ORIGINAL ARTICLE

Genetic diversity for attributes of biological nitrogen fixation in Abyssinian field pea (*Pisum sativum var. Abyssinicum*) germplasm accessions

Gemechu Keneni[1], Fassil Assefa[2], M. Imtiaz[3], and Endashaw Bekele[2]

[1]Holetta Agricultural Research Center, P. O. Box 2003, Addis Ababa, Ethiopia

[2]Department of Biology, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

[3]International Center for Agricultural Research in the Dry Areas (ICARDA), P. O. Box 5466, Aleppo, Syria

ABSTRACT

Breeding of Abyssinian field pea (*Pisum sativum* var. *Abyssinicum*) genotypes effective for biological nitrogen fixation (BNF) has considerable economic and ecological significance. An experiment was conducted to study the magnitude of genetic diversity for attributes of biological nitrogen fixation. The experiment was carried out in 2007 in the greenhouse on Vertisol and Nitisol soils at Addis Ababa University using a randomized complete block design with three replications, where the Nitrogen difference technique with Yellow Dodolla, a variety of *Brassica carinata* A Braün as a reference crop, was used to estimate the proportions of nitrogen derived from soil and atmosphere. Differences among the accessions for 14 traits studied were significant on both soil types. However, genotype by soil type interaction effects were significant only for early vigor, number of nodules, nodule dry weight, foliage phosphorus content (%), phosphorus derived from fertilizer (%) and phosphorus uptake efficiency (%). This indicated that the two soils were distinctly different or the accessions responded differently to soil type for these traits. Cluster analysis for average performances on the two soils grouped the accessions into four different classes, and Mahalanobis's D^2 analyses showed significant genetic distances between most of the clusters. Five accessions, namely MColl-7/07, MColl-8/07, TKColl-6/07, MCColl-4/07 and TKColl-3/07 were identified as best fixers of nitrogen under both Vertisol and Nitisol soils with a fixation range of 41- 45 % and these accessions could be exploited in future breeding endeavors to develop BNF efficient genotypes. Additionally, there was no clear interrelationship between the origins of accessions and the pattern of genetic diversity as there were genotypes from the same source of origin fell into different clusters and *vice versa*.

Key words: *Abyssinian* field pea, genetic diversity, germplasm accession, *Pisum sativum var. Abyssinicum*

INTRODUCTION

Field pea (*Pisum sativum* L.) is known to be grown in Ethiopia since antiquity (Dawit *et al*., 1994). Currently, the crop is the third most important pulse crop in Ethiopia, preceded only by faba bean and chickpea in terms of both area coverage and total national production (CSA, 2005). There are two botanical cultivars of *Pisum sativum* known to grow in Ethiopia, namely *P. sativum var Sativum* and *P. sativum* var *Abyssinicum* (Westphal, 1974). The botanical cultivar *P. sativum var Sativum* dominates the production system in the highlands of Ethiopia (Messiaen *et al*., 2004) while *P. sativum var Abyssinicum* is limited to sporadic growth in some pockets, particularly in Wello and Tigray in the north and Arsi in the southeast (Westphal, 1974; Hagedorn, 1984). Like all other cool-season food legumes, the productivity of field pea in general and that of the Abyssinian field pea in particular is very low in Ethiopia compared to many other continents and countries of the world (Kelley *et al*., 2000), which, among many other factors, may be attributed to poor soil fertility (Getachew *et al*., 2006).

It is obvious that crop yields can be improved by application of commercial fertilizers but such practices, apart from the environmental concerns, must be repeated each season and hence are expensive particularly for totally neglected crops like the Abyssinian field pea which are grown by the resource-poor farmers under marginal conditions. Soil fertility problems, particularly nitrogen deficiency, can be alleviated through the development of integrated plant nutrition systems involving both organic and inorganic fertilizers, use of nitrogen efficient genotypes and developing efficient cultivars for biological nitrogen fixation in legumes (Graham, 1988; Bowen and Zapata, 1991; FAO, 1995) including field pea (Beringer *et al*., 1988; Clark *et al*., 1988; Schmidt, 1988; Witty *et al*., 1988; FAO, 1995). It is known that the majority of the farmers in Ethiopia do not apply fertilizer to legumes (Asfaw and Angaw, 2006) and, out of the total land area currently under pulse crops, the proportion of the fertilized area is insignificant as compared to cereals (Messiaen *et al*., 2004). Field pea is known to fix more nitrogen than some of the other legumes like chickpea and lentil but less than the others like faba bean (Schmidt, 1988). The most stable option could, therefore, be achieved most likely from selection of compatible genotypes with effective *Rhizobium* strains (Beringer *et al*., 1988; Clark *et al*., 1988; Schmidt, 1988; Witty *et al*., 1988) which, in turn, should be based on a sound prior characterization of the available germplasm for symbiotic association and biological nitrogen fixation.

Even though information on the magnitude of genetic diversity has been generated for *Rhizobium* strains collected from different legume species in Ethiopia (Endalkachew *et al*., 2004; Endalkachew *et al*., 2005; Asfaw and Angaw, 2006), including those of field pea (Aregu, 2006; Asfaw and Angaw, 2006), the role of host plants is not yet well studied (Takalign and Asgelil, 1994; Asfaw and Angaw, 2006). On the other hand, a number of authors elsewhere reported possibilities for improving biological nitrogen fixation through breeding in a number of legumes including field pea (Beringer *et al*., 1988; Clark *et al*., 1988; Schmidt, 1988; Witty *et al*., 1988; FAO, 1995). This experiment was carried out to study the magnitude of genetic diversity for traits related to nodulation and biological nitrogen fixation among the Abyssinian field pea germplasm accessions treated with an effective *Rhizobium leguminosarum* var *Viceae* isolated from field pea.

MATERIALS AND METHODS

The study was conducted under controlled condition in the greenhouse at Addis Ababa University on two soil types, Nitisol and Vertisol, taken from the upper 20 cm of the top soil from around Holetta Agricultural Research Center and Adadi Sub-Center, respectively. Nitisols and Vertisols are among the dominant soil types in Ethiopia, making 12.5 % and 10.0% of the land area (EMA, 1988; IFPRI and CSA, 2006), particularly in the highlands where field pea is among the most dominant pulses (Mussa *et al*., 2006). The soil taken from Holetta is red-brown clay Nitisol with a pH of 6.32 while the one from Adadi is dark-clay loam Chromic Vertisol soil with a pH of 7.62. The physico-chemical properties of the soils are given in Table 1. The greenhouse had an average temperature of 20.31^{o}C and relative humidity of 67.01 %. While various growing media have been suggested in the greenhouse to study biological nitrogen fixation, the comparative advantage of using soil as a substrate was realized earlier as it simulates the actual production environment in the field (Vicent, 1970).

