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ISOZYME VARIATION AND NUTRITIONAL ANALYSIS IN FIELD PEA (*PISUM SATIVUM* L.) POPULATIONS FROM ETHIOPIA

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ABSTRACT: Isozyme variation and nutrient composition of 41 field pea (*Pisum sativum* L.) populations were analysed to determine the genetic and nutrient variability within and between the populations. The isozyme analysis showed that of the six loci (AAT-1, AAT-2, EST-1, EST-2, LAP-1, LAP-2) recorded, three were polymorphic (EST-1, EST-2, AAT-2). The mean number of alleles per locus for all populations was 1.4. The mean percentage of polymorphic loci and mean value of expected heterozygosity recorded was 39.8 and 0.132, respectively. The mean F_{ST} = 0.26 indicated the existence of moderate level of variation between populations, although most of the variation occurred within populations. Altitudinal class 4 (2601-2800 m a.s.l.) showed the highest mean heterozygosity (Ho = 0.017, He = 0.189). Higher genetic diversity was observed in Welo (Ho = 0.138, He = 0.201) followed by Tigray (Ho = 0.051, He = 0.189) regions though these regions were represented by relatively few samples. In addition to the isozyme variability within and between the populations, nutrient composition in association with region, altitude, soil texture and soil colour were also studied. The values, on dry weight basis, ranged from 22.82 to 27.90% for protein, 0.67 to 1.06% for fat, 7.37 to 8.77% for fibre, 2.54 to 3.24% for minerals, 7.75 to 9.05% for moisture and 59.85 to 65.12% for carbohydrate. Principal component analysis showed that the accessions that were collected from different regions had similar percentage of nutrient composition and the cluster analysis showed that there was little effect of soil texture, soil colour, altitude and geographical zones (regions) on nutrient composition.

Key words/phrases: Allelic frequency; Genetic diversity; Isozyme; Nutrition; *Pisum sativum*.

INTRODUCTION

Field pea, *Pisum sativum* L., (2n = 14) probably originated in south-western Asia, possibly north-western India, Pakistan or adjacent areas of the former USSR and Afghanistan and spread to the temperate zones of Europe (Kay, 1979; Makasheva, 1983). Important production areas of the world include France, Russia, Ukraine, Denmark and United Kingdom in Europe; China and India in Asia; Canada and USA in North America; Chile in South America; Ethiopia in Africa, and Australia (FAO, 1994). Based on genetic diversity, four centers of origin, namely, Central Asia, the Near East,

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Abyssinia/Ethiopia and the Mediterranean have been recognized (Gritton, 1980). Field pea is a nutritious foodstuff and it is among the four important cultivated legumes next to soybean, groundnut and beans (Hulse, 1994).

According to Harlan (1968), field pea is one of the pulses that were introduced from West Asia to Ethiopia and still possesses wide genetic diversity. It is the third most important staple legume among the highland pulses in rural Ethiopia (Asfaw Telaye *et al.*, 1994). It requires cool, relatively humid climate with temperature range between 13-18°C and is cultivated in the highlands of Ethiopia (1800 to 3000 m a.s.l.), being most common in the temperate zone (2000 to 2500 m a.s.l.) of the central pulse zone. Productivity in the tropics, below 1200 m a.s.l., is usually low (Purseglove, 1987; Asfaw Telaye, 1988). Field pea ranks second in area of production and yield when compared to other pulses (Hailu Beyene *et al.*, 1994). Statistical data from private holdings indicated that 120,000 tons of field pea was produced in the main rainy season and 5.2 tons in the small rainy season in the year 1999/2000 (CSA, 2000).

Diversity existing in a crop of interest is important for crop improvement. Most of the genetic diversity present in peas is reported to be available in the Near East, Mediterranean region, Central Asia and Ethiopia (Van der Maesen *et al.*, 1988).

Reliable information on the existing genetic variation is needed for selection, breeding and conservation programs of genetic resources. Genetic variation that is believed to exist between or within a species can be determined by using morphological, biochemical and/or molecular markers.

Isozymes provide a relatively simple and inexpensive method of obtaining genetic information (Kephart, 1990). Isozyme data allow quantification of the similarity, or difference between populations and groups of populations (Gottlieb, 1977). This method has been used for cultivar identification of commercial peas (Posvec and Griga, 2000), to test polymorhphism in core collection of *Pisum* (Swieciki *et al.*, 2000), to study phylogenetic affinities in *Vicia* (Jaaska, 1997) and to study the genetic diversity in grass pea (Chowdhaury and Slinkard, 2000).

The nutrient composition of field pea makes it suitable for a particular end use. Green peas are marketed fresh, and are eaten cooked as vegetables. They are also prepared as canned or frozen, while ripe dried peas are used whole, split or made into flour (Davies *et al.*, 1985). In Ethiopia, field pea is used in the traditional dish as "Shiro" and "Kik" wot (a sauce made of

powder of roasted pea and split pea, respectively), "Nifro" (boiled peas mixed with other cereals) and soup.

Native soil fertility varies greatly from soil to soil, and so is the way it has been managed over the years. Yield potential, soil types and production inputs must be considered when determining nutrient needs (Benson *et al.*, 1994). In field pea, the rate and duration of plant growth, as well as final grain composition and quality are influenced by prevailing environmental conditions. These include variables such as temperature, day length, water supply, and the availability of soil minerals (Duffus *et al.*, 1993).

