Phenotypic Diversity in Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm Accessions for Phosphorus Uptake and Use Efficiency

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Abstract

*Ethiopia is known as the secondary center of diversity for chickpea (***Cicer arietinum** *L.). Plant breeders primarily interested in utilizing the available germplasm for improving phosphorus uptake and use efficiency have no background information on the genetic diversity for this attribute. A field study involving 155 chickpea genotypes was undertaken at Ambo and Ginchi, Ethiopia, in 2009/2010 to characterize the genotypes for nutrient uptake and use efficiencies. Cluster analysis grouped the genotypes into five clusters in the absence and six clusters in the presence of phosphorus. The higher number of clusters when the crop was grown with phosphorus may be a manifestation of more genetic diversity due to the application of phosphorus. The Mahalanobis's* D^2 *statistics mostly showed significant genetic distances between clusters constituted local landraces on the one hand and introduced genotypes on the other. This indicated that there were distinct multivariate differences between landraces and introduced genotypes. No clear interrelationship was observed between the origins of the landraces within Ethiopia and the pattern of genetic diversity. Different characters had different contribution to the total differentiation of the populations in all the cases. The result of this study suggests existence of adequate genetic diversity for attributes of phosphorus uptake and use efficiency in these chickpea genotypes, which should be exploited in future breeding.*

Keywords: Cluster analysis, dendrogram, Ethiopia, genetic diversity, principal component analysis.

Introduction

Abiotic stresses cause more economic losses to crop plants than the biotic ones (Slater *et al*., 2003) and low nutrient stress, which ranks the second after drought, may be among the most important abiotic stresses (Singh, 2002; Cattivelli *et al*., 2008). Nitrogen and phosphorus deficiency stresses are important problems worldwide, particularly in the tropics and the subtropics (Beebe *et al*., 2006; Gunes *et al*., 2006; Ojo *et al*., 2006). The problem is widespread in East Africa (Sanchez, 2002) and Ethiopia is no exception. Most legume crops perform poorly under low phosphorus level (Beebe *et al*., 2006; Ojo *et al*., 2006; McKnight Foundation, 2008).

Crop productivity can basically be improved by genetic modification of crops or by altering the growing environments (Wallace and Yan, 1998). In order to overcome the problem of phosphorus deficiency stress, environmental manipulation through the application of phosphorus fertilizer from external sources has been widely adopted. The use of phosphorus fertilizer has been increasing across time (World Phosphate Institute, 2006) may be because many countries inspire to intensify crop production as a result of the increasing demand for food with population growth. However, in addition to the immediate costs, the continued use of high fertilizer input accelerated depletions of the non-renewable raw materials and energy resources required for fertilizer production (FAO, 1984; Syers *et al*., 2008).

Genetic modification of crops for developing nutrient use efficient genotypes is often preferred to the continual manipulation of the growing environment not only because of cost but also because of concerns over food safety and agricultural sustainability (Burger *et al*., 2008; Löschenberger *et al*., 2008; Wolfe *et al*., 2008). Nutrient use efficient genotypes are commonly defined as genotypes that are able to mobilize the limiting nutrients in greater amounts and better use of the absorbed nutrient for yield formation (Beebe *et al*., 2006; Liao *et al*., 2008). Examples of success stories in terms of varietal identification and/or release for nutrient use efficiency have also never been inexistent. For instance, phosphorus efficient genotypes of chickpea (Singh, 1990) and haricot bean (Aráujo *et al*., 1998) were identified and improved varieties of soybean with potentials of doubling yield without additional nutrients have been released to farmers in China and Africa (McKnight Foundation, 2008). A number of studies on different legume crops including chickpea elsewhere also showed existence of genetic diversity as sources of initial materials for breeding traits related to phosphorus efficiency (Aráujo *et al*., 1998; Walley *et al*., 2005; Srinivasarao *et al*., 2006; Vesterager *et al*., 2006).

Landraces have considerable role in crop improvement under marginal management and soil fertility levels as they contain valuable adaptive genotypes to different circumstances (Ceccarelli, 1994; Bunder *et al*., 1996; Chahal and Gosal, 2002). Although large number of chickpea landrace collections is available in Ethiopia, most of these collections have not yet been characterized and evaluated for important attributes like nutrient use efficiency, despite the significant economic and ecological importance of such studies. The objectives of this study were, therefore, to assess the magnitude and pattern of genetic diversity among the Ethiopian chickpea germplasm for attributes of economic and ecological importance including agronomic characters and phosphorus uptake and use efficiency.