Thirty-three random samples of Abyssinian field pea germplasm accessions collected from northern Ethiopia (North Wello and South Tigray) were considered for the study. Two improved varieties of *P. sativum var Sativum*, Adi (G22763–2C X 305PS210813-3) and Holetta-90 (a selection from a local collection), were included to make references when necessary, however, excluded from the statistical analysis to avoid their overruling effects on the genetic differences among the test accessions. Plastic pots with a volume of 4 liters were filled with dry soils of 3.5 kg each. Seeds of each accession were surface-sterilized by

soaking in 95 % ethanol alcohol for 3 minutes followed by 0.2 % acidified mercuric chloride for a brief period of time and then rinsing 5 times with distilled water (Vincent, 1970). The seeds were germinated on tissue paper with distilled water for 4 days. Then 8 undamaged clean germinated seeds of the accessions were planted in each pot and they were thinned to 5 seedlings per pot within a few days after development into normal seedlings. The inocula of an effective strain of field pea *Rhizobium leguminosarum* var *Viceae*, designated as NSRIFP 5, identified earlier as one of the best competitive from among the Ethiopian isolates (Aregu, 2006) was mass produced in Erlemeyer flasks containing 100 ml of yeast extracts manitol (YEM) broth by inoculating a loop full of the selected strain. The inocula were then kept at an ambient temperature of 23 ^{o}C on a shaker at 120 rpm for three days or 72 hours to make approximately 10^{9} cells ml^{-1} (Clark *et al*., 1998).

Each seedling was inoculated with 1 ml of the inocula twice, the first inoculation being at planting and the second two weeks thereafter. Yellow Dodolla, a variety of *Brassica carinata*, was included as a non-fixing reference crop to estimate the proportion of nitrogen fixed from the atmosphere by the difference method (Hauck and Bremner, 1976; Smith *et al.* 1984; Zapata, 1990). Nitrogenous fertilizer was totally omitted in all test treatments. To calculate phosphorus use efficiency, one Abyssinian field pea germplasm accession with modest growth was grown with and without triple supper phosphate (TSP) fertilizer for the determination of the proportion of phosphorus absorbed by the crop from fertilizer, with the assumption that all accessions derive proportionally equal amount of phosphorus from the soil and the fertilizer. A blanket basal application of phosphorus was made to all other pots in the form of TSP at the rate of 1 g P pot^{-1}. Each pot received equal amount of tap water every other day approximately at around two-thirds of the water-holding capacity of the soil. All other crop management practices were applied uniformly to all pots as required so that the test genotypes could express their full genetic potential for the traits under consideration. The experiment was laid down in a randomized complete block design (RCBD) with 3 replications. The accessions were assigned to pots at random within each block. After 40 days of growth (shortly before flowering), all plants from each pot were carefully pulled out and their root systems washed free of soils with water running over a sieve.

Table 1. Physico-chemical properties of the soils

Parameter	Source of soil	
	Adadi	Holetta
Type	Vertisol	Nitisol
% Clay	61.19	46.42
% Silt	25.91	32.75
% Sand	12.66	20.83
Organic C (%)	1.16	1.79
N (%)	0.15	0.16
P (ppm*)	8.70	23.67
K (ppm)	39.75	36.56
pH (H_2O)	6.32	7.62
EC (µS)	405.63	697.67

*ppm = parts per million; µS = micro siemens

Data were collected on early vigor (1-5 scale; 1 being with low vigor and 5 with very high vigor), leaf senescence (1-5; 1 being with low senescence and 5 with very high senescence), plant height (cm), number of nodules plant^{-1}, nodule dry weight (mg plant^{-1}), root dry weight (mg plant^{-1}), shoot dry weight (mg plant^{-1}), total dry weight (mg plant^{-1}), foliage nitrogen content (%), total nitrogen derived from fixation (%), nitrogen fixation efficiency (%), total phosphorus content (%), phosphorus derived from fertilizer (%) and phosphorus uptake efficiency (%). The determination of phosphorus content was made using the wet digestion technique (AOAC, 1970) at Holetta Soil Science Research Laboratory. Based on the nitrogen contents, the following parameters were calculated:

Amount of N fixed in shoot = Nfg (%) - Nrg (%)

$$\textbf{N fixation efficiency} = \frac{(Nfg - Nrg)\ X\ 100}{Nfg}$$

Where Nfg = amount of nitrogen in shoot of fixing genotype and Nrg = amount of nitrogen in shoot of non-fixing reference.

Amount of P derived from fertilizer (%) = Pfg (%) - Png (%)

Where Pfg = amount of P in shoot of P treated genotype and Png = amount of P in shoot of non-fertilized genotype.

Phosphorus uptake efficiency (%) was calculated based on the following proportion:

$$PPFF\ (\%) = \frac{(SUPTP - SUPUTP)\ X\ 100}{PATP}$$

Where PPFF = proportion of P from fertilizer (%), SUPTP = shoot uptake of P in treated plants, SUPUTP = shoot uptake of P in untreated plants, and PATP = P applied to treated plants.

Representative bulk shoot samples from each pot were oven-dried to constant moisture, ground to pass through 1 mm size mesh sieve for determination of nitrogen and phosphorus contents using the conventional Kjeldahl and the dry ashing techniques, respectively (AOAC, 1970; Sahlemedhin and Taye, 2000) at Holetta Soil Science and Plant Nutrition Research Laboratory. The amount of nitrogen derived from fixation was estimated based on the difference in nitrogen content of the field pea accessions and Yellow Dodolla and, similarly, the amount of phosphorus derived from fertilizer was estimated based on the proportional difference in phosphorus content of the field pea accession grown with and without phosphorus fertilizer as given above.