Research has indicated that it would be possible to increase protein content by about 2-3% by selection (Kay, 1979). Differing percentages of total protein and ratios of protein components (and of amino acids) can also be modified by breeding and improving the nitrogen fertility of the soil (Stanley *et al.*, 1994).

The aim of this work was to assess the variation existing within and between *Pisum sativum* populations collected from different regions and altitudinal zones of Ethiopia using isozyme markers, and to evaluate nutrient composition in terms of region, altitude, soil texture and soil colour.

MATERIALS AND METHODS

Plant Materials

A total of 41 *P. sativum* accessions from the collection of the Institute of Biodiversity Conservation (IBC) were used for isozyme and nutritional analyses. These accessions were collected from six former administrative regions of Ethiopia, namely, Shewa, Arsi, Harerge, Tigray, Gonder and Welo at different altitudinal zones. Details on collection data are given in Table 1.

Isozyme Analysis

Enzyme Assays and Electrophoresis

For each accession, 20 seeds, germinated at room temperature for 5 days, were used for isozyme analysis. Enzyme extraction was made manually on plexi-glass wells with plexi-glass rod from roots and shoots of germinated seeds, using 2 drops of 0.1 M Tris-HCl, pH 7.0 plus 0.14 M β-mercaptoethanol (Haputli and Jain, 1978). This yielded enough extract to saturate a filter paper 10 mm x 4 mm size. Electrophoresis was performed on 12% starch gels. The gel and the electrode buffer used were slightly

modification from that described by Ashton and Braden (1961).

Table 1 IBC accession number (Acc. No.), regions, altitude (in meters), soil texture and soil color.

Code	Acc. No.	Region	Altitude (m)	Soil texture	Soil color
1	223330	Hararge	2150	clay loam	black
2	223331	Hararge	2130	clay loam	black
3	229213	Shewa	2250	sand	brown
4	229210	Shewa	2340	nd	nd
5	229217	Shewa	2580	nd	brown
6	229214	Shewa	2110	nd	nd
7	229219	Shewa	2550	sandy loam	brown
8	229218	Shewa	2550	sandy loam	red
9	229222	Shewa	2680	clay	black
10	229225	Shewa	2720	clay	black
11	229226	Shewa	2760	clay	black
12	231260	Arsi	2330	sandy loam	brown
13	231261	Arsi	2330	sandy loam	brown
14	231262	Arsi	2520	sandy loam	brown
15	231263	Arsi	2030	sandy loam	brown
16	231265	Arsi	2200	sandy loam	brown
17	231266	Arsi	2190	clay loam	black
18	231272	Arsi	2520	clay loam	black
19	234999	Welo	2840	clay loam	black
20	235002	Shewa	3100	silt	yellow
21	235004	Shewa	3000	sand	orange
22	235005	Shewa	3040	sand	orange
23	235006	Shewa	2950	sand	brown
24	235749	Gonder	2700	loam	brown
25	235750	Gonder	2590	sandy loam	brown
26	235751	Gonder	2000	sand	brown
27	235752	Gonder	1920	loam	brown
28	235898	Gonder	2940	sandy loam	brown
29	235899	Gonder	2940	clay loam	brown
30	235900	Gonder	2870	sandy loam	brown
31	236202	Welo	2920	sandy loam	red
32	236415	Shewa	2860	loam	brown
33	236898	Shewa	2790	clay loam	brown
34	236900	Shewa	2670	loam	red
35	237059	Arsi	2575	clay loam	black
36	237060	Arsi	2600	sandy loam	brown
37	237061	Arsi	2300	sandy loam	brown
38	237064	Arsi	2100	sandy loam	brown
39	237066	Arsi	2350	clay loam	black
40	237067	Arsi	2400	clay loam	black
41	237509	Tigray	1500	nd	nd

nd: not determined.

The gel buffer used was 0.045 M Tris-base and 0.006 M monohydrous citric acid, pH 8.3 and the electrode buffer was 0.192 M boric acid and 0.028 M lithium hydroxide, pH 8.3. Electrophoresis was carried out under +4°C at 70 milliampere (mA). Three enzyme systems, esterase (EST, E.C. 3.1.1.-), aspartate aminotransferase (AAT, E.C. 2.6.1.1), and leucine aminopeptidase (LAP, E.C. 3.4.11.1) were assayed. After electrophoresis, the gels were stained with the enzyme-specific staining solutions and incubated in the dark at 37°C for 20-25 minutes for esterase and aspartate aminotransferase

and 35-40 minutes for leucine aminopeptidase.

Seed Traits

Twenty seeds were selected randomly and were examined for five seed traits. The characters recorded were, seed color [black (1), brown (2), green (3), yellow (4), white (5), or other (6)]; seed size [small (1), medium (2), or large (3)]; seed shape [flattened/dented (1), angular (2), round (3), or mixed (4)]; hilum color [brown (1), black (2), light green (3), mixed (4), or other (5)] and seed texture [wrinkled (1), smooth (2), mixed (3) or, other (4)].

Nutritional Analysis

Approximately 30 g of seed from each accession were ground in ultra centrifugal mill ZM 1000 with 0.5 mm sieve and analyzed in duplicate. Data was recorded for six nutritional compositions, namely, crude protein, crude fat, crude fiber, mineral ash, moisture and carbohydrate.