Materials and Methods

A total of 155 chickpeas were evaluated. They include 139 accessions from different geographical regions of Ethiopia kindly provided by the Ethiopian Institute of Biodiversity Conservation (IBC), 5 improved genotypes provided by ICRISAT, 8 originally introduced commercial cultivars released in Ethiopia and three genetically non-nodulating genotypes received from ICRISAT and ICARDA. These chickpeas, called hereafter as "genotypes" for experimental purpose, are described in Table 1. The map of the areas of collection of the Ethiopian accessions is also given elsewhere (Keneni *et al*., 2012). Therefore, only statistical analysis unique to this part is presented here in detail.

The experiment was conducted under field conditions at two locations (Ginchi and Ambo) in central part of Ethiopia for one year during the main cropping season of 2009/10 (September to January). The two locations are characterized by Vertisol soils (Dibabe *et al*., 2001) and assumed to represent the major chickpea production areas in Ethiopia. Chickpea is mostly grown on Vertisol soils with residual moisture in Ethiopia. Detailed information related to the test locations, the climatic and edaphic characteristics, the strain of *Rhizobium* and method of inoculation, experimental design and layout, crop management and protection practices have been presented in Keneni *et al.* (2015).

Table 1. Description of the test genotypes

Geographical origin	No of Genotypes	Name of genotypes (serial numbers in bracket stand for designation in this study)
Arsi	13	Acc. No. 231327 (1), Acc. No. 231328 (2), Acc. No. 209093 (3), Acc. No. 208829 (4), Acc. No. 209094 (5), Acc. No. 209092 (6), Acc. No. 209096 (7), Acc. No. 209097 (8), Acc. No. 209098 (9), Acc. No. 41002 (10), Acc. No. 207761 (11), Acc. No. 207763 (12), Acc. No. 207764 (13)
East Gojam	13	Acc. No. 41268 (14), Acc. No. 41026 (15), Acc. No. 41074 (16), Acc. No. 41075 (17), Acc. No. 41073 (18), Acc. No. 41076 (19), Acc. No. 41021 (20), Acc. No. 41027 (21), Acc. No. 41222 (22), Acc. No. 207734 (23), Acc. No. 41103 (24), Acc. No. 41320 (25), Acc. No. 41029 (26)
West Gojam	13	Acc. No. 41015 (27), Acc. No. 41271 (28), Acc. No. 41272 (29), Acc. No. 41276 (30), Acc. No. 207745 (31), Acc. No. 41275 (32), Acc. No. 41277 (33), Acc. No. 207743 (34), Acc. No. 207744 (35), Acc. No. 41273 (36), Acc. No. 41274 (37), Acc. No. 207741 (38), Acc. No. 207742 (39)
North Gonder	13	Acc. No. 41316 (40), Acc. No. 41298 (41), Acc. No. 41311 (42), Acc. No. 41313 (43), Acc. No. 41280 (44), Acc. No. 41312 (45), Acc. No. 41315 (46), Acc. No. 41308 (47), Acc. No. 41299 (48), Acc. No. 41046 (49), Acc. No. 41047 (50), Acc. No. 41304 (51), Acc. No. 41303 (52)
South Gonder	12	Acc. No. 41295 (53), Acc. No. 41296 (54), Acc. No. 41289 (55), Acc. No. 41290 (56), Acc. No. 41284 (57), Acc. No. 41291 (58), Acc. No. 41297 (59), Acc. No. 41293 (60), Acc. No. 41019 (61), Acc. No. 41048 (62), Acc. No. 41049 (63), Acc. No. 41053 (64)
West Harargie	11	Acc. No. 41054 (65), Acc. No. 41052 (66), Acc. No. 209082 (67), Acc. No. 209083 (68), Acc. No. 209084 (69), Acc. No. 209091 (70), Acc. No. 209087 (71), Acc. No. 209088 (72), Acc. No. 209089 (73), Acc. No. 209090 (74), Acc. No. 209081 (75)
East Shewa	13	Acc. No. 41159 (76), Acc. No. 41160 (77), Acc. No. 41161 (78), Acc. No. 207661 (79), Acc. No. 207667 (80), Acc. No. 207666 (81), Acc. No. 41141 (82), Acc. No. 207665 (83), Acc. No. 41134 (84), Acc. No. 41128 (85), Acc. No. 41168 (86), Acc. No. 41129 (87), Acc. No. 41130 (88)
North Shewa	13	Acc. No. 41110 (89), Acc. No. 207657 (90), Acc. No. 41111 (91), Acc. No. 41106 (92), Acc. No. 207658 (93), Acc. No. 41142 (94), Acc. No. 41207 (95), Acc. No. 41215 (96), Acc. No. 41216 (97), Acc. No. 41066 (98), Acc. No. 41011 (99), Acc. No. 41007 (100), Acc. No. 41008 (101)
West Shewa	13	Acc. No. 41186 (102), Acc. No. 209035 (103), Acc. No. 41176 (104), Acc. No. 41175 (105), Acc. No. 41174 (106), Acc. No. 209027 (107), Acc. No. 41170 (108), Acc. No. 41171 (109), Acc. No. 41185 (110), Acc. No. 209036 (111), Acc. No. 41190 (112), Acc. No. 41195 (113), Acc. No. 41197 (114)
Tigray	12	Acc. No. 207150 (115), Acc. No. 207151 (116), Acc. No. 207563 (117), Acc. No. 207564 (118), Acc. No. 207894 (119), Acc. No. 207895 (120), Acc. No. 213224 (121), Acc. No. 219797 (122), Acc. No. 219799 (123), Acc. No. 219800 (124), Acc. No. 219803 (125), Acc. No. 221696 (126)
South Wello	13	Acc. No. 41114 (127), Acc. No. 212589 (128), Acc. No. 41113 (129), Acc. No. 207659 (130), Acc. No. 207660 (131), Acc. No. 41115 (132), Acc. No. 225878 (133), Acc. No. 225873 (134), Acc. No. 225874 (135), Acc. No. 225877 (136), Acc. No. 207645 (137), Acc. No. 207646 (138), Acc. No. 225876 (139)
ICRISAT	5	ICC 5003 (140), ICC 4918 (141), ICC 4948 (142), ICC 4973 (143), ICC 15996 (144)
National releases	8	Shasho (ICCV 93512) (145), Arerti (FLIP 89-84C) (146), Worku (DZ-10-16-2) (147), Akaki (DZ-10-9-2) (148), Ejere (FLIP-97–263 C) (149), Teji (FLI 97–266 C)(150), Habru (FLIP 88-42c)(151), Natoli (ICCX-910112-6)(152)
Non-nodulating checks	3	ICC 19180 (153), ICC 19181 (154), PM 233 (155)