Scales were pre-transformed into percentage values and all percentage values were ARCSINE transformed for statistical analysis as suggested by little and Hills (1978). Separate and pooled analyses of variance (ANOVA) were computed to quantify the total variation among the accessions using the following model of separate and pooled analysis of variance (ANOVA), respectively, as:

$$\rho_{ik} = \mu + a_i + b_k + e_{ik}$$

$$\rho_{ijk} = \mu + a_i + s_j + (as)_{ij} + (bs)_{jk} + e_{ijk}$$

Where ρ_{ik} (ρ_{ijk}) = phenotypic observation on accession i in block *k* (on soil j) (i = 1…*A*, *j* = 1…*S*, and *k* = 1…B) and A, S and B = number of accessions, soil type and block, respectively, μ = grand mean, a_i = the effect of accession i, s_j the effect of soil type j, $(as)_{ij}$ = the interaction effect between accession i and soil type, j, $(bs)_{jk}$ = the effect of block k (within soil type j) and e_{ijk} = error.

Clustering of accessions was performed by average linkage method of SAS software (SAS Institute, 1996). The pseudo F statistic and the pseudo t2 statistic were examined to decide the number of clusters. Genetic distances between clusters as standardized Mahalanobis's D2 statistics were calculated as:

$$D^2ij = (xi - xj)' \, cov\text{-}1(xi - xj)$$

Where, D^2ij = the distance between cases i and j; xi and xj = vectors of the values of the variables for cases i and j; and cov-1 = the pooled within groups variance-covariance matrix. Principal components based on correlation matrix were calculated using the same software as in clustering.

The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi-square (X^2) and were tested for significance both at 1% and 5% probability levels against the tabulated value of X^2 for 'P' degree of freedom, where P is the number of characters considered (Singh and Chaudhary, 1985). The dendrograms were built using Euclidian distance with the SPSS software.

RESULTS AND DISCUSSION

Performances of the accessions

Separate analysis of variance revealed highly significant differences ($P < 0.05$) among the accessions for all characters at both soil types and between the two soil types except for early vigor. Biological nitrogen fixation among the *P. sativum var. Abyssinicum* accessions ranged between 38.60 % and 46.10 %, the average on the two soils being 42.39 %. From the improved varieties of *P. sativum var. Sativum,* Holetta-90 with a fixation efficiency of 45.37 % was in the top list not only for fixation but for most of the associated attributes while Adi with a fixation of 50.19 % was apparently superior to all the test accessions. This may be attributed to: (1) the botanical cultivar *P. sativum var. Sativum* may be inherently superior for biological nitrogen fixation to *P. sativum var. Abyssinicum,* (2) the past genetic improvement efforts for other traits may inadvertently improved biological nitrogen fixations in Adi and Holetta-90, and/or (3) there might be better association of the strain with *P. sativum var. Sativum* than it is with *P. sativum var. Abyssinicum* due to host specificity, as the test strain was originally selected for best association with the former (Aregu, 2006). The five best fixers of nitrogen from among the test accessions under both Vertisol and Nitisol with a fixation ranging from 41 to 45 % of the total nitrogen in foliar tissue include MColl-7/07, MColl-8/07, TKColl-6/07, MCColl-4/07 and TKColl-3/07, three of them from South Tigray and the other two from North Wello (Table 2).

Table 2. Average performance of the accessions for biological nitrogen fixation and the component characters

Accession	EV*	LS	PH	NN/P	NDW/p (mg)	RDW/p (mg)	SDW/p (mg)	TDW/p (mg)	TN	NDFfa	RNFE	TP	PDFf	PUTE
MColl-1/07	66.60 b**	60.00 ab	106 ab	18.30 ab	40.40 a	61.67 abcd	1008 a	1070 a	2.75 bc	1.26 bc	41.60 ab	0.53 ab	0.28 ab	45.60 ab
MColl-2/07	60.00 bcd	56.60 abc	104 abc	16.40 abcd	32.21 ab	59.67 abcdef	912 abcd	971 abcd	2.77 abc	1.28 abc	42.70 ab	0.58 ab	0.33 ab	46.70 ab
MColl-3/07	36.60 h	50.00 bc	95 bcd	10.37 abcdef	19.03 bcdef	39.33 cdefghij	597 hij	636 ghi	2.57 c	1.08 c	38.60 b	0.56 ab	0.31 ab	47.30 ab
MColl-7/07	56.60 cde	56.60 abc	105 abc	16.90 abc	29.67 abcd	52.33 abcdefgh	840 abcdef	893 abcde	3.35 a	1.86 a	46.10 a	0.50 b	0.24 b	49.60 b
MColl-8/07	63.40 bc	60.00 ab	102 abc	19.00 a	32.20 ab	78.00 a	933 abcd	1011 abc	2.96 abc	1.47 abc	45.00 ab	0.55 ab	0.30 ab	46.70 ab
MColl-10/07	56.60 cde	53.40 bc	104 abc	18.77 a	40.67 a	47.00 bcdefghij	949 abc	996 abcd	2.82 abc	1.33 abc	42.10 ab	0.56 ab	0.30 ab	47.30 ab
KColl-1/07	63.40 bc	56.60 abc	103 abc	17.13 abc	27.62 abcde	68.67 ab	888 abcde	957 abcd	2.64 bc	1.15 bc	40.40 ab	0.52 b	0.27 b	45.60 ab
KColl-2/07	60.00 bcd	53.40 bc	105 abc	18.80 a	28.14 abcde	66.00 abc	973 ab	1039 ab	2.85 abc	1.36 abc	43.30 ab	0.55 ab	0.30 ab	46.70 ab
KColl-3/07	80.00 a	73.40 a	110 a	15.73 abcde	30.76 abc	62.33 abcd	1025 a	1087 a	2.60 bc	1.11 bc	40.40 ab	0.56 ab	0.31 ab	47.30 ab
KColl-5/07	60.00 bcd	50.00 bc	101 abc	18.15 ab	24.36 bcdef	49.33 bcdefghi	746 defghi	795 defgh	2.74 bc	1.25 bc	41.60 ab	0.55 ab	0.29 ab	46.70 ab
AWColl-1/07	60.00 bcd	53.40 bc	105 abc	15.70 abcde	18.38 bcdef	41.00 cdefghij	777 cdefgh	818 cdefg	2.59 bc	1.10 bc	39.20 b	0.54 ab	0.29 ab	46.70 ab
AWColl-3/07	53.40 def	53.40 bc	97 bcd	12.90 abcdef	19.82 bcdef	36.00 defghij	687 fghi	723 efgh	2.66 bc	1.17 bc	38.60 b	0.56 ab	0.31 ab	47.30 ab
AWColl-4/07	50.00 efg	56.60 abc	99 abcd	8.57 bcdef	16.60 bcdef	29.67 ghijk	611 ghij	641 ghi	2.76 bc	1.27 bc	41.00 ab	0.54 ab	0.29 ab	46.70 ab
MCColl-1/07	46.60 fg	53.40 bc	96 bcd	7.90 ef	20.77 bcdef	22.33 ijk	572 ij	594 hi	2.69 bc	1.20 bc	41.60 ab	0.63 a	0.38 a	50.20 a
MCColl-2/07	56.60 cde	60.00 ab	99 abcd	9.70 bcdef	15.57 cdef	40.67 cdefghij	627 ghij	668 efghi	2.90 abc	1.41 abc	43.90 ab	0.59 ab	0.34 ab	49.00 ab
MCColl-3/07	56.60 cde	53.40 bc	100 abcd	12.63 abcdef	13.32 ef	34.33 defghij	606 ghij	640 ghi	2.77 abc	1.28 abc	45.00 ab	0.56 ab	0.31 ab	47.30 ab
MCColl-4/07	50.00 efg	53.40 bc	94 cd	10.50 abcdef	20.31 bcdef	43.00 bcdefghij	633 ghij	676 efghi	2.89 abc	1.40 abc	45.00 ab	0.58 ab	0.33 ab	47.90 ab
MCColl-5/07	56.60 cde	53.40 bc	103 abc	9.77 bcdef	19.87 bcdef	33.33 fghijk	677 fghi	710 efgh	2.73 bc	1.24 bc	40.40 ab	0.58 ab	0.33 ab	48.40 ab
MCColl-6/07	56.60 cde	40.00 c	97 bcd	9.03 cdef	19.88 bcdef	28.00 hijk	647 fghij	675 efghi	3.17 ab	1.68 ab	45.00 ab	0.57 ab	0.32 ab	47.90 ab
MCColl-7/07	43.40 gh	50.00 bc	90 d	5.50 f	9.03 f	21.00 jk	473 jk	494 ij	2.64 bc	1.15 bc	40.40 ab	0.55 ab	0.30 ab	47.30 ab