All analyses were done according to official methods of analysis of AOAC (Association of Official Analytical Chemists) international. The protein content was determined by taking 1 g sample using Kjeldahl method. Fat content was determined by extracting 3 g sample with petroleum benzene on a Soxtec extraction unit. Fiber content was determined by taking 1 g sample and digesting with boiling dilute sulfuric acid (0.128 M) and potassium hydroxide (0.223 M) and washing with acetone consecutively, drying and washing. Mineral content was determined by taking 3 g samples and incinerating in a muffle furnace at 550°C. Moisture content was determined by taking 2 g sample and putting it in an oven at 135°C for 2 hrs. Carbohydrate was calculated by difference [carbohydrate = 100 - (protein + fat + mineral + moisture)]. All were calculated on dry weight basis (dwb).

Statistical Analysis

Isozyme

Diversity was measured at population, region and altitudinal class levels. The altitude range of the collection for the samples used in this study ranged from 1500 to 3100 m a.s.l. and were arbitrarily classified into five groups (<2200, 2200-2400, 2401-2600, 2601-2800 and >2800 m a.s.l). Genetic variation measurements were calculated from allele frequencies using Biosys-1 release 1.7 (Swofford and Selander, 1981). The mean number of alleles per locus (A), the percentage of polymorphic loci (P) (95% criterion), the observed heterozygosity (Ho) and the expected heterozygosity (He) were measured for each population and classified groups. Allele frequencies

at 6 loci were used to compute unbiased genetic identity coefficients between regions and altitudinal classes (Nei, 1978). Genetic differentiation was analyzed by F-statistics (Wright, 1978). Cluster analysis was carried out by the unweighted pair-group method with arithmetic average (UPGMA) of Sneath and Sokal (1973).

Seed Traits Analysis

Shannon's index of diversity $H = \sum_{i=1}^{n} Pi \ln Pi$, where Pi is the proportion of the total number of entries in the i^{th} class, and n is the number of phenotypic classes for a given character, was calculated for seed characters of each population, populations pooled by altitudinal classes and region. Since different number of phenotypic classes were recognized for the five seed characters, H was standardized by converting it to the relative index, $H' = H/H_{max}$ where $H_{max} = ln\ n$.

Nutrition

Data for the nutrient composition of the accessions were analysed using SAS (SAS, 1999). Univariate and correlation analyses were used to test for normality of the distribution of the six nutritional compositions, and simple correlation among the nutrients was performed using SPSS 10.0 (1999). The population mean of the quantitative value of nutrients was used as the input for cluster analysis. Principal component analysis using correlation matrix was performed to define the existing pattern of variation based on nutritional composition between the accessions and place of origin.

RESULTS AND DISCUSSION

Isozyme Analysis

Population Genetic Diversity

Six loci were separated from the three enzymes systems (AAT-1, AAT-2, EST-1, EST-2, LAP-1, and LAP-2). The banding patterns obtained were fewer in number when compared to those reported by Posveck and Griga (2000) on commercial peas. This could be due to different methodology used and the inclusion of wider range of plant parts in their case. In the polymorphic loci, two alleles were recorded at EST-1 and EST-2 loci and three alleles were recorded at AAT-2 locus. The percentage of polymorphic loci ranged from 16.7 to 50.0 with a mean of 39.8. The average number of alleles per locus ranged from 1.2 to 1.7 with a mean of 1.4. The observed

and expected heterozygosity values ranged from 0.000 to 0.042 with a mean of 0.01, and from 0.032 to 0.21 with a mean of 0.13, respectively (Table 2). Population 5 showed the highest value in all the genetic variability measurements followed by population 3 and 32, all from Shewa region. Previous studies indicated that dicots showed 31.2% polymorphic loci, and 1.69 and 0.14 mean number of alleles per locus and mean heterozygosity, respectively (Hamric *et al.*, 1979), which was not far from the result shown by field pea samples used in this study. Population 38, which showed uniformity in seed trait analysis, revealed some level of polymorphism in one of the isozyme locus.

A comparison of F-statistics (Wright, 1978) for all populations is given in Table 3. F_{ST} (genetic variability among populations) compares the ratio between population component of diversity and the total diversity. In this study, F_{ST} for all populations was estimated as 0.26 indicating that 74% of the genetic diversity resided within a population. High F_{ST} value was recorded at EST-1, suggesting that the between-population differentiation is most developed at this locus.

The matrix of Nei's unbiased minimum distance between pair-wise combinations of the field pea populations ranged from 0.00 to 0.27 (data not shown). The highest value was exhibited between populations 15 and 37. In general, population 37 showed the highest distance when compared against all other populations.

Allele frequency is also used to cluster the populations by applying single linkage algorithms using SPSS (Fig. 1). It formed six major groups that encompassed one to eleven populations. The first group contained four populations, two from Arsi and two from Harerge. They all showed polymorphism only at EST-2 locus. Six of the seven populations from Gonder fell in the second group with other four populations from Shewa and one from Tigray. Except population 23 which was found between groups one and two, populations in this group show significant polymorphism at AAT-2 and EST-2 loci. The data might be insufficient to conclude that populations from Gonder were genetically similar. Other studies, which had employed isozyme method for variation analysis, indicated that populations from the same region often did not cluster in the same group (Harris, 1997; Meglecz, 1997; Chamberlain, 1998), which was also partially observed in populations included in this analysis. The third group contained two populations from Welo, three from Arsi and six from Shewa regions.

Almost all the populations in this group showed polymorphism at EST-1,

EST-2 and AAT-2 loci. In most cases, the allelic variation at AAT-2 locus in this group was very low. Group 4 contained populations that showed low allelic variation in all the polymorphic loci and only 10% variation was observed at AAT-2 locus. Only one population was found in the fifth group. It showed high allelic variation at AAT-2 and EST-1 loci. The sixth group contained three populations from Arsi, one from Gonder and one from Shewa.