Phosphorus application and experimental layout

The experiment was laid down in a randomized complete block design with 2 replications. Each block was divided into two adjacent sub-blocks to accommodate both the phosphorus fertilized and unfertilized plots. The sub-blocks were separated 1.5 m apart. Whole set of genotypes were planted separately in alternating adjacent sub-blocks with and without phosphorus in side-by-side pairs. Undamaged clean seeds of each genotype selected to a reasonably uniform size by hand sorting were planted on the seedbeds. Plot size was 1 row 4m long. One sub-block in each block received basal application of phosphorus in the form of triple supper phosphate (TSP) containing 46% P_2O_5 in water soluble form at the recommended rate (calculated as 20 gm for a single row of 4 meters) and not to the other sub-block. The accessions were assigned to plots at random within each sub-block. As a source of nitrogen, all genotypes were inoculated with an effective isolate of *Rhizobium* for chickpea, CP EAL 004, originally isolated by the National Soil Laboratory from a collection of Ada'a District of East Shewa Zone, Ethiopia. The isolate was found to be efficient in nodulation and symbiotic nitrogen fixation in previous studies (Hailemariam and Tsige, 2006). The inoculum was received at the concentration of approximately 10^9 cells gm^{-1} of peat carrier. The concentration and purity of the inoculum was confirmed in the Soil Microbiology Laboratory at Holetta Agricultural Research Center immediately before planting. Seeds of all genotypes were coated with the inoculant at the rate of approximately 2 gm of inoculum for 80 seeds using 40% gum Arabic as an adhesive. All other crop management practices were applied uniformly to all treatments as required so that the test genotypes could express their genetic potentials for the traits under consideration.