TKColl-1/07	56.60 cde	53.40 bc	103 abc	13.87 abcdef	29.14 abcde	61.00 abcde	793 bcdefgh	854 bcdef	2.78 abc	1.29 abc	42.10 ab	0.53 ab	0.28 ab	46.10 ab
TKColl-2/07	36.60 h	40.00 c	96 bcd	7.57 ef	8.73 f	8.67k	395 k	403 j	2.98 abc	1.50 abc	43.90 ab	0.52 b	0.27 b	45.00 b
TKColl-3/07	56.60 cde	60.00 ab	100 abcd	9.70 bcdef	23.46 bcdef	42.33 bcdefghij	700 efghi	743 efgh	2.89 abc	1.40 abc	43.90 ab	0.52 b	0.27 b	46.10 ab
TKColl-4/07	50.00 efg	56.60 abc	94 cd	10.73 abcdef	14.64 def	45.67 bcdefghij	661 fghij	707 efgh	2.79 abc	1.30 abc	42.70 ab	0.52 b	0.27 b	45.60 ab
TKColl-5/07	63.40 bc	53.40 bc	97 bcd	14.67 abcde	21.58 bcdef	55.67abcdefg	752 defghi	808 cdefg	2.68 bc	1.19 bc	41.10 ab	0.58 ab	0.32 ab	47.90 ab
TKColl-6/07	60.00 bcd	60.00 ab	105 abc	15.77 abcde	27.62 abcde	50.00bcdefgh	742 defghi	792 defgh	2.93 abc	1.44 abc	43.90 ab	0.53 ab	0.28 ab	46.70 ab
TKColl-7/07	60.00 bcd	60.00 ab	100 abcd	13.67 abcdef	26.96 abcde	43.00 bcdefghij	769 cdefghi	812 cdefg	2.84 abc	1.35 abc	43.30 ab	0.57 ab	0.32 ab	47.90 ab
TKColl-8/07	60.00 bcd	56.60 abc	102 abc	12.60 abcdef	20.88 bcdef	44.67 bcdefghij	758 cdefghi	803 defgh	2.82 abc	1.33 abc	42.70 ab	0.56 ab	0.30 ab	46.70 ab
TKColl-9/07	56.60 cde	50.00 bc	103 abc	10.50 abcdef	31.32 abc	52.00bcdefgh	803bcdef	855 bcdef	2.66 bc	1.17 bc	41.00 ab	0.57 ab	0.32 ab	47.90 ab
TKColl-10/07	60.00 bcd	53.40 bc	103 abc	11.13 abcdef	18.04 bcdef	35.33 defghij	648 fghij	684 efghi	2.86 abc	1.37 abc	42.70 ab	0.54 ab	0.29 ab	46.10 ab
TKColl-11/07	60.00 bcd	50.00 bc	103 abc	11.93 abcdef	29.07 abcde	43.00 bcdefghij	749 defghi	792 defgh	2.80 abc	1.31 abc	42.70 ab	0.52 b	0.27 b	45.00 b
TKColl-12/07	63.40 cd	50.00 bc	102 abc	13.40 abcdef	19.67 bcdef	45.67 bcdefghij	842 abcdef	888 abcde	2.88 abc	1.39 abc	43.30 ab	0.52 b	0.27 b	45.60 ab
TKColl-13/07	60.00 bcd	45.00 bc	109 a	10.35 abcdef	18.59 bcdef	25.50 hijk	779 cdefgh	805 defg	2.90 abc	1.41 abc	43.90 ab	0.56 ab	0.31 ab	46.70 ab
Mean	56.85	54.10	100.97	12.96	23.28	44.43	744.61	789.09	2.81	1.32	42.39	0.55	0.30	47.02
CV (%)	12.26	23.59	7.85	47.49	47.82	43.12	18.84	19.09	14.75	31.42	18.17	12.63	23.22	10.90

*EV = early vigor, LS = leaf senescence, PH = plant height, NN/P = number of nodules/plant, NDW/p = nodule dry weight per plant, RDW/p = root dry weight per plant, SDW/p = shoot dry weight per plant, TDW/p = total dry weight per plant, TN = total foliage nitrogen content, NDFfa = proportion of total nitrogen derived from atmospheric fixation, RNFE = rate of nitrogen fixation efficiency, TP = total phosphorus content, PDFf = phosphorus derived from fertilizer and PUTE = phosphorus uptake efficiency

**Means in a column not sharing the same letter are significantly different ($P \leq 0.05$).

Likewise, differences for P uptake efficiency were significant but only among a few accessions, ranging between 45.00 and 50.20 %. This probably indicates that the accessions were more variable for biological nitrogen fixation than they were for P uptake efficiency.