Table 2 Genetic variability in 41 populations (accessions) of field pea from Ethiopia at 6 isozyme loci.

Acc. No.	N	A	P	Ho	He	H'
223330	20.0	1.3	33.3	0.008	0.048	0.65
223331	20.0	1.2	16.7	0.000	0.064	0.73
229213	20.0	1.5	50.0	0.000	0.198	0.76
229210	20.0	1.5	50.0	0.008	0.113	0.83
229217	20.0	1.5	50.0	0.025	0.208	0.72
229214	20.0	1.3	33.3	0.000	0.121	0.85
229219	20.0	1.5	50.0	0.000	0.158	0.69
229218	20.0	1.5	50.0	0.008	0.167	0.83
229222	20.0	1.5	50.0	0.000	0.138	0.72
229225	20.0	1.3	16.7	0.008	0.093	0.74
229226	20.0	1.5	50.0	0.008	0.172	0.59
231260	20.0	1.5	50.0	0.008	0.122	0.74
231261	20.0	1.5	50.0	0.000	0.172	0.68
231262	20.0	1.5	50.0	0.000	0.166	0.63
231263	20.0	1.3	33.3	0.000	0.032	0.36
231265	20.0	1.2	16.7	0.000	0.064	0.60
231266	20.0	1.5	50.0	0.008	0.111	0.61
231272	20.0	1.5	50.0	0.025	0.145	0.62
234999	20.0	1.3	33.3	0.000	0.128	0.67
235002	20.0	1.5	50.0	0.017	0.180	0.66
235004	20.0	1.3	33.3	0.017	0.156	0.66
235005	20.0	1.5	33.3	0.008	0.068	0.59
235006	20.0	1.3	33.3	0.000	0.062	0.50
235749	20.0	1.3	33.3	0.033	0.140	0.76
235750	20.0	1.3	33.3	0.033	0.157	0.52
235751	20.0	1.5	50.0	0.000	0.111	0.84
235752	20.0	1.3	33.3	0.033	0.167	0.67
235898	20.0	1.3	33.3	0.025	0.129	0.70
235899	20.0	1.3	33.3	0.042	0.153	0.69
235900	20.0	1.5	33.3	0.000	0.139	0.49
236202	20.0	1.5	50.0	0.008	0.170	0.75
236415	20.0	1.5	50.0	0.017	0.198	0.57
236898	20.0	1.5	50.0	0.042	0.167	0.77
236900	20.0	1.5	50.0	0.008	0.122	0.71
237059	20.0	1.5	50.0	0.000	0.187	0.51
237060	20.0	1.5	50.0	0.008	0.143	0.62
237061	20.0	1.2	16.7	0.000	0.044	0.54
237064	20.0	1.2	16.7	0.000	0.072	0.00
237066	20.0	1.2	16.7	0.000	0.085	0.72
237067	20.0	1.5	50.0	0.000	0.173	0.49
237509	20.0	1.7	50.0	0.025	0.184	0.57
Mean		1.40	39.8	0.010	0.132	0.64

N: Mean sample size per locus, A; Mean number of alleles per locus, P: Percentage of polymorphic loci, (95% criterion) Ho: Mean observed heterozygosity, He: Mean expected heterozygosity, H': genetic diversity index (for seed characteristics).

Table 3 Summary of F-statistics at all loci among 41 populations, populations pooled by region and by altitude. F_{IS} – genetic variability within populations, F_{IT} - total genetic variability, F_{ST} - genetic variability among populations.

Locus	Population			Region			Altitude		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
AAT-2	.911	.923	.144	.394	.503	.181	.922	.923	.016
EST-1	.936	.960	.375	.971	.974	.101	.958	.960	.038
EST-2	.927	.944	.239	.910	.920	.110	.939	.940	.017
Mean	.925	.944	.260	.760	.792	.132	.941	.942	.023