Shoot and grain phosphorus analysis

Representative shoot and grain samples were collected at 90% physiological maturity and oven-dried to constant moisture at 70°C for 18 hours and ground to pass through 1 mm size mesh sieve. The determination of phosphorus content was made using the wet digestion technique (AOAC, 1970) at Holetta and Debre Zeit Soil Science Research Laboratories. Phosphorus uptake and use efficiency was estimated by a combination of the difference, balance and partial factor productivity methods (Cassman *et al*., 1998) following Syers *et al.* (2008) as:

The apparent use of P from fertilizer and soil sources (APUfs %) =

$$\frac{\text{Biomass uptake of }P\text{ in treated plants }(g/5\text{ plants})\ X\ \ 100}{P\text{ applied to treated plants }(g/5\text{ plants})}$$

The apparent P fertilizer recovery efficiency (APUf %) =

$$\frac{[\text{Biomass uptake of }P\text{ in treated plants }(g/5\text{ plants})\ -\text{Biomass uptake of }P\text{ in untreated plants }(g/5\text{ plants})]\ X\ \ 100}{P\text{ applied to treated plants }(g/5\text{ plants})}$$

The apparent use of P from soil (APUs %) = APUfs – APUf

Phosphorus yield efficiency (PYE) =

$$\frac{\text{Grain yield of treated plants }(g/5\text{ plants})}{P\text{ applied to treated plants }(g/5\text{ plants})}$$

Phosphorus physiological efficiency (PYE) =

$$\frac{\text{Grain yield in treated plants }(g/5\text{ plants})}{P\text{ in treated plants }(g/5\text{ plants})}$$

Plant phosphorus yields were obtained by multiplying their tissue phosphorus concentration by dry matter yield as follows:

Grain P yield = Grain P content × grain yield

Shoot P yield = Shoot P content × shoot yield

Biomass P yield = Grain P yield × shoot P yield

The phosphorus harvest index (PHI), i.e. the ratio of the amount of the element in the grain relative to the amount of the element in the total above-ground biomass of the plant, was estimated as:

$$\text{PHI} = \frac{\text{Grain P yield}}{\text{Biomass P yield}}$$

Relative reductions of phosphorus related and agronomic characters in phosphorus untreated plants relative to the respective phosphorus treated plants were calculated to evaluate the sensitivities of the characters to phosphorus unavailability at both locations (Pimratch et al., 2008) as:

$$\text{Relative reduction} = 1 - \left(\frac{\text{performance without P}}{\text{Performance with P}}\right)$$

Data collection

Data were collected either on plot basis or from randomly selected five plants mostly based on the descriptor developed by IBPGR, ICRISAT and ICARDA (1993). Data were recorded on phosphorus related traits which include: shoot P content (SPC, g 5 plants^{-1}), grain P content (GPC, g 5 plants^{-1}), biomass P content (BMPC, g

5 plants^{-1}), shoot P yield (SPY, mg 5 plants^{-1}), grain P yield (GPY, mg 5 plants^{-1}), biomass P yield (BMPY, mg 5 plants^{-1}), phosphorus harvest index (PHI), apparent use of P from fertilizer and soil (APUfs, %), apparent use of P from fertilizer (APUf, %), apparent use of P from soil (APUs, %), phosphorus yield efficiency (PYE, GY P applied^{-1}), phosphorus physiological efficiency (PPE, GY P in plant^{-1}), days to 50% flowering (DTF), days to 90% maturity (DTM), grain filling period (GFP), No. of pods (NP, 5 plants^{-1}), No. of seeds (NS, 5 plants^{-1}), shoot dry matter weight (SDMW, g 5 plants^{-1}), total biomass weight (BMWT, g 5 plants^{-1}), harvest index (HI), grain production efficiency (GPE, g 5 plants^{-1}), biomass production rate (BPR, %), economic growth rate (EGR, %), thousand seed weight (TSW, g) and grain yield (YLD, g 5 plants^{-1}).

Statistical analysis

Means on all traits were pre-standardized to means of zero and variances of unity before clustering to avoid bias due to differences in measurement scales (Manly, 1986). Clustering of accessions was performed by average linkage method of SAS software (SAS Institute, 1996) for both symbiotic and agronomic traits. Points where local peaks of the pseudo F statistic join with small values of the pseudo t^2 statistic followed by a larger pseudo t^2 for the next cluster fusion were examined to decide the number of clusters (SAS Institute, 1996). A dendrogram was built by Ward's agglomerative hierarchical minimum variance method (Ward, 1963) using the MINITAB 14 statistical package. Genetic distances between clusters as standardized Mahalanobis's D^2 statistics were calculated as:

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

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 and were tested for significance both at 1% and 5% probability levels against the tabulated values of X^2 for 'P' degree of freedom, where P is the number of characters considered (Singh and Chaudhary, 1985).