Genotype by soil type interaction effects were significant for early vigor, nodule number and weight and total phosphorus content, phosphorus derived from fertilizer and phosphorus uptake efficiency. This clearly showed that the two soil types were distinctly different or the genotypes responded differently to the two soil types for these traits or both (Annicchiarico, 2002). Hence, for traits with significant genotype by soil type interaction, the best genotype under one soil type may not be the best under the other (van Oosterom *et al*., 1993). Fortunately, however, genotypes by soil type interaction effects were non-significant for a number of other component traits including the ultimate biological nitrogen fixation itself. This indicated that genotypes selected under one of the soils may repeat similar performance for biological nitrogen fixation under the other soil type as well.

The present experiment indicated that significant performance differences were observed for different traits due to both genotype and soil types. However, better level of average biological nitrogen fixation was generally observed under Nitisol (45.01 %) than it was under Vertisol (39.29 %) and this could somehow be associated with the variable characteristics of the two soil types particularly the difference in drainage of excess moisture (Tekalign and Asgelil, 1994) as the impeded drainage in Vertisols may reduce the level of nitrogen fixation (Hebblethwaite *et al*., 1983).

Magnitude of genetic diversity

The accessions were grouped into four diversity classes under Vertisol, three under Nitisol and four clusters for average performance on the two soils (Figure 1 A-C). Different members within a cluster being assumed to be more closely related in terms of the traits under consideration with each other than those members in different clusters. Similarly, members in clusters with non-significant distances are assumed to have more close relationships with each other than they are with those in significantly distant clusters. Cluster C_1 was the largest constituting over 45 % of accessions under Vertisol, close to 67 % under Nitisol and over 36 % of the total population for combined cluster performance under the two soils. The rest of the clusters also constituted from a single or two accessions to over one-third of the populations.

The pair wise generalized squared distances (D^2) among the clusters showed that the

maximum distances were found between clusters C_2 and C_3 under both Vertisol (D^2 = 310.92) and Nitisol (D^2 = 109.19) and between clusters C3 and C4 for average performances combined over Vertisol and Nitisol (D2 = 461.71). The genetic divergence between all possible pairs of clusters were also highly significant ($P < 0.01$) except between clusters C_1 and C_2 under Nitisol (D^2 = 14.89) (Table 3). Maximum genetic recombination and variation in the subsequent generation may be expected from crosses that involve parents derived from the clusters characterized by significant distances (Reddy, 1988; Wallace and Yan, 1998; Chahal and Gosal, 2002; Singh, 2002). Crosses between lines selected from these clusters are, therefore, expected to provide relatively better genetic recombination and segregation in their progenies.

There was no clear association between geographic origin and genetic diversity but collections from North Wello showed tendencies to fall into different clusters with non-significant distances while those from South Tigray were better distributed allover the clusters and this may be related to the limited number of samples from North Wello as compared to those from South Tigray. The fact that some of the accessions from the same places of origin fell into different clusters and vice versa might show possibilities for the presence of genetic diversity among accessions for the traits studied regardless of the similarities in places of origin. On the other hand, there were accessions from different places of origin fell into the same clusters, indicating that accessions may show genetic similarities regardless of the differences in places of origin (Figure 1). This may be related to the fact that the Abyssinian field pea exists in confined adjacent regions that could be considered similar in terms of both agro-ecology and crop production system under which distinct pattern of evolution may not be expected.

Table 3. Pairwise generalized squared distances (D^2) among 33 Abyssinian field pea accessions grown under Vertisol and Nitisol

Cluster	C_1	C_2	C_3	C_4
		Vertisol		
C_1	0	101.58**	114.79**	30.20**
C_2		0	310.92**	164.84**
C_3			0	31.72**
C_4				0
		Nitisol		
C_1	0	14.89NS	47.27**	--
C_2		0	109.19**	--
C_3			0	--
		Average (Vertisol and Nitisol)		
C_1	0	34.65**	159.14**	96.94**
C_2		0	52.98**	219.52**
C_3			0	461.71**
C_4				0