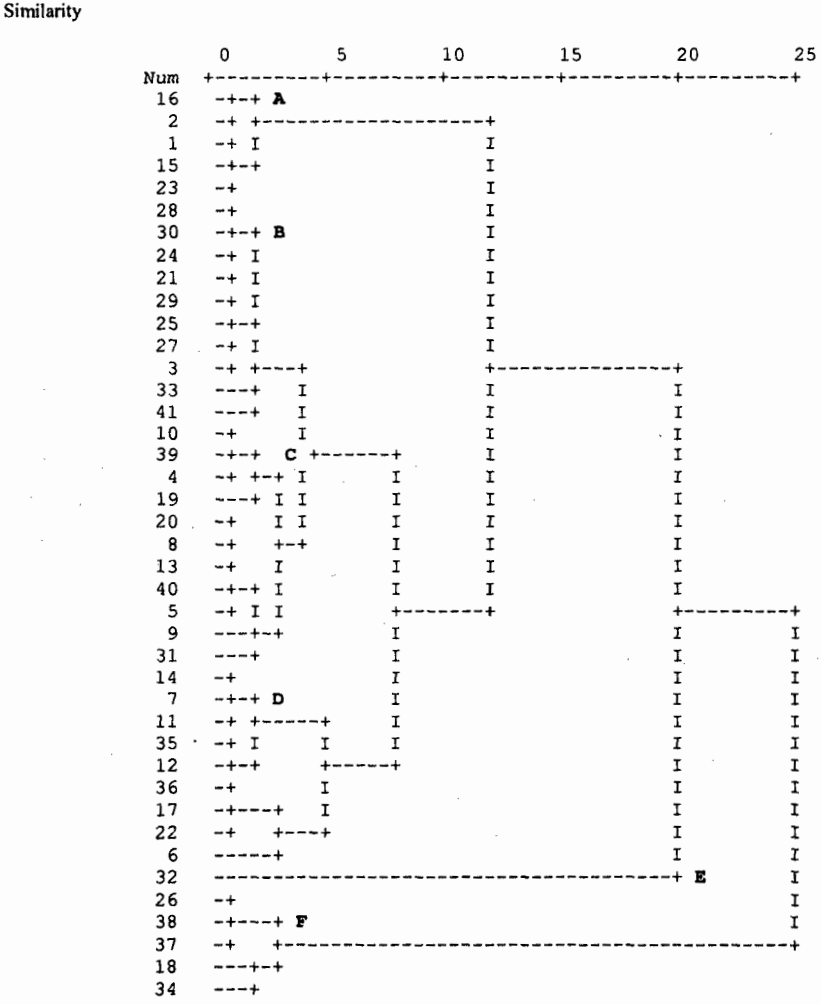

Fig. 1. UPGMA dendrogram based on 6 isozyme loci in 41 field pea population. (See Table 1 for population codes).

Populations in this group showed higher polymorphism at EST-1 locus when compared with the other loci though low range of allelic variation was scored at EST-2 and AAT-2 loci in some of them.

Altitudinal Class Diversity

Genetic diversity statistics for all populations pooled by altitudinal class is given in Table 4. There was no difference in the percentage of polymorphic loci in any of the altitudinal classes and the mean number of alleles per locus was similar in all classes, except in class 1 (<2200 m a.s.l.). The mean observed and expected heterozygosity values varied from 0.002 to 0.017 and 0.169 to 0.189, respectively.

Table 4 Genetic variability at six loci for all populations pooled by altitudinal classes (standard errors in parenthesis).

Altitudinal class	Altitudinal range	N	A	P	Ho	He	H'
1	<2200	180.0 (.0)	1.7 (.3)	50.0	0.008 (.005)	0.172 (.085)	0.59
2	2200-2400	160.0 (.0)	1.5 (.2)	50.0	0.002 (.001)	0.164 (.083)	0.67
3	2401-2600	160.0 (.0)	1.5 (.2)	50.0	0.013 (.005)	0.185 (.008)	0.64
4	2601-2800	120.0 (.0)	1.5 (.2)	50.0	0.017 (.009)	0.189 (.089)	0.71
5	>2800	200.0 (.0)	1.5 (.2)	50.0	0.013 (.006)	0.169 (.087)	0.63

N: Mean sample size per locus, A: Mean number of alleles per locus, P: Percentage of polymorphic loci, (95% criterion) Ho: Mean observed heterozygosity, He: Mean expected heterozygosity, H': Genetic diversity index (for seed characters).

Zemede Asfaw (1989), working on Ethiopian barley employing biochemical and morphological characters, indicated the existence of strong association between morphology and hordeins, morphology and altitude and hordeins and altitude. In this study, little or no outweighing effect of altitude has been detected. Low level of genetic distance was depicted between the altitudinal classes as shown by the matrix of genetic similarity (Nei, 1978). The highest distance was shown between altitudinal class 5 and 3 (Table 5). Altitudinal class 5 showed the highest distance when compared against the others. This class contained those populations that were produced on yellow and orange soil colors. The abiotic ecological forces of climate, location and soil were some of the factors that determined genetic diversity within any species of land plants (Frankel *et al.*, 1995). Though it was not strongly reflected, the observed diversity between the altitudinal classes might be the effect of these factors.

Table 5 Matrix of unbiased minimum distance (Nei, 1978) for populations pooled by altitude.

Altitude (m)	1	2	3	4	5
1) <2200	-				
2) 2200-2400	.001	-			
3) 2400-2600	.007	.003	-		
4) 2601-2800	.001	.001	.000	-	
5) >2800	.003	.007	.017	.006	-

Wright's Fixation indices grouped by altitude showed relatively low level of differentiation between the different classes compared to differentiation at individual level and populations grouped by region (Table 3). Only 2.4% of the variation came from differentiation between the different altitudinal classes. The high value of F_{ST} was recorded at EST-1 indicating that between the altitudinal classes, differentiation was most developed at this locus.

Regional Diversity

The genetic variability measurement data by geographic region are summarized in Table 6. The mean number of alleles per locus ranged from 1.3 (Hararge) to 1.7 (Tigray). The percentage of polymorphic loci ranged from 16.7 to 50. The observed and expected heterozygosity estimates ranged from 0.004 to 0.138 and 0.054 to 0.201, respectively. Hararge region showed the lowest value in all the variability estimates.

Table 6 Genetic variability at six loci for all populations pooled by regions (standard errors in parentheses).