Results and Discussion

Magnitude of phenotypic diversity

Analysis of variance (ANOVA) indicated that there were significant differences ($P < 0.01$) among the genotypes for all characters (Table 2). The location effects and interaction terms at different levels were also significant for a number of characters but the detail is not discussed here. The existence of significant differences among the genotypes for all characters may confirm the presence of adequate

Table 2. Combined analysis of variance (over locations and phosphorus level) for attributes of phosphorus use efficiency and agronomic performance in 155 chickpea genotypes tested at two locations in Ethiopia

Character	Mean square[1]							CV (%)
	L	G	P	G × L	G × P	P × L	G × L × P	
Phosphorus contents and yields								
Shoot P content (SPC, g/5 plants)	**	**	**	**	*	NS	NS	22.04
Grain P content (GPC, g/5 plants)	**	**	**	NS	NS	**	NS	23.29
Biomass P content (BMPC, g/5 plants)	**	**	**	**	NS	NS	NS	25.04
Shoot P yield (SPY, mg/5 plants)	**	**	**	**	*	NS	NS	27.47
Grain P yield (GPY, mg/5 plants)	**	**	**	**	NS	**	NS	24.31
Biomass P yield (BMPY, mg/5 plants)	**	**	**	**	NS	NS	NS	21.24
Phosphorus harvest index	**	**	**	*	NS	NS	NS	11.53
Phosphorus uptake and use efficiency								
Apparent use of P from fertilizer and soil (APUfs, %)	**	**	---	*	---	---	---	19.86
Apparent use of P from fertilizer (APUf, %)	NS	**	---	NS	---	---	---	24.95
Apparent use of P from soil (APUs, %)	**	**	---	NS	---	---	---	21.91
Phosphorus yield efficiency (PYE, GY/P applied)	NS	**	---	NS	---	---	---	24.95
Phosphorus physiological efficiency (PPE, GY/P in plant)	**	**	---	NS	---	---	---	15.98
Agronomic characters								
Days to 50% flowering (DTF)	**	**	NS	**	NS	NS	NS	3.92
Days to 90% maturity (DTM)	**	**	NS	**	NS	NS	NS	2.95
Grain filling period (GFP)	**	**	NS	**	NS	NS	NS	6.89
No of pods (NP, 5 plants^{-1})	**	**	**	**	NS	NS	NS	21.58
No of seeds (NS, 5 plants^{-1})	NS	**	**	**	NS	NS	NS	23.35
Shoot dry matter weight (SDMW, g 5 plants^{-1})	**	**	**	*	NS	NS	NS	24.61
Total biomass weight (BMWT, g 5 plants^{-1})	**	**	**	*	NS	NS	NS	21.04
Harvest index (HI)	**	**	NS	NS	NS	NS	NS	16.03
Grain production efficiency (GPE, g 5 plants^{-1})	**	**	**	**	NS	NS	NS	22.37
Biomass production rate (BPR, %)	**	**	**	**	NS	NS	NS	20.68
Economic growth rate (EGR, %)	NS	**	**	*	NS	NS	NS	21.12
Thousand seed weight (TSW, g)	NS	**	NS	*	NS	NS	NS	18.43
Grain yield (YLD, g 5 plants^{-1})	NS	**	**	*	NS	NS	NS	24.95

[1] L = location, G = genotype, P = phosphorus level; **=highly significant ($P \leq 0.01$), * = significant ($P \leq 0.05$) and NS = non-significant ($P > 0.05$)

Table 3. Clustering of one hundred fifty five chickpea genotypes grown without and with phosphorus into clusters using mean of seventeen response characters to phosphorus and agronomic performance

