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Genetic Diversity in Ethiopian Field Pea (*Pisum sativum* L.) Germplasm Collections as Revealed by SSR Markers

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አህፅርኦት

አተር ከጥንት ጀምሮ በኢትዮጵያ ውስጥ ለምግብነት የሚመረት የጥራጥሬ ሰብል ነው። በአገሪቱ ውስጥ ከ1500 በላይ ተለያይነት ያላቸዉ የአተር ስብስቦች ቢኖርም፣ በተለያይነት ሁኔታ እና መጠን ላይ በሞለኪውላር ደረጃ በተለይ በSSR ማርከር ጥቂት ጥናቶች ብቻ ተደርጓል። በዚህ ጥናት ውስጥ, 142 ተቃራኒ የኢትዮጵያ አተር germplasm ጄኔቲክ ስብጥር SSR ማርከር በመጠቀም ጥናት ተደርጓል። Euclidean የርቀት ማትሪክስ በመጠቀም germplasm በሰባት የተለያዩ ኢጅቦች ወስጥ ተመድበዋል። ሃያ በአንደኛው ምድብ ውስጥ፣ 11 በሁለተኛው፣ 5 በሦስተኛው፣ 41 በአራተኛው፣ 17 በአምስተኛው፣ 18 በስድስተኛው እና 30 በሰባተኛው ምድብ ውስጥ ታጅበዋል። የመጀመሪያ፣ ሁለተኛ እና ሦስተኛ principal components በቅደም ተከተል 76.85%, 6.89% እና 6.06% በስብስቦቹ መካከል ያለውን ልዩነት አሳይተዋል። በሞለኪዩል ስብጥር እና በስብስብ ዞኖች መካከል ወጥ የሆነ ግንኙነት አልታየም፣ ይህም የሚያሣየው በስባስቦቹ ውስጥ እና መካከል ከፍተኛ ተለያይነት በgenotypes ውስጥ መኖሩን ነው። በዚህ ጥናት ውስጥ ጥቅም ላይ የዋለው SSR ማርከር ከ0.33-0.95 polymorphic ያለው ሲሆን ይህም በአንፃራዊ ሁኔታ ከፍተኛ የተለያይነት መረጃ ይዘት (PIC) አሳይተዋል። ይህም የሞለኪውላር ማርከር ጄኔቲካዊ ልዩነት ትንተና ለአተር ጠቃሚ እንደሚሆን ያመለክታል። ጥናቱ ለመስክ አተር ማዳቀል እና ማምበር እንቅስቃሴዎች ላይ ጥቀም ላይ ለማዋል የበዛ ጄኔቲክ ብዝሃነት ሀብት እንዳለ አመላክቷል።

Abstract

Field pea is an ancient legume crop grown mainly for food in Ethiopia. Even though, there are over one thousand five hundred field pea collections, only a few studies has been conducted on the magnitude and pattern of genetic diversity at molecular level particularly with SSR markers. In this study, genetic diversity of 142 contrasting Ethiopian field pea germplasm were investigated using SSR markers. Euclidean Distance Matrix clustered the collections into seven distinct groups. There were 20 collections in Cluster I, 11 in Cluster II, 5 in Cluster III, 41 in Cluster IV, 17 in Cluster V, 18 in Cluster VI and 30 in Cluster VII. The first, second and third principal components accounted for variation of 76.85%, 6.89% and 6.06%, respectively. There was no definite relationship between pattern of molecular diversity and collection zones, enlightening high levels of intra and inter-genetic diversity of the germplasm. The SSRs used in this study showed relatively higher polymorphic information content (PIC), ranging from 0.33 to 0.95. This indicates that markers used in this study would be useful for genetic diversity analysis of pea. The study exposed that there is wealth of genetic diversity in the gene pool to exploit in field pea breeding and conservation endeavors.

Introduction

Field pea (*Pisum sativum* L.) was the original model organism used in Mendel's discovery of the laws of inheritance, making it the foundation of modern plant genetics (Smýkal *et al*., 2012). Field pea belongs to the Leguminosae family (Genus; Pisum, subfamily; Faboideae, tribe; Fabeae). Field pea possess fourteen (2n =14) chromosomes. Its area of origin and initial domestication lies in the Mediterranean region, primarily in the Middle East (Davies, 1976; Hagedorn 1984). Field pea is one of the ancient legume grown in Ethiopia, where two botanical cultivars are known to grow, namely *P. sativum var Sativum* and the native *P. sativum var Abyssinicum*, (Westphal, 1974).