** = highly significant ($P \leq 0.01$); NS = non-significant

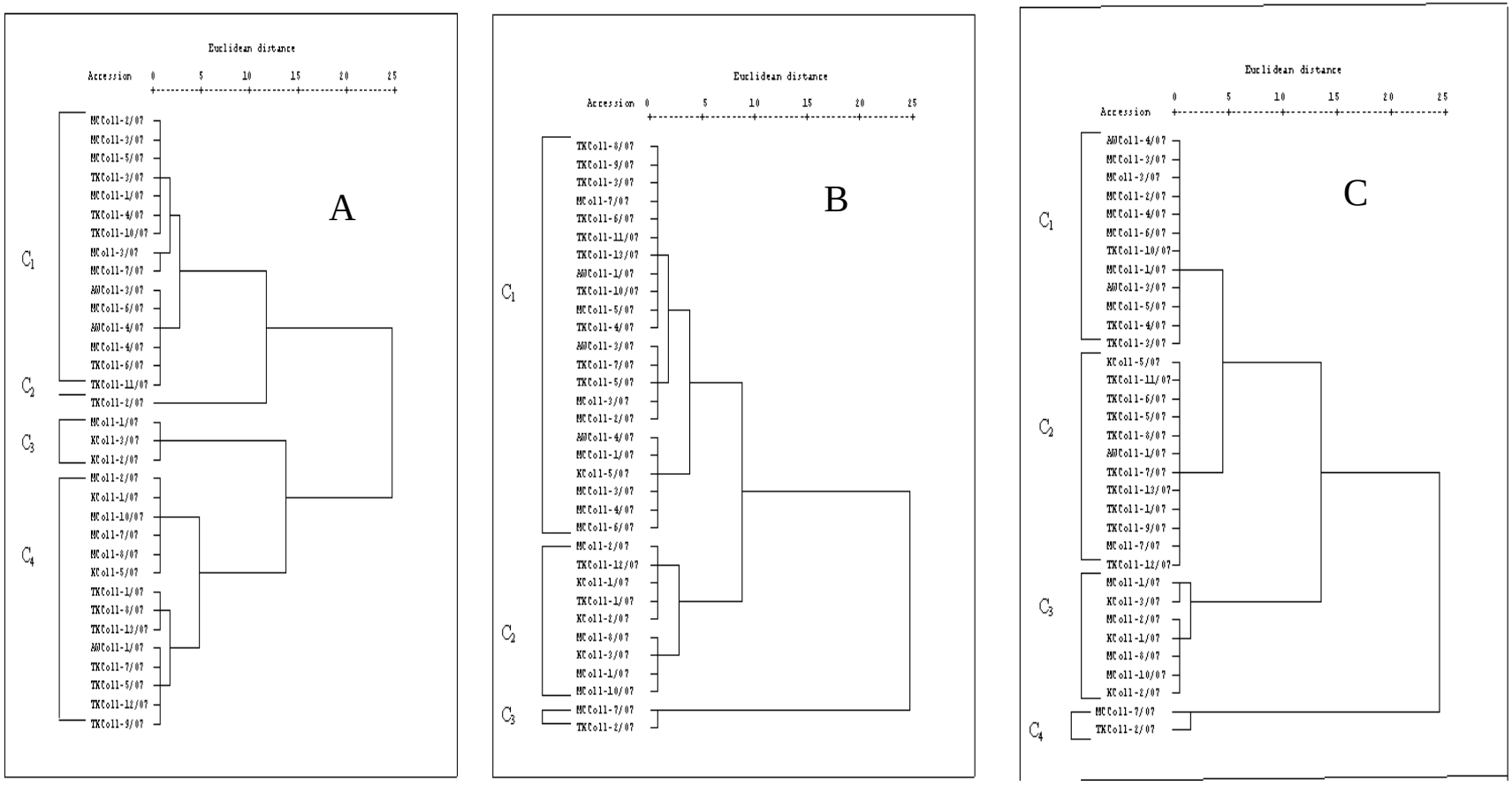

Figure 1 A-C. Dendrograms of 33 Abyssinian field pea accessions grown on (A) Vertisol, (B) Nitisol and (C) average of Vertisol and Nitisol based on average linkage cluster analysis between groups.

A study previously conducted on *P. sativum* var Sativum also suggested that differences in eco-geographic origin might not necessarily suggest presence of genetic divergence for other morpho-agronomic traits (Gemechu *et al*., 2005; Mussa *et al*., 2006).

Relative contribution of the component characters to genetic diversity.

Principal component analysis (PCA) for average performance over the two soil types showed that the first four principal components accounted for slightly over 86 % of the total variation, of which 66 % was contributed by the first two principal components (PC1 and PC2) (Table 4). The first principal component contributed slightly over 42 % while the second contributed over 23 % of the total variation. It is normally assumed that characters with larger eigenvector values closer to unity within the first principal component influence the clustering more than those with lower values closer to zero (Chahal and Gosal, 2002). Accordingly, characters related to total plant biomass and nodule biomass and number had higher relative contribution to the total diversity and, therefore, the differentiation of the accessions into different clusters was rather determined by the cumulative effects of a number of characters than individual contribution by specific traits. The second principal component illustrated primarily the variation for total nitrogen and phosphorus contents and nitrogen fixation and phosphorus uptake efficiencies. A graph of three-dimensional plot (Figure 2) of the first three principal components (PC1, PC2 and PC3) for performances on both Vertisol and Nitisol also revealed more or less consistent pattern with the output of cluster analysis. The existence of accessions with positive values for the first and the second principal components on both Vertisol and Nitisol may suggest favorable association among the components for simultaneous improvement of nitrogen fixation and P uptake efficiencies (Figure 2).

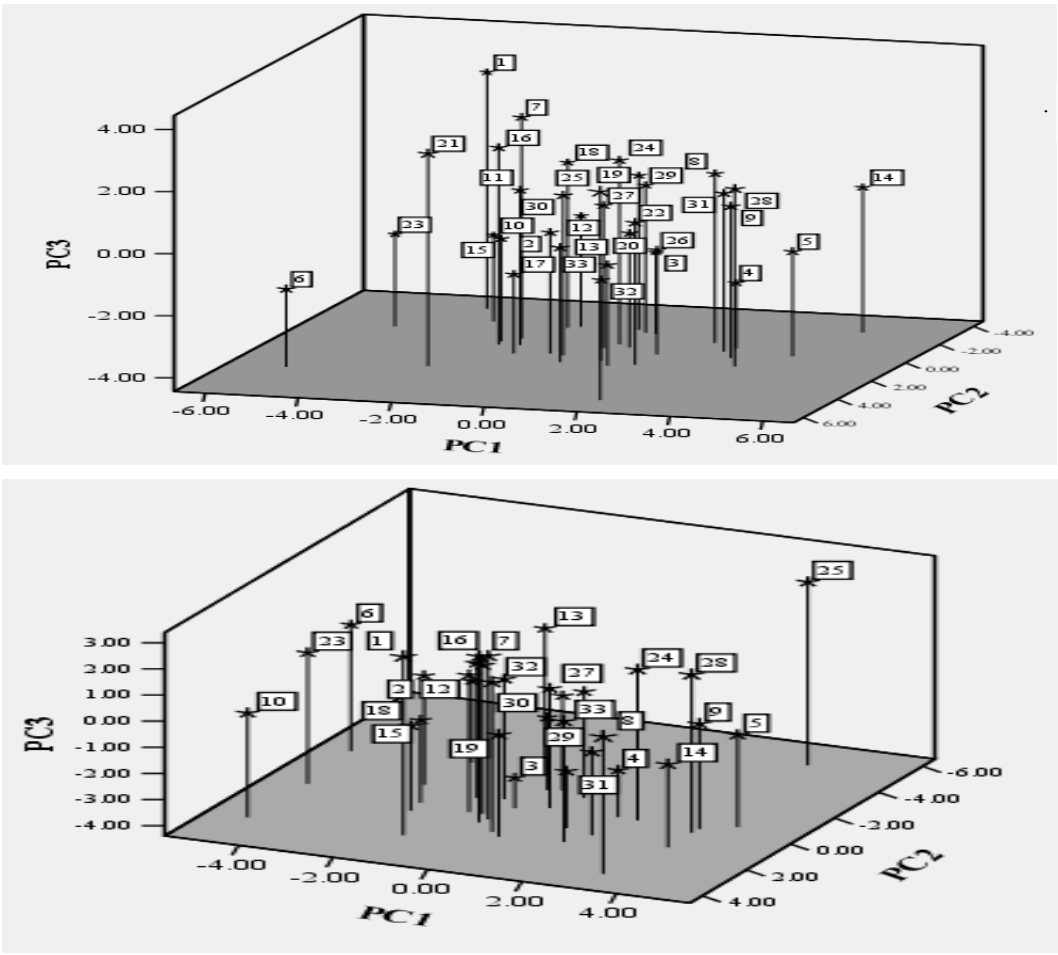

Figure 2. Three-dimensional plot of the first three principal components (PC1, PC2 and PC3) of 33 Abyssinian field pea accessions grown on (A) Vertisol and (B) Nitisol