Cluster	No of genotypes	Genotypes included in the cluster
Without phosphorus		
C_1	106	231327, 208829, 209092, 209097, 209098, 41002, 207761, 207763, 207764, 41074, 41075, 41073, 41076, 41021, 41027, 41271, 41276, 207745, 41275, 41277, 207744, 41273, 207741, 41316, 41298, 41311, 41315, 41308, 41299, 41304, 41303, 41295, 41296, 41290, 41291, 41019, 41048, 41049, 41053, 41054, 41052, 209082, 209083, 209084, 209087, 209088, 209089, 209090, 209081, 41159, 41160, 207661, 207667, 207666, 41141, 41128, 41168, 41129, 41130, 41106, 41142, 41207, 41216, 41011, 41007, 41008, 41186, 209035, 41176, 41175, 41174, 209027, 41171, 41195, 41197, 207151, 207564, 207895, 213224, 219797, 219800, 219803, 221696, 212589, 41113, 207659, 207660, 41115, 225878, 225873, 225874, 225877, 207645, 207646, 225876, ICC 5003, ICC 4918, ICC 4948, ICC 4973, ICC 15996, Shasho, Arerti, Worku, Akaki, Habru, Natoli
C_2	42	231328, 209094, 41268, 41026, 41222, 207734, 41103, 41320, 41029, 41015, 41272, 207743, 41274, 207742, 41313, 41280, 41312, 41046, 41047, 41289, 41284, 41297, 41293, 209091, 41161, 207665, 41134, 41110, 207657, 41111, 207658, 41215, 41066, 41170, 41185, 209036, 41190, 207150, 207563, 207894, 219799, 41114
C_3	2	209093, 209096
C_4	3	Ejere, Teji, ICC 19180
C_5	2	ICC 19181, PM 233
With phosphorus		
C_1	54	231327, 209098, 207761, 207763, 207764, 41073, 41027, 207734, 41103, 41015, 41276, 207745, 207743, 41273, 41274, 207742, 41298, 41311, 41047, 41295, 41289, 41284, 41019, 41049, 41053, 41052, 209083, 209084, 209091, 41160, 207667, 41141, 207665, 41128, 41168, 41111, 41106, 207658, 41215, 41216, 41066, 41176, 41171, 41185, 207563, 219800, 219803, 221696, 212589, 207659, 225877, 207645, 207646, ICC 4948
C_2	88	231328, 209093, 208829, 209094, 209092, 209096, 209097, 41002, 41268, 41026, 41074, 41075, 41076, 41021, 41222, 41320, 41029, 41271, 41272, 41277, 207744, 207741, 41316, 41313, 41280, 41312, 41315, 41308, 41046, 41304, 41296, 41290, 41291, 41297, 41293, 41048, 41054, 209082, 209087, 209088, 209089, 209090, 209081, 41159, 41161, 207661, 207666, 41134, 41129, 41130, 41110, 207657, 41142, 41207, 41011, 41007, 41008, 41186, 209035, 41175, 41174, 209027, 41170, 209036, 41190, 41195, 41197, 207150, 207151, 207564, 207894, 207895, 213224, 219797, 219799, 41114, 41113, 207660, 41115, 225878, 225873, 225874, 225876, ICC 5003, ICC 15996, Arerti, Worku, Akaki
C_3	6	ICC 4918, Ejere, Teji, Habru, Natoli, ICC 19180
C_4	1	41275
C_5	4	41299, 41303, ICC 4973, Shasho
C_6	2	ICC 19181, PM 233