There are wide range of field pea germplasm that puts the country as one of the secondary centers of genetic diversity (Gemechu et al., 2012). Scholars considered high elevation of Ethiopia within the range of the center of origin of the crop (Hagedorn, 1984). In areas where mono cropping is a dominant practice the crop has double advantage in terms of fixing atmospheric nitrogen and it serves as a "break crop" to diseases and pests when rotated with cereals (Gemechu et al., 2016). In Ethiopia, field pea is mainly used to prepare "shiro wet", a stew eaten with local bread made of tef, i.e. "Injera". The crop commonly grown in association with Faba bean (*Viciafabae*), and is important food, cash and "hunger break" crop in highlands of the country (Asfaw *et al*., 1997).

Knowledge of magnitude and pattern of genetic diversity in a given gene pool is a prerequisite for effective breeding. A number of investigators studied the magnitude and pattern of genetic diversity in pea germplasm, including cultivated species and wild relatives (Samec and Našinec, 1995; Zong *et al*., 2008a) using morphological, biochemical and molecular parameters (Smýkal *et al*., 2008). Molecular markers have enormous potential to explore genetic diversity thereby improve the efficiency and precision of conventional plant breeding. Simple sequence repeat markers (SSRs) are among the most widely used markers in crop species (Blair et al., 2007; Sarıkamış *et al*., 2009) which are cost effective, abundant, co-dominant in inheritance, multi-allelic and easily detected by PCR.

Despite the large number of filed pea accessions held in the genebank of Ethiopia, information on the magnitude and pattern of genetic diversity are scanty at molecular level. Only one EST based SSR study conducted on 46 Ethiopian field pea accessions and the study recommended further SSR based studies (Abel *et al*., 2015). Therefore, the objective of this study was to characterize genetic diversity of 142 contrasting Ethiopian field pea

genotypes using SSR markers, thereby depict the magnitude and pattern of genetic diversity for genetic conservation and advancement of field pea breeding improvement.

Materials and Methods

Plant Materials and DNA Preparation

One hundred forty two contrasting field pea germplasm, which were obtained from Holetta Agricultural Research Center, was considered in the study. The germplasm were collected from various altitudes (ranging from 1520-3140 m. above sea level (Figure 1 and Table 1). Genomic DNA was extracted from 100 mg leaves of two weeks old plants. The samples were grinded using geno grinder (Roach Miller, Germany). Modified CTAB/Chloroform-Isoamyl Alcohol Protocol was used for Genomic DNA extraction (Doyle and Doyle, 1987). The quantity and quality of the DNA was determined using a NanoDrop ND-8000 8-position Spectrophotometer (Thermo Scientific) and by horizontal electrophoresis on 1% agarose before setting PCR.

SSR Primers and PCR Amplification

Nine polymorphic EST-derived polymorphic SSR markers for field pea were used in the study (Table 2).The SSR markers was developed from pea ESTs based on the National Center for Biotechnology Information (NCBI) database. The unigenes were used for identifying SSRs via simple sequence repeat identification tool (SSRIT) software and The primers were designed using the Primer Premier 5.0 software with length of 17–24 bp (Gong et, al., 2010).

Primers' working solution with the concentration of to10 pmoles/µl was used for PCR. PCR amplification was performed in 25µl containing 2.5µl of 10x PCR buffer with 15mM $MgCl_2$, 2µl of 10 mM dNTPs (0.2 mM of each nucleotide dNTPs), 0.5 pmoles/µl of each primers, 0.125µl of 5U taq polymers and 30-50 ng/µl gDNA as a template. PCR reactions were done on Eppendorf Thermal Cycler (MJ Research, Waltham, MA, USA) with an initial 3 min of denaturation at 94°C, followed by 30 cycles of 94°C for 30 s, at appropriate annealing temperature of each primers for 30 s, 72°C for 1 min, and with final extension at 72°C for 10 min. Vertical electrophoresis was performed with 6% polyacrylamide gel containing 11ml of 10x TBE, 6.6 g of 19:1 bisacrylamide, 66 µl TEMED, 1.1 ml of 10% APS with final volume of 110 ml by adding nucleus free water. Gel was run for 2:30 h at 150 V and 60 mA and gel red was used to visualize the marker fragments under Bench top UV Trans Illuminator (UVP). PAGE results were

manually scored as present (1) and absent (0) for each SSR band based on the profiles produced by vertical gel system. The score result was used for the diversity analysis and PIC calculation.

Statistical Analysis

Euclidean distance was used for the cluster analysis, i.e. the square distance between two vectors. Principal component analysis (PC) was done by princomp using the R statistical software. Package cluster, NbClust (Charrad et al., 2014) was employed for analysis and visualization, and to determine the optimum number of clusters. Dindex was used to gain intra-cluster inertia and to measure the degree of homogeneity between data associated with a cluster (Lebart et al. 2000). Polymorphic information content (PIC) of SSRs was calculated using the formula: $PIC = 1- \Sigma Pi^2$, where pi represents number of band frequency to characterize the efficiency of each primer to show polymorphic loci.

