclusions

Generally, the presence of genetic diversity within a given breeding population provides primary resources of potentially used traits. Methods of identification of useful heritable traits could play an essential role in designing better breeding strategies for genetic improvement aimed at solving the needs of the producers. For such reasons, genetic diversity is a resource that can contribute to the well-being of the present and future generations, if useful traits can be identified and incorporated into gerplasms. This study revealed considerable amount of genetic variation between cultivated faba bean varieties in Ethiopian. However, some varieties were quite more similar which points to the need to broaden the genetic base. Moreover, there should be efforts to maintain and improve gene pool of released faba bean cultivars of Ethiopia by involving divergent parents in the crossing program. The observed genetic variation showed an opportunity of using these materials in a future faba bean breeding program via introgression with other germplasm resources (from either introduction or landrace materials).

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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