Table 4. Percentage and cumulative variances and eigenvectors on the first four principal components (PC) on 14 characters in 33 Abyssinian field pea accessions for average performance under Vertisol and Nitisol

Parameter	PC1	PC2	PC3	PC4
Eigen value	5.94	3.30	2.21	0.70
% variance	42.45	23.55	15.82	5.00
Cumulative variance	42.45	66.00	81.82	86.82
Character	Eigenvectors			
Early vigor (1-5 scale)	0.323	0.088	0.094	0.425
Leaf senescence (1-5 scale)	0.238	0.204	0.117	0.649
Plant height (cm)	0.324	0.042	0.031	0.221
No. of nodules plant $^{-1}$	0.363	0.027	0.003	0.258
Nodule dry weight (mg plant^{-1})	0.347	0.018	0.102	0.411
Root dry weight (mg plant^{-1})	0.361	0.035	0.002	0.152
Shoot dry weight (mg plant^{-1})	0.399	0.034	0.045	0.128
Total dry weight (mg plant^{-1})	0.401	0.034	0.042	0.131
Total nitrogen content (%)	0.000	0.486	0.304	0.005
Nitrogen derived from fixation (%)	0.002	0.487	0.302	0.007
Nitrogen fixation efficiency (%)	0.013	0.421	0.334	0.121
Total phosphorus content (%)	0.108	0.365	0.440	0.161
Phosphorus derived from fertilizer (%)	0.118	0.370	0.420	0.129
Phosphorus uptake efficiency (%)	0.081	0.154	0.545	0.033

CONCLUSIONS

The present study, even though conducted with a limited number of accessions under controlled conditions, showed that there is adequate level of genetic diversity among the Abyssinian field pea accessions for biological nitrogen fixation, with different levels of contribution towards the total diversity by different component characters. Nevertheless, accessions from different origins might be closely related and vice versa, indicating absence of definite pattern of correspondence between geographic origin and genetic diversity, which might emanate from the proximity to one another between the origins of collection. This indicated that the accessions may be considered as source population for the enhancement of biological nitrogen fixation through selection for superior genotypes. This finding, coupled with the existence of high *Rhizobium* strain diversity reported in Ethiopia (Endalkachew *et al.*, 2004;

Endalkachew *et al*., 2005) including in field pea (Aregu, 2006), should be considered as an opportunity enabling the exploitation of the synergic effects of host strain interaction. However, it should be noted that this investigation could provide only baseline information which need to be verified under field condition and probably with a large number of collections for identification of highly efficient genotypes. In addition, future in depth study of the morphological, physiological, agronomic and molecular basis for differences in biological nitrogen fixation and mechanism of effective host-strain synergy could further help BNF efficient varietal and strain development processes.

Acknowledgements

We would like to acknowledge the Biology Department of Addis Ababa University and Holetta Agricultural Research Center for material and technical supports and Mussa Jarso for assistance in statistical analysis. We also thank Hailu Regassa for allowing us to flexibly carry out the chemical analysis of plant tissue and soil samples.

REFERENCES

Annicchiarico, P. 2002. Genotype x Environment Interaction: Challenges and Opportunities for Plant Breeding and Cultivar Recommendation. FAO Plant Production and Protection Paper No. 174. Food and Agriculture Organization, Rome.

Agegnehu, G, Fikre, A, and Tadesse, A. 2006. Cropping systems, soil fertility and crop management research on cool-season food legumes in the Central Highlands of Ethiopia. In Kemal Ali, Gemechu Keneni, Seid Ahmed, Rajendra Malhotra, Surendra Beniwal, Khaled Makkouk and M.H. Halila (eds.). Food and forage legumes of Ethiopia: Progress and prospects. Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria. pp. 135-145.

Aregu Amsalu 2006. Symbiotic and phenotypic characterization of Rhizobium leguminosarium bv viciae isolates of field pea (*Pisum sativum*) from different growing regions of Ethiopia. M.Sc Thesis, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

Association of Official Agricultural Chemists (AOAC) 1970. Official Methods of Analysis (11th ed.), Washington, D.C.

Beringer, JE, Bisseling, TA and Larue, TA. 1988. Improving symbiotic nitrogen fixation through the genetic manipulation of *Rhizobium* and legume host plants. In R.J. Summerfield (ed.). World crops: Cool season food legumes. Kluwer Academic Publishers, Dordrecht. pp. 691-702.

Bowen, GD and Zapata, F. 1991. Efficiency in uptake and use of nitrogen and phosphorus by plants. In stable isotopes in plant nutrition, soil fertility and environmental studies. Proceedings of an international symposium on the use of isotopes in plant nutrition, soil fertility and environmental studies, 1-5 Oct. 1990, IAEA, Vienna. pp. 349-362

Chahal, GS and Gosal, SS. 2002. Principles and procedures of plant breeding: biotechnological and conventional approaches. Narosa Publishing House, New Delhi.

Clark, KW, Brockwell, J and Thompson, JA. 1988. Role of inoculants in improving nitrogen fixation in legumes. In R.J. Summerfield (ed.). World crops: Cool season food legumes. Kluwer Academic Publishers, Dordrecht. pp. 731-743.

CSA 2004. Area under cultivation, yield and production of major crops in the Meher season. Central Statistics Authority (CSA), Addis Ababa, Ethiopia.

EMA 1988. National Atlas of Ethiopia. Ethiopian Mapping Authority (EMA), Addis Abeba.

FAO. 1995. Sustainable dryland cropping in relation to soil productivity - FAO soils bulletin 72. Food and Agriculture Organization of the United Nations (FAO). Rome.

Graham, RD. 1988. Development of wheats with enhanced nutrient efficiency: Progress and potential. In Klatt A.R. (ed.). Wheat production constraints in tropical environments. Mexico, D.F.: CIMMYT. pp. 305-320.

Hagedorn, DJ. 1984. Compendium of pea diseases. The American Phytopathological Society, Minnesota, USA.

Hauck, RD and Bremner, JM. 1976. Use of tracers in soil and fertilizer nitrogen and phosphorus research. *Advances in Agronomy* 28:219-266.