Table1. List of the test field pea germplasm collections, zones, localities and altitudes of origin

SN	Name of collection	Collection Zone*	Locality	Altitude (m)	SN	Genotypes	Collection Zone	Locality	Altitude (m)
1	FPColl-207/99	NS	WushaWishi	2860	36	FPColl-52/99	SW	Ababoru	Market
2	FPColl-74/00	AR	Bile	2400	37	FPColl-49/00	AR	Shire Kombolcha	2820
3	FPColl-36/99	NW	WoteyeGiorgis	2400	38	FPColl-103/99	NG	Ambezu	2660
4	FPColl-75/00	AR	Bile	2400	39	FPColl-53/99	SW	Grume	2870
5	FPColl-45/00	AR	Chofra Debora	2960	40	FPColl-182/99	NS	Zanjera	2740
6	FPColl-46/00	AR	Gedebasasa	2740	41	FPColl-38/99	NW	Terara	2460
7	FPColl-213/99	NS	MehalDerie	2900	42	FPColl-84/00	AR	Kurofta	2500
8	FPColl-196/99	NS	Gedembo	2820	43	FPColl-67/00	AR	Juyna	2330
9	FPColl-122/99	NG	Afaf	2700	44	FPColl-35/99	NG	Yedogit Michael	2820
10	FPColl-125/99	NG	ArbaTensa	2660	45	FPColl-115/99	NG	Kuara	2600
11	FPColl-47/99	NW	Goshmeda	2490	46	FPColl-129/99	NG	Birkach	2500
12	FPColl-98/99	NG	GonderZuria	2500	47	FPColl-198/99	NS	MentaDebir	2920
13	FPColl-201/99	NS	Begochgat	3100	48	FPColl-66/00	AR	Jeyna	2330
14	FPColl-30/00	AR	Dosaha	2490	49	FPColl-108/99	NG	Baldegie	2600
15	FPColl-193/99	NS	Kitela	2820	50	FPColl-192/99	AR	Bash	2820
16	FPColl-116/99	NG	Tentanie	2680	51	FPColl-68/00	AR	Bulkobalkesa	2400
17	FPColl-191/99	NS	TiftefDingay	2890	52	FPColl-107/99	NG	Hiywetbadema	2700
18	FPColl-195/99	NS	Emego	2820	53	FPColl-78/00	AR	Jedda	2450
19	FPColl-40/99	NW	GurbaGiorgis	2700	54	FPColl-36/00	AR	Digelu Bora	2680
20	FPColl-112/99	NG	Chemelgie	2640	55	FPColl-38/00	AR	Gobesa	2380
21	FPColl-130/99	NG	Selmgie	2300	56	FPColl-71/00	AR	Malkicho	2420
22	FPColl-100/99	NG	Degoma	2500	57	FPColl-65/00	AR	SibeMeraro	2390
23	FPColl-45/99	NW	Boda	2960	58	FPColl-66/99	AR	-	-
24	FPColl-185/99	NS	Keyit	2840	59	FPColl-111/99	NG	Gedebeye	2700
25	FPColl-183/99	NS	Kinbo Ager	2800	60	FPColl-53/00	AR	Tulujebi	1520
26	FPColl-131/99	NG	Selamgie	2300	61	FPColl-70/00	AR	Marfogora	2420
27	FPColl-47/00	AR	Gedebasasa	2450	62	FPColl-79/00	AR	Jedda	2450
28	FPColl-105/99	NG	Wondgate	2900	63	FPColl-40/00	AR	Gobesa	2380
29	FPColl-41/99	NW	Mushmender	FS	64	FPColl-30/00	AR	Dosaha	2490
30	FPColl-58/00	AR	Gadogedemsa	2340	65	FPColl-200/99	NS	Gragne	3140