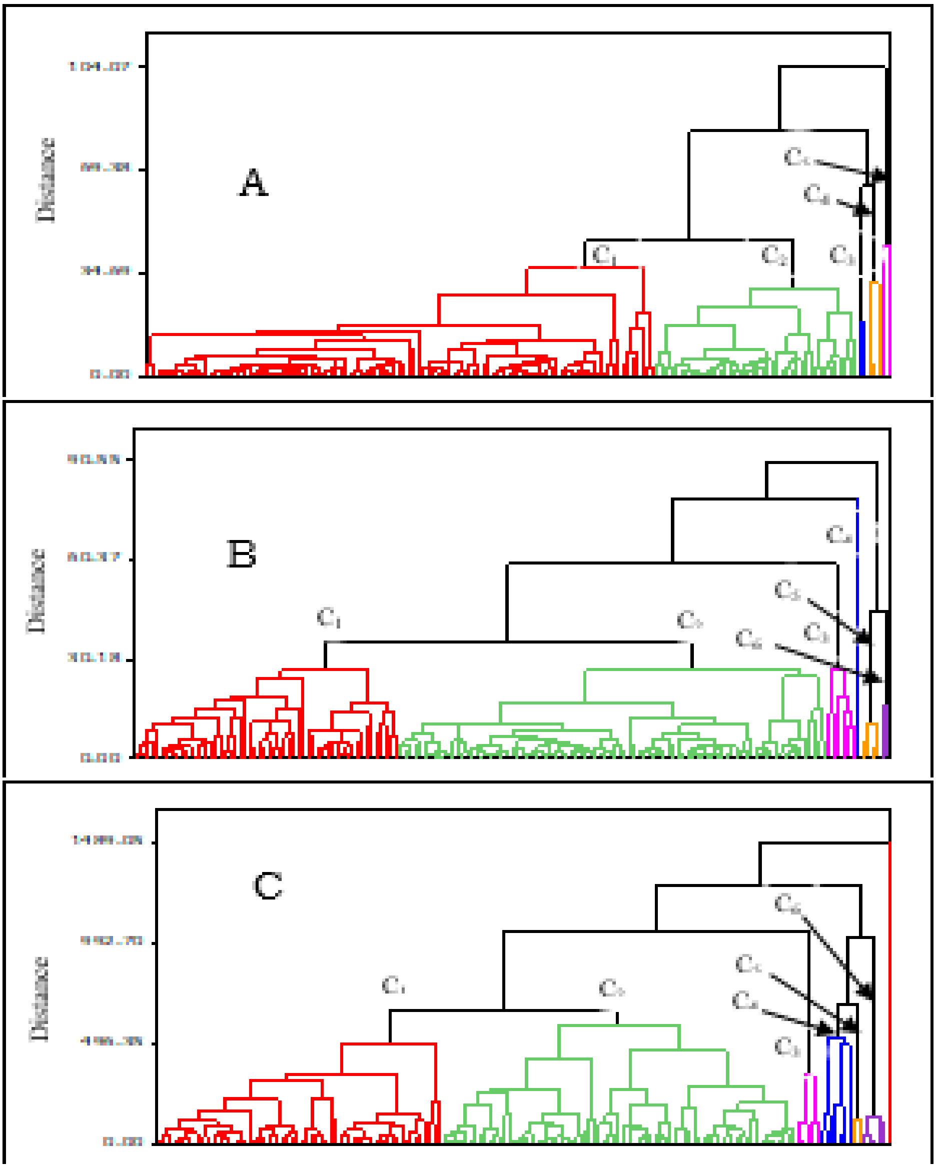

Figure 1. Dendrogram of hundred fifty five chickpea genotypes grown (A) without phosphorus, (B) with phosphorus and dendrogram (C) was built based on attributes of phosphorus uptake and use efficiency parameters (i.e. apparent use of phosphorus fertilizer and/or soil sources, phosphorus physiological and yield efficiencies). The dendrogram were developed by average linkage and Squared Euclidian distance using mean of 17 agronomic

genetic distances (D^2) of the 155 chickpea genotypes grown without and with phosphorus are presented in Table 4. Inter-cluster D^2 values ranged from 11 (between clusters C_1 and C_2) to 132 (between clusters C_4 and C_5) when the crop is grown in the absence of phosphorus and from 10 (between clusters C_1 and C_2) to 162 (between clusters C_4 and C_6) were obtained with phosphorus. The maximum pairwise generalized squared distances (D^2) were found between clusters C_4 and C_5 (D^2 = 132) without phosphorus and between C_4 and C_6 (D^2 = 132) with phosphorus. It is interesting to note that C_5 and C_6 constituted the non-nodulating (i.e. ICC 19181 and PM 233) references without and with phosphorus, respectively.

The second most divergent groups in the absence of phosphorus were clusters C_3 and C_4 (D^2 = 97) constituting local landraces and introductions, respectively. In the presence of phosphorus, the second most divergent groups were in clusters C_4 and C_5 (D^2 = 152), i.e. between a single local accession versus two local accessions and two introductions, respectively. The genetic divergences between other clusters were also highly significant (Table 4).

Maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances. Therefore, crosses between lines to be extracted from the landraces with introduced genotypes constituted in divergent clusters are expected to provide relatively better genetic recombination and segregation in their progenies.

Selection of parents should, however, consider the special advantages of each cluster and each genotype within a cluster depending on the specific objectives of hybridization. Therefore, this study revealed that the desirable relationship between parental lines to be developed from landrace collections and exotic introductions tends to be mutually complementary. The minimum inter-cluster distances between C_1 and C_2 indicated that members of these clusters were closely related whether they were grown in the presence or absence of phosphorus.