Region	N	A	P	Ho	He	H'
Shewa	320.0 (.0)	1.5(.2)	50.0	0.010 (.005)	0.185 (.087)	0.69
Gonder	140.0 (.0)	1.5 (.2)	50.0	0.024 (.016)	0.175 (.088)	0.67
Hararge	40.0 (.0)	1.3 (.2)	16.7	0.004 (.004)	0.054 (.045)	0.69
Welo	40.0 (.0)	1.5 (.2)	50.0	0.138 (.138)	0.201 (.099)	0.71
Tigray	20.7 (.7)	1.7 (.3)	50.0	0.051 (.035)	0.189 (.106)	0.57
Arsi	260.0 (.0)	1.5 (.2)	50.0	0.004 (.002)	0.167 (.089)	0.55

N: Mean sample size per locus, A: Mean number of alleles per locus, P: Percentage of polymorphic loci, Ho: Mean observed heterozygosity, He: Mean expected heterozygosity, H': Genetic diversity index (for seed characteristics).

The extent of differentiation between regional groupings was tangible. Regional differentiation was most developed at AAT-2 and the level of diversity within regions was high (Table 3). As revealed by the matrix of genetic distance between regions, accessions from Gonder and Shewa show the lowest distance while the highest dissimilarity was observed between population from Welo and Hararge (Table 7). The result of cluster analysis showed that field pea accessions from Shewa, Gonder and Tigray regions were most closely related (Fig. 2). The rest of the regions fell into separate groups.

Table 7 Matrix of genetic distance coefficients for all populations pooled by regions. Below diagonal: Nei (1978) unbiased minimum distance.

Region	1	2	3	4	5	6
1. Shewa	-					
2. Gonder	.002	-				
3. Hararge	.039	.032	-			
4. Welo	.025	.017	.081	-		
5. Tigray	.007	.002	.036	.016	-	
6. Arsi	.006	.017	.063	.049	.025	-

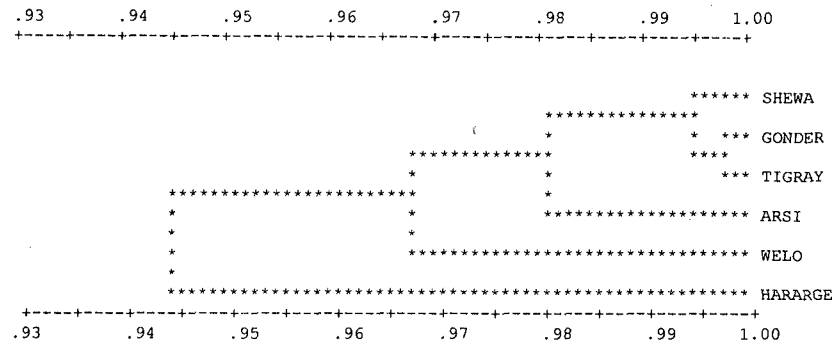

Fig. 2. Genetic similarity dendrogram among the populations in the six regions.

Diversity Index for Seed Traits

Estimation of diversity for populations, populations pooled by regions and altitudinal classes was determined. The mean H' value for populations ranged from 0.00 to 0.85 and averaged 0.64 (Table 2). The highest mean diversity index pooled over traits was shown by populations from Welo, H' = 0.71 (Table 6) and altitudinal class 4 (2601-2800), H' = 0.71 (Table 4). Diversity index values pooled over all traits for regional and altitudinal classes showed a lower magnitude of differences when compared with individual populations. A similar trend was observed in Ethiopian barley (Abebe Demissie and Bjornstand, 1996), although wide ranges of morphological traits were assessed in their study.

Nutrition

In terms of nutritive value, field pea showed a range of variation. The protein content of pea can range from about 19.7% to as high as 28% (Kay, 1979). In this study, the value, on dry weight basis, ranged from 22.82 to 27.90% with a mean of 25.23% for protein. The other nutrient composition showed a range from 0.67 to 1.06% with a mean of 0.92% for fat, 7.37 to 8.77% with a mean of 8.17% for fiber, 2.54 to 3.24% with a mean of 2.83% for mineral ash, 7.75 to 9.05% with a mean of 8.43% for moisture and 59.85 to 65.12% with a mean of 62.58% for carbohydrate (Table 8).

The analysis for univariate normality for all nutrient composition indicated that all the nutrients had good approximation of normal distribution ($p < 0.01$). The data was standardized before handling the multivariate analysis.

Cluster analysis was found to group the accessions into six main clusters (Fig. 3). Average linkage clustering technique that finds cluster of equal

variance was found to give the most satisfactory clustering result (Millgan, 1981).

Table 8 Nutrient composition of *Pisum sativum* populations.