31	FPColl-99/99	NG	GonderZuria	2500	66	FPColl-126/99	NG	Dequa	2600
32	FPColl-41/00	AR	Biritifarachu	3000	67	FPColl-190/99	NS	Kuromider	2780
33	FPColl-205/99	NS	Gudoberet	2860	68	FPColl-132/99	NG	Woken	2620
34	FPColl-127/99	NG	Abay	2500	69	FPColl-60/00	AR	HiraroSibe	2400
35	FPColl-37/00	AR	Lemu Eddo	2800	70	FPColl-34/00	AR	Digalu Bora	2680
71	FPColl-194/99	NS	Angawa	2840	107	FPColl-187/99	NS	Liymush	2900
72	FPColl-59/00	AR	SibeMeraro	2390	108	FPColl-76/00	AR	Askalo	2420
73	FPColl-186/99	NS	Aba Mute	2900	109	FPColl-33/99	NW	Yewetet	2800
74	FPColl-54/99	SW	Tenta Michael	2780	110	FPColl-42/00	AR	Guji	2800
75	FPColl-215/99	NS	Lemi	Market	111	FPColl-203/99	NS	Debremarkos/M	2900
76	FPColl-51/00	AR	Fajimeti	2310	112	FPColl-210/99	AR	Kura Mariam	3000
77	FPColl-189/99	NS	Ezawiene	2940	113	FPColl-120/99	NG	Miligebsa	3000
78	FPColl-50/99	SW	Gishen	FS	114	FPColl-209/99	NS	Adgo Ager	3000
79	FPColl-54/00	AR	Tulujebi	2700	115	FPColl-188/99	NS	Gudo Beret	2960
80	FPColl-55/99	SW	SibeMeraro	2390	116	FPColl-101/99	NG	GindMetaya	2300
81	FPColl-42/99	NW	Chegoma	2820	117	FPColl-62/00	AR	Kako	2410
82	FPColl-72/00	AR	Bamo Genet	2440	118	FPColl-104/99	NG	Koseya	2800
83	FPColl-52/00	AR	Munesa	2200	119	FPColl-44/99	NW	Yilana	2900
84	FPColl-56/00	AR	JeynaBarbuko	2360	120	FPColl-217/99	NS	-	-
85	FPColl-110/99	NG	Workdemo	2680	121	FPColl-128/99	NG	Chenchit	1540
86	FPColl-69/00	AR	Marfogoragora	2400	122	FPColl-106/99	NG	AmbaGiorgis	2840
87	FPColl-37/99	NW	Yekorit	2350	123	FPColl-121/99	NG	Amanamba	3100
88	FPColl-208/99	NS	Debel	3000	124	FPColl-216/99	NS	-	-
89	FPColl-211/99	NS	Ankober	2940	125	FPColl-73/00	AR	Darole	2420
90	FPColl-35/00	AR	Digelu Bora	2660	126	FPColl-83/00	AR	wadogomsa	2450
91	FPColl-81/00	AR	Jida Town	2460	127	FPColl-31/00	AR	AleltuShala	2590
92	FPColl-30/99	NW	Betehor	2800	128	FPColl-102/99	NG	Ambezu	2660
93	FPColl-44/00	AR	Shashe	2900	129	FPColl-57/99	NG	Godo (Chacha)	FS
94	FPColl-64/00	AR	DenbelKilisa	2400	130	FPColl-197/99	NS	ChirocheAmba	2820
95	FPColl-32/99	NW	Betehor	2810	131	FPColl-114/9	NG	Shimelako	2580
96	FPColl-204/99	NS	Seladingay	2880	132	FPColl-117/99	NG	Dildiye	2800
97	FPColl-214/99	NS	Aleyo	2840	133	FPColl-31/99	NW	Betehor	2810
98	FPColl-212/99	NS	WelieDeneba	2940	134	FPColl-55/00	AR	Efa Lode	2680
99	FPColl-32/00	AR	TemensaGugesa	2520	135	FPColl-57/00	AR	JeynaBarbuko	2360
100	FPColl-80/00	AR	Jidda Jim	2440	136	FPColl-43/00	AR	Guji	2800

101	FPColl-82/00	AR	Wadogomsa	2450	137	FPColl-123/99	NG	Limalimo	2700
102	FPColl-184/99	NS	Arthside	2800	138	FPColl-219/99	NS	-	-
103	FPColl-39/00	AR	Gobesa	2380	139	FPColl-206/99	NS	HausiniAmba	2840
104	FPColl-51/99	SW	Kundi	FS	140	FPColl-34/99	NW	Kon	2820
105	FPColl-61/00	AR	Endeto	2420	141	FPColl-119/99	NG	Kumbel	2960
106	FPColl-33/00	AR	DigeluKidame	2600	142	FPColl-48/99	NW	Goshmeda	2200

** AR = Arsi, NG = North Gonder, NS = North Shewa, NW = North Wollo, SW = South Wollo)*

Table 2. Lists of SSR markers for diversity study: PM1 is code of polymorphic SSR primer one, accession number, SSR motif, primer sequence, expected allele size in bp.

Marker code	Accession no. putative homology	SSR motif	Primer sequence 5'→3'	Allele size range (bp)
PM1	32542470-13; 2542362-1	(tatt)4	F: GCCGAGGTACAAAAGAAGT R: CTGGAAACCAAGAAAAGTG	323
PM2	32544169-1; 32542559	(aac)6	F: CAATGATGGGTGGAAGATG R: AGGCAGTGATTCAGACGGT	337
PM3	32543080-1	(ttc)7	F: GAGCAGCATTTTGTTGGA R: CTGGAGGAGGCTTTCATT	178
PM4	32543524-1	(gaa)5	F: ATCCAGAACTCACAACAT R: TAGAATCAAAACACGACC	242
PM5	2537373-1	(aat)7	F: AAGAGAGGTGTGGTTCA R: ATTTCGTTTTGGTTACG	254
PM6	90646231-1	(tc)10	F: CAACAACACAAATCCAT R: AGTCTCACAACAGCACC	352
PM7	32542612-1	(aac)5	F: ATGAAGCACATGAAA AAT R: TGGTGAGGAGGAAACTAT	212
PM8	32545076-1	(ttgat)3	F: CTCCATCTCAAGAAATCC R: CACATAACTAAAAAACCC	383
PM9	90646520-1	(gca)5	F: CTCTCCCTTTTCATTCCAT R: TTTCGCTTGTCTCCTTGTT	155