Hailemariam, A and Sigie, A. 2006. Biological nitrogen fixation research on food legumes in Ethiopia. In Kemal Ali, Gemechu Keneni, Seid Ahmed, Rajendra Malhotra, Surendra Beniwal, Khaled Makkouk and M.H. Halila (eds.). Food and forage legumes of Ethiopia: Progress and prospects. Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria. pp. 172-176.

Hebblethwaite, PD, Hawtin, GC and Latman, JW. 1983. The husbandry of establishment and maintenance. In P.D. Hebblethwaite (ed.). The Faba Bean (*Vicia faba* L.). A basis for improvement, Cambridge University Press, UK. pp. 271-312.

International Food Policy Research Institute (IFPRI) and Central Statistics Agency (CSA) 2006. Atlas of the Ethiopian rural economy. Addis Ababa, Ethiopia.

Jarso, M, Wolabu, T and Keneni, G. 2006. Review of Field Pea (*Pisum sativum* L.)

Genetics and Breeding Research in Ethiopia. In Kemal Ali, Gemechu Keneni, Seid Ahmed, Rajendra Malhotra, Surendra Beniwal, Khaled Makkouk and M.H. Halila (eds.). Food and forage legumes of Ethiopia: Progress and prospects. Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria. pp. 67-79.

Kelley, TG, Rao, PP and Grisko-Kelley, H. 2000. The Pulse Economy in the Mid-1990s: A Review of Global and Regional Development. In Knight R. (ed.). Linking Research and marketing opportunities for pulses in the 21st Century. Proceeding of the third international Food Legumes Research Conference. Kluwer academic Publishers, Dondrecht. pp. 1-29.

Keneni, G, Jarso, M, Wolabu, T and Dino, G. 2005. Extent and pattern of genetic diversity for morpho-agronomic traits in Ethiopian highland pulse landraces. I. Field pea (*Pisum sativum* L.). *Genet. Resour Crop Evol.* 52: 539-549.

Little, TM and Hills, FJ. 1978. Agricultural Experimentation: Design and analysis. John Wiley and Sons. New York.

Mamo, T and Dibabe, A. 1994. Soil microbiology research. In Asfaw Telaye, Geletu Bejiga, M.C. Saxena, and M.B. Solh. (eds.). Cool-Season Food Legumes of Ethiopia.

Proceedings of the First National Cool-SeasonFood Legumes Review Conference, 16-20 December 1993, Addis Ababa, Ethiopia. ICARDA/IAR. ICARDA: Aleppo, Syria. pp 293-311.

Messiaen, CM., Seif, AA, Jarso, M and Keneni, G. 2006. *Pisum sativum* In Brink M. and Belay G. (eds.). Plant Resources of Tropical Africa 1: Cereals and Pulses. PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands. pp. 151-158.

Messiaen, C M, Seif, AA, Jarso, M and Keneni, G. 2004. *Pisum sativum* In Grubben G.J.H and Denton O.A. (eds.). Plant Resources of Tropical Africa 2: Vegetables. PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands. pp. 419-425

Reddy, PS. 1988. Genetics, Breeding and Varieties. In Reddy P.S. (ed.), Groundnut. Publication and Information Division, Indian Council of Agricultural Research, Krishi Anusandhan Bhavan, Pusa, New Delhi. pp. 200-317.

SAS Institute 1996. SAS/STAT guide for personal computers, version 6.12 edition. Cary, NC: SAS Institute Inc.

Schmidt, EL. 1988. Competition for legume nodule occupancy: a down-to-earth limitation on nitrogen fixation. In R.J. Summerfield (ed.). World crops: Cool season food legumes. Kluwer Academic Publishers, Dordrecht. pp. 663-674.

Serstu, S and Bekele, B. 2000. Procedures for soil and plant analysis. Technical Paper No. 74. National Soil Research Center, Ethiopian Agricultural Research Organization, Addis Ababa.

Singh, BD. 2002. Plant breeding: principles and methods. Kalyani Publishers, New Delhi-Ludhiana.

Singh, RK and Chaudhary, BD. 1985. Bioemetrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi-Ludhiana.

Smith, A, Elmes, AE, Howard, RS and Franklin, MF. 1984. The uptake of soil and fertilizer-nitrogen and phosphorus by barley growing under Scottish climatic condition. *Plant and Soil* (76): 49-57.

Tadesse, D, Tilaye, A and Bejiga, G. 1994. Genetic resources in Ethiopia. In Asfaw Tilaye, Geletu Bejiga, M. C. Saxena, and M. B. Solh (eds.) Cool-season Food Legumes of Ethiopia. Proceeding of the first national cool-season food legumes review conference, 16-20 December 1993, Addis Ababa, Ethiopia. ICARDA/IAR. ICARDA, Syria. pp. 79-96.

van Oosterom, EJ, Kleijn, D, Ceccarelli, S and Nachit, MM. 1993. Genotype by environment interaction of barley in the Mediterranean Region. *Crop Science* 33: 669-674.

Vincent, JM. 1970. A manual for the practical study of root nodule bacteria. Blackwell Sci. Publ. Oxford.

Wallace, DH and Yan, W. 1998. *Plant Breeding and Whole-System Crop Physiology*. University Press, Cambridge, UK.

Westphal, E. 1974. Pulses in Ethiopia: Their Taxonomy and Significance. College of Agriculture, Haile Sellessie I University Ethiopia/ Agriculture University, Wageningen, The Netherlands.

Witty, JF, Rennie, RJ and Atkins, CA. 1988. ^{15}N addition methods for assessing N_2 fixation in legumes. In R.J. Summerfield (ed.). World crops: Cool season food legumes. Kluwer Academic Publishers, Dordrecht. pp. 715-730.

Wolde-meskel, E, Terefework, Z, Frostegard, A and Lindstrom, K. 2005. Genetic diversity and phylogeny of Rhizobia isolated from agroforestry legume species in Southern Ethiopia. *International Journal of Systematic and Evolutionary Microbiology 55: 1439-1452*.

Wolde-meskel, E, Terefework, Z, Lindstrom, K and Frostegard, A. 2004. Metabolic and genomic diversity of Rhizobia isolated from field standing native and exotic woody legumes in Southern Ethiopia. *Systematic and Applied Microbiology* 27: 603-611.

Zapata, F. 1990. Field experimentation in isotope-aided studies. In use of nuclear techniques in soil-plant relationships. In Hardarson G. (ed.). Training course series No 2, IAEA, Vienna. pp. 61-127.