Code	Accession no.	% crude protein	% crude fat	% crude fiber	% mineral ash	% moisture	% carbohydrate
1	223330	27.13	0.91	8.77	2.82	8.51	60.63
2	223331	26.58	0.92	8.06	2.82	8.62	61.63
3	229213	26.19	0.96	7.81	2.69	8.56	61.60
4	229210	25.71	0.84	8.58	2.83	9.05	61.57
5	229217	24.10	0.99	8.08	2.94	8.52	63.45
6	229214	25.98	0.85	8.74	3.10	8.50	61.57
7	229219	25.48	0.93	7.49	2.70	8.53	62.36
8	229218	24.66	0.99	8.34	2.84	8.52	62.99
9	229222	26.31	0.94	8.05	2.69	8.63	61.43
10	229225	25.68	0.94	8.27	2.72	8.56	62.10
11	229226	26.29	0.97	8.34	2.80	8.54	61.40
12	231260	25.57	0.97	8.06	2.77	8.39	62.30
13	231261	25.15	0.89	7.78	2.80	8.40	62.76
14	231262	27.90	0.67	7.80	3.24	8.34	59.85
15	231263	24.31	0.94	8.54	2.92	7.97	63.86
16	231265	23.38	0.84	8.15	2.91	8.03	64.84
17	231266	24.39	0.82	8.43	3.02	8.01	63.76
18	231272	26.33	0.86	8.64	2.96	8.27	61.58
19	234999	25.46	0.94	8.54	2.84	8.36	62.40
20	235002	23.24	0.95	8.30	2.74	8.33	64.74
21	235004	25.62	0.81	7.37	2.74	8.32	62.51
22	235005	26.00	0.92	8.05	2.75	8.34	61.99
23	235006	24.75	0.93	8.49	2.79	8.46	63.07
24	235749	26.09	0.98	8.02	2.73	8.39	61.81
25	235750	26.20	0.90	8.35	3.00	8.42	61.48
26	235751	27.19	0.94	8.23	3.02	8.34	60.51
27	235752	24.00	0.98	8.41	3.07	8.26	63.69
28	235898	25.06	0.97	8.32	2.84	8.93	62.20
29	235899	25.70	0.91	8.18	2.97	8.81	61.61
30	235900	25.48	0.97	8.77	2.76	8.79	62.00
31	236202	25.26	0.92	7.48	2.68	8.87	62.27
32	236415	24.95	0.94	8.38	2.75	8.40	62.96
33	236898	25.48	0.92	8.42	2.86	8.48	62.26
34	236900	23.38	0.94	8.67	2.83	8.50	64.35
35	237059	23.22	1.00	8.19	2.78	8.50	64.50
36	237060	25.02	0.92	7.78	2.82	7.75	63.49
37	237061	25.36	0.87	7.74	2.83	8.36	62.58
38	237064	24.81	0.82	7.38	2.54	8.21	63.62
39	237066	22.82	0.98	7.93	2.74	8.34	65.12
40	237067	24.25	0.95	7.90	2.61	8.38	63.81
41	237509	24.11	1.06	8.24	2.91	8.34	63.58

Cluster 1 is characterized by those populations that exhibit similar percentage of protein (27.13 to 27.19%), fat (0.91 to 0.94%), fiber (8.23 to 8.77%), mineral (2.82 to 3.02%), moisture (8.34 to 8.51%) and carbohydrates (60.63 to 60.51%). These populations were collected from different regions (Gonder and Harerge) and altitudes (2150 and 2000 m a.s.l., respectively). They were produced on different types of soil texture (clay loam and sand) and soil color (brown and black).

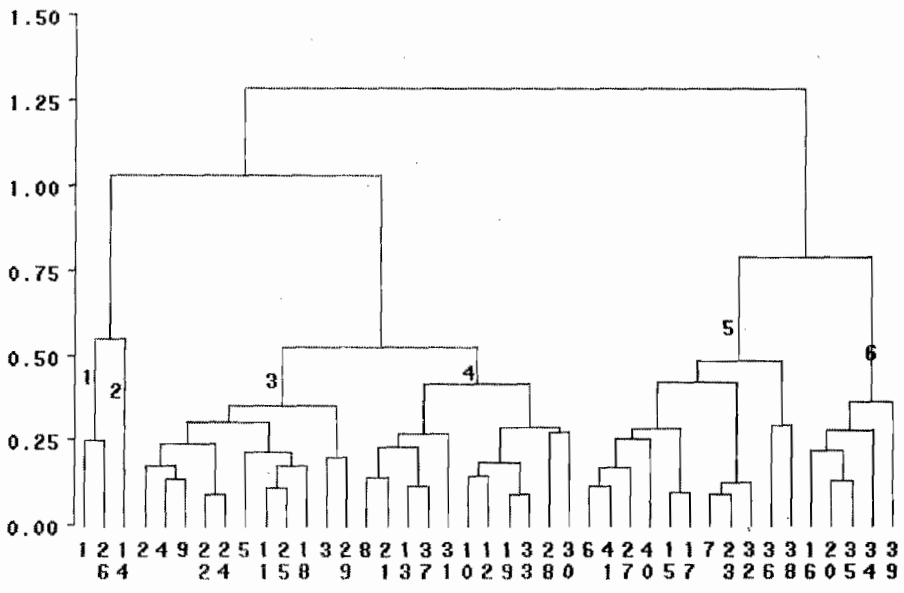

Fig. 3. Dendrogram of the 41 field pea populations using the average linkage clustering based on their chemical composition (See Table 8 for population codes).

Cluster two was characterized by one population that exhibited maximum protein (27.9%) and maximum mineral (3.24%). It also exhibited minimum fat (0.67%). This relationship was clearly explained in correlation analysis result (Table 10). This population was collected from Arsi at an altitude of 2520 m a.s.l. and produced on brown, sandy loam soil.

Cluster three was characterized by those populations that exhibited similar percentage of protein (25.7-26.0%), fat (0.84-0.98%), fiber (7.81-8.74%), mineral (2.69-3.10%), moisture (8.27-9.05%) and carbohydrate (61.40-61.99%). Here also it was observed that the populations were collected from different regions (Shewa, Gonder, Harerge and Arsi) and altitudes (2110-3040 m a.s.l.) and were planted on different types of soil texture (loam, sandy loam, clay loam and sand) and soil color (brown and black). The other clusters had also the same effect as cluster three. The observed effect of places of origin (region) and altitude on nutrient composition was insignificant.