Results and Discussion

Magnitude of genetic diversity

The study revealed the presence of high genetic variation among field pea accessions used in this study. Field pea genotypes were clustered into seven distinct groups (I-VII), illuminating the presence of high genetic diversity within and among collection zones (Figure 2). The number of genotypes contained in each cluster varied from 5 in cluster II to 41 in cluster V (Table 3 and Figures 2 and 3).

Genetic distance (GD) values ranged from the minimum of 0.883 (between collections from Arsi and North Wollo) to 3.324 (between collections from North Shewa and South Wollo). The magnitude of genetic distances among genotypes originated from different eco-geographical origins showed more differentiations between collection from South Wollo and the rest, i.e. Arsi, North Gonder, North Shewa and North Wollo. The second largest inter-regional distance range was observed between accessions from Arsi and North Shewa (GD = 1.999) followed by North Wollo and North Gonder (GD = 1.840) (Table 4). Genetic distances between germplasm collections from different eco-geographical origins may be attributed to the nature and degree of both human and natural selection after introduction and/or specificities of ecological and agricultural conditions as major forces of evolution (Ford-Lloyd and Jackson, 1986; Spagnoletti and Qualset, 1987).

Table 4. Population Euclidean genetic distance showing the magnitude of genetic differentiation among field pea populations from different collection zones

Collection Zone	Arsi	North Gonder	North Shewa	North Wollo	South Wollo
Arsi	0.000				
North Gonder	1.238	0.000			
North Shewa	1.999	1.174	0.000		
North Wollo	0.883	1.840	1.424	0.000	
South Wollo	3.318	2.802	3.324	3.285	0.000

Pattern of genetic diversity

Genotypes collected from Arsi and North Gonder were distributed across all clusters, whereas genotypes collected from North Wollo were distributed overall clusters except cluster III, which comprises only a few genotypes (Table 3). This may indicate the presence of genetic differences among genotypes collected from the same places of origin. Genotypes collected from the same areas grouped into different clusters. This describes that genotypes could show genetic similarities regardless of the differences in their places of origin, the probable reason being the fact that Ethiopian farmer's not only do habitually exchange seeds within and outside their vicinity but also select for common traits of interest like grain yield, yield stability, seed color, and abiotic and biotic stresses resistance.

Generally, this study showed that there was no definite relationship between the pattern of genetic diversity and eco-geographic origins of collections. Accordingly, enlightening high levels of intra and inter genetic diversity among the genotypes. Similarly, research results on genetic diversity of morpho-agronomic traits of field pea genotypes (Gemechu *et al.*, 2005) and molecular diversity analysis using EST-SSR markers (Abel *et al.*, 2015) revealed that, despite the high intra and inter-genetic variation among pea accessions, there was no clear relationship between accessions and geographic locations (Figure 3).