It seems that the impact of soil texture and color on nutritional composition is minimal for these populations because similar percentage of protein, fat, fiber, etc. is observed on populations grown on different types of soil texture and soil color. Earlier studies also showed that field pea could be grown on

a wide range of soil types, from light sandy loams to heavy clay with good drainage, as field pea did not tolerate soggy or water-soaked conditions (Oelke *et al.*, 1991).

Principal Component Analysis

A summary of the composition of variables of the three components, eigenvalues and loading are given in Table 9.

Table 9 Result of principal component analysis.

Character	PC1	PC2	PC3
Protein	0.61	0.11	-0.12
Fat	-0.34	0.44	0.40
Fiber	0.07	-0.12	0.80
Mineral	0.28	-0.58	0.37
Moisture	0.22	0.64	0.23
Carbohydrate	-0.62	-0.18	0.005
Eigenvalue	2.40	1.41	1.33
% Total variation	40	24	22
% Cumulative variance	40	64	86

The first principal component (PC1) explains about 40% of the total variability. In this principal component, protein has a significant positive loading and carbohydrate has a significant negative loading. The second Principal component (PC2) explains about 24% of the total variability. Moisture and fat have a positive significant loading and mineral has a significant negative loading in this component. The third PCA explains about 22% of the total variability. This principal component is strongly and positively associated with fiber, mineral and fat.

Figure 4 shows the principal component scores of the first and second component for the populations using the six nutritional compositions. Most of the populations collected from Shewa, Gonder and all from Harerge and some from Welo had a positive loading in the first principal component. Most of the populations collected from Arsi, Tigray and some from Welo had negative loading in the second principal component. However, these studies had shown that the populations that were collected from, Shewa, Harerge and Gonder were grouped together and Arsi, Welo and Tigray were grouped together. Populations within the group might probably have similar genetic background. Moreover, population migration from the north and central highlands to the south and eastern regions of Ethiopia and trade relationship could have contributed to the similar groupings of the populations and their spreading from Gonder to Shewa to Harerge and Arsi.

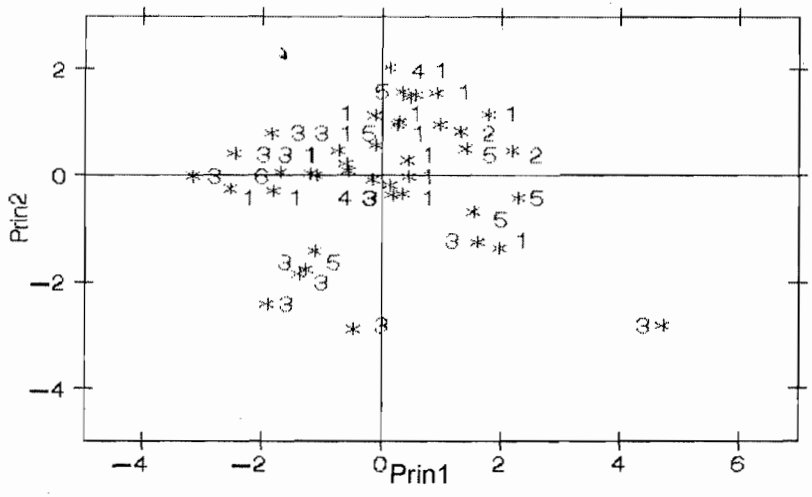

Fig. 4. Regional ordination of the 41 field pea populations using plot of the first two principal components.

Correlation Analysis

It was observed that protein was highly and negatively correlated with carbohydrate at $P \leq 0.01$, and was negatively correlated with fat at $p \leq 0.05$ level (Table 10). Fat was correlated negatively with mineral and positively with carbohydrate at $P \leq 0.05$ level. Fiber was highly and positively correlated with mineral at $p \leq 0.01$ level and carbohydrate was highly and negatively correlated with moisture at $p \leq 0.01$ level.

Knowledge of the correlation coefficients within the nutrient compositions of the field peas is beneficial for breeders to select better quality, like high protein variety, high mineral variety, etc. and other end users like industries.

Table 10 Correlation coefficient between nutritional compositions.

	Protein	Fat	Fiber	Mineral	Moisture	Carbohydrate
Protein	1					
Fat	-0.389[a]	1				
Fiber	-0.023	0.211	1			
Mineral	0.216	-0.339[a]	0.407[b]	1		
Moisture	0.247	0.174	0.130	-0.176	1	
Carbohydrate	-0.975[b]	0.312[a]	-0.065	-0.262	-0.414[b]	1

[a] correlation is significant at the 0.05 levels

[b] correlation is significant at the 0.01 levels

CONCLUSION

Variation of field pea populations from Ethiopia was studied by using isozyme analysis, seed traits and nutrient composition. It is important to analyse and identify nutritional composition since it helps considerable public and private users to develop knowledge of using the right germplasm for the right purpose. The isozyme analysis shows that most of the variation occurs within rather than between the populations and the classifying criteria. Minimum effect of location, altitude, soil type and soil color was observed on nutrient composition of the accessions. This suggests that each population has something to offer to field pea improvement in terms of yield, disease resistance, stress tolerance, end use quality, etc. Although isozymes can be useful markers, they have limitations, because existing isozyme markers are few and they cannot span the whole genome. Therefore, in order to determine the genetic relationship with precision, it is recommended that further studies of the populations using molecular methods be conducted. The variation observed in this study in terms of seed traits, isozymes and nutrient composition in the field pea populations can be a useful guide for further studies in conservation, breeding, selection and other works. Those regions that show higher diversity need to be explored further in order to collect and conserve maximum variation.

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