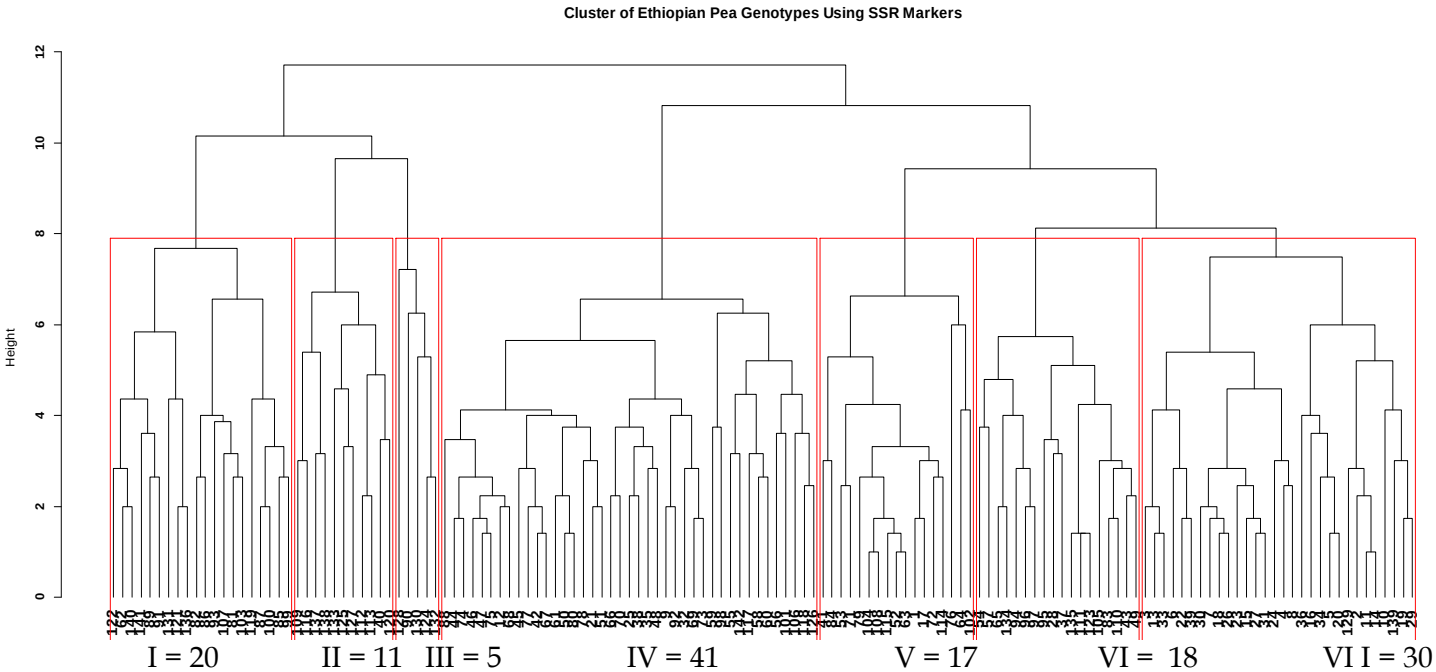

Figure 2. Dendrogram showing the genetic relationships of 142 pea genotypes based on Euclidean Distance Matrix by hclust package analysis using nine polymorphic SSR markers data. Cluster and genotypes per cluster, numbers in each cluster represent serial number (SN) of the genotypes as indicated in table 1.

Table 3. Cluster, genotype serial number (SN), number of genotypes per cluster, collection zone and number of genotypes from each collection zone, and cluster percentage.

Cluster	Genotype SN	Number of genotypes	Collection zone and number of genotype from each collection zone	Cluster percentage
I	(122, 62, 140, 141, 89, 91, 131, 121, 136, 82, 86, 93, 107, 81, 103, 119, 87, 100, 85, 99)	20	(Arsi (9), North Wollo (4), North Gonder(5), North Shewa(2))	14
II	(109, 116, 137, 138, 133, 125, 127, 112, 113, 40 120)	11	(Arsi (3), North Wollo(2), North Gonder(3), North Shewa(3))	8
III	(128, 90, 130, 124, 132)	5	(Arsi(1), North Gonder(2), North Shewa(2))	4
IV	(88, 44, 74, 46, 47, 75, 12, 68, 45, 77, 42, 67, 61, 50, 80, 78, 21, 51, 66, 70, 25, 38, 35, 48, 9, 92, 32, 69, 73, 59, 98, 55, 142, 117, 58, 60, 56, 101, 106, 118, 126)	41	(Arsi(16), North Wollo (2), North Gonder(11), North Shewa(9), South Wollo (3))	29
V	(41, 84, 53, 71, 79, 104, 108, 115, 52, 63, 1, 17, 72, 114, 76, 64, 102)	17	(Arsi(9), North Wollo(1), North Gonder(1), North Shewa(5), South Wollo(1))	12
VI	(54, 57, 65, 134, 94, 96, 97, 95, 28, 37, 135, 111, 123, 105, 83, 110, 43, 49)	18	(Arsi(10), North Wollo(1), North Gonder(3), North Shewa(4))	13
VII	(3, 13, 33, 6, 22, 39, 30, 7, 18, 26, 23, 15, 27, 31, 24, 4, 81, 36, 16, 34, 5, 20, 129, 2, 11, 14, 10, 139, 19, 29)	30	(Arsi(7), North Wollo(5), North Gonder(8), North Shewa(8), South Wollo(2))	21

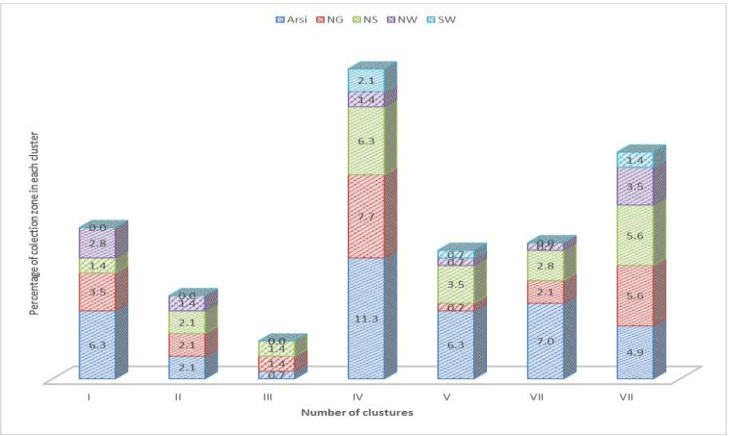

Figure 3. Pattern of distribution of genotypes originated from different eco-geographic regions over the seven distict clusters (AR= Arsi, NG = North Gonder, NS = North Shewa, NW = North Wollo, SW = South Wollo)

Similarly, it has been deduced from polymorphic information content (PIC) that there is genetic variation among germplasm, which ranges from 0.33 to 0.95, the average being 0.79. SSR marker PM6 has shown maximum PIC value, whereas PM2 has shown small PIC values. The range of allele per locus is form 2 to 10, which is similar to SSR studies on field pea (Yang, et al.; 2015) with the average

being 3.027 (Abel et al., 2015). This indicates that the microsatellite markers used in this study are informative and would be useful for genetic diversity analysis in field pea (Figure 4). Genetic variation within and among germplasm was evaluated using PIC value as an index, as it is reported by Botstein et al. (1980). Therefore, genetic diversity of germplasm at each locus is determined using polymorphism information content (PIC), and markers can be classified as informative when PIC value is ≥ 0.5.

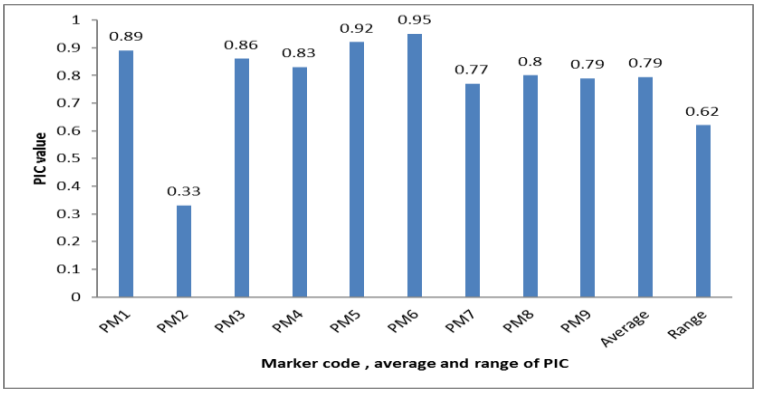

Figure 4. PIC value, average PIC value and range of PIC by SSR markers

Principal component analysis

Principal component (PC) analysis showed that the first three PCs accounted for 76.85, 6.89 and 6.06% of the total variation in that order. Hence, the percent cumulative proportions of the first three PCs explained 90% of the variation of original variables. Likewise, the first five eigenvalues each contributed greater than unity of the original variation (Table 5). Thus, according to the Kaiser's Criterion and purpose of data reduction in factor analysis, in this study the first 5 PCs showed variation in the genetic analysis as much as original variables. Kaiser Criterion suggests retaining factors with eigenvalues greater than unity, in essence that a factor should extract equivalent to unity of one original variable (Kaiser, 1960). Additionally, based on scree plot analysis, which was originally proposed by Cattell (1966), had an elbow turn break supporting that 5 eigenvalues elucidated as much as variation of the original variables. This is in agreement with the ordination methods used by Messmer et al (1993), in which PC has been used in combination with cluster analysis. In this study, the diversity analysis PC1 attributed for higher genetic diversity (76.85%) and about 90% of the information contained in the data retained by the first three principal components (Table 5).

Table 5. Components, eigenvalues, proportion of variance, cumulative proportion and percent cumulative proportion.

Component	Eigenvalues	Proportion of variance	Cumulative proportion	% Cumulative proportion
PC1	1.716	0.769	0.769	76.85
PC2	1.434	0.069	0.837	83.74
PC3	1.070	0.061	0.90	90.00
PC4	1.009	0.032	0.93	92.99
PC5	1.005	0.022	0.951	95.14

Conclusion

This study revealed the existence of adequate genetic variation in Ethiopian field pea germplasm collections. The pea germplasm, which were collected from different collection zones were found to cluster together, indicating that differences in eco-geographic origins may not necessarily guarantee existence of genetic diversity in pea genotypes. Thus, not only genetic diversity among collection zones, but also within collection zones should be considered for pea improvement and conservation plans. The SSRs markers employed in this study were found to be informative and important for marker-based studies of field pea in the future. Field pea breeders could explore the genetic variation in those field pea genotypes. Moreover, the genetic distance revealed in the Ethiopian filed pea genetic materials could also definitely help the species to increase adaptability of the crop to the changing environmental conditions.

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References

Abel Teshome, B Tomas, Kifle Dagne and Mulatu Geleta .2015. Assessment of genetic diversity in Ethiopian field pea (*Pisum sativum* L.) accessions with newly developed EST-SSR markers. BMC Genetics 16:102

Asfaw Negassa, Abdissa Gemeda, Tesfaye Kumsa and Gemechu Gedeno. 1997. Agro ecological and socio-economical circumstances of farmers in east Wolloga zone of Oromiya region. Research report No. 32. Institute of Agriculture Research (IAR). Addis Ababa.

Blair MW, JM Diaz, R Hidalgo, LM Diaz. 2007. Microsatellite characterization of Andean races of common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 116: 29-43.

Botstein D, RL White, M Skolnick RW Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 32(3):314

Cattell RB. 1966. The screen test for the number of factors. Multivariate Behavioral Research, 1, 245-276.

Charrad M, N Ghazzali, V Boiteau, and A Niknafs. 2014. NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. Journal of Statistical Software October 2014, Volume 61, Issue 6.

Davies DR. 1976. Peas. In: Simmonds N.W. (ed.), Evolution of crop plants. Longman, London, pp. 172–174.

Doyle JJ and JL Doyle .1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 19:11-15.

Ford-Lloyd B and M Jackson .1986. Plant Genetic Resources: An Introduction to their Conservation and Use. Edward Arnold, Australia.

Gemechu Keneni, Asnake Fikre and Million Eshete.2016. Reflections on Highland Pulses Improvement Research in Ethiopia. Ethiopian Journal of Agricultural Sciences Special Issue: 17-50

Gemechu Keneni, Endashaw Bekele, Fassil Assefa , Muhammad Imtiaz , Tolessa Debele , Kifle Dagne and Emana Getu. 2012. Phenotypic diversity for symbio-agronomic characters in Ethiopian chickpea (Cicer arietinum L.) germplasm accessions. African Journal of Biotechnology Vol. 11(63), pp. 12634-12651.

Gemechu Keneni, Musa Jarso, Tefera Wolabu, and Getnet Dino .2005. Extent and pattern of genetic diversity for morpho-agronomic traits in Ethiopian highland pulse landraces: I. Field pea (*Pisum sativum* L.). Genetic Resources and Crop Evolution 52: 539–549.

Gong Y, S Xu, W Mao, Q Hu, G Zhang, J Ding, and Y Li. 2010. Developing new SSR markers from ESTs of pea (*Pisum sativum* L.).Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 11(9):702-707.

Hagedorn DJ. 1984. Compendium of pea diseases. University of Wisconsin-Madison.

Kaiser HF. 1960.The application of electronic computers to factor analysis. Educational and Psychological Measurements, 20, 141-151.

Lebart L, A Morineau, and M Piron .2000. Statistique Exploratoire Multidimensionnelle. Dunod, Paris.

Messmer MM, AE Melchinger, RG Herrmann, and J Boppenmaier. 1993. Relationships among early European maize inbreds: II. Comparison of pedigree and RFLP data. Crop Sci. 33: 944-950.

Sarikamiş G, FYasar, M Bakir, and K Kazan. 2009. Genetic characterization of green bean (*Phaseolus vulgaris)* genotypes from eastern Turkey. Genet. Mol. Res. 8: 880-887.

Smýkal P, G Aubert, J Burstin, CJ Coyne, NTH. Ellis, A J Flavell, R Ford, M Hýbl, J Macas, P Neumann, KE McPhee, RJ Redden, D Rubiales, JL Weller, and TD Warkentin. 2012. Pea (*Pisum sativum* L.) in the Genomic Era. Agronomy2: 74-115

Smýkal P, J Horácèk, R Dostálová, and M Hýbl .2008. Variety discrimination in pea (Pisumsativum L.) by molecular, biochemical and morphological markers. J. Appl. Genet. 49: 155-166.

Spagnoletti, PL and CO Qualset. 1987. Geographical diversity for quantitative spike characters in a world collection of durum wheat. Crop Science 27:235-241.

Westphal E. 1974. Pulses in Ethiopia. Wageningen: Centre for Agricultural Publishing and Documentation.

Yang TI, F Li, Z Xiaoyan, H Jinguo, B Shiying, H Junjie, Ling Li, H Yuhua, J Junye, W Fang, T Shufang, Z Xuxiao. 2015. High-Throughput Development of SSR Markers from Pea (*Pisum sativum* L.) Based on Next Generation Sequencing of a Purified Chinese Commercial Variety. PLoS ONE 10(10).

Zong XX, JP Guan, SMWang, and QC. Liu. 2008. Genetic diversity among Chinese Pea *(Pisum sativum* L.) landraces as revealed by SSR markers. ActaAgron. Sinica 34: 1330-1338.