Comparison of D^2 values in the absence and presence of phosphorus showed that, not only the number of clusters increased from five to six with the application of phosphorus, but also the D^2 values between some clusters tended to increase under the latter. More number of clusters and higher cluster distances were obtained when the crop was grown with phosphorus compared to when it was grown without phosphorus. It is generally believed that more conducive environments may be expected to result in better expression of the genetic potential of the genotypes for the traits under consideration (Rosielle and Hamblin, 1981; Simmonds, 1991; Singh, 2002) despite the controversy that there may be no interrelationship between the type of the environment and the magnitude of genetic variation (Ceccarelli and Grando, 1996).

Table 4. Pair-wise generalized squared distances (D^2) values between clusters constituting 155 chickpea genotypes grown in the absence and presence of phosphorus fertilizers

Clusters	Clusters**					
	C_1	C_2	C_3	C_4	C_5	C_6
			Without phosphorus			
C_1	0.00	11.02NS	65.46**	74.06**	60.69**	---
C_2		0.00	42.24**	68.70**	65.26**	---
C_3			0.00	97.05**	57.92**	---
C_4				0.00	132.18**	---
C_5					0.00	---
			With phosphorus			
C_1	0.00	9.94	80.42**	42.05**	50.83**	78.97**
C_2		0.00	97.01**	84.09**	22.90 NS	64.37**
C_3			0.00	116.65**	115.58**	140.52**
C_4				0.00	152.84**	161.88**
C_5					0.00	47.97**
C_6						0.00

** = highly significant, $P \leq 0.01$, NS = non-significant, $P > 0.05$

Pattern of phenotypic diversity

The pattern of distribution of the genotypes from different origins over different clusters was apparently random, showing that there was no clear association between geographic sources of origin and genetic diversity. Some genotypes from the same places of origin fell into different clusters and *vice versa* (Table 5). This is as opposed to genetic diversity and cluster analysis of the same genotypes using microsatellite markers which showed definite association between pattern of genetic diversity and geographic sources of origin as discussed in Keneni *et al*. (2012).

The distribution of local accessions over the clusters was almost entirely limited to clusters C_1 and C_2, with the exception of two accessions from Arsi which fell in cluster C_3 when grown without phosphorus and one accessions from West Gojam and other two from North Gonder which fell into clusters C_4 and C_5 when grown with phosphorus. The rest of the genotypes which were grouped into clusters C_3-C_5 without phosphorus and C_3-C_6 with phosphorus trace their genetic background back into introductions from ICARDA or ICRISAT. Despite this distinct pattern of variation in a number of cases, however, overlappings were found among local landraces and introductions mostly in clusters C_1 and C_2. The partial overlapping of genotypes across geographical boundaries is an indication that geographical isolation was not the only factor that caused genetic diversity (Sharma and Mehta, 1990).

It should be noted here that the non-nodulating references were separately grouped from all the other genotypes into cluster C_5 when grown without phosphorus and cluster C_6 with phosphorus. This may be related to their inferior multi-trait performance, except for seed size. Even if relatively more distinct pattern of variation was revealed between the introduced genotypes and the local accessions, it may be not possible to rule out morpho-agronomical similarities among genotypes regardless of the differences in places of origin.

Table 5. Clustering pattern of 155 chickpea genotypes from different origins over six clusters based on mean performance of 17 characters

Origin	No. of genotypes	No. of genotypes in each cluster					
		C_1	C_2	C_3	C_4	C_5	C_6
Without phosphorus							
Arsi	13	9	2	2	-	-	-
East Gojam	13	6	7	-	-	-	-
West Gojam	13	8	5	-	-	-	-
North Gonder	13	8	5	-	-	-	-
South Gonder	12	8	4	-	-	-	-
West Haragie	11	10	1	-	-	-	-
East Shewa	13	10	3	-	-	-	-
North Shewa	13	7	6	-	-	-	-
West Shewa	13	9	4	-	-	-	-
Tigray	12	8	4	-	-	-	-
South Wello	13	12	1	-	-	-	-
Introduction (ICARDA and ICRISAT)	16	11	-	-	3	2	-
Total	155	106	42	2	3	2	-
With phosphorus							
Arsi	13	5	8	-	-	-	-
East Gojam	13	4	9	-	-	-	-
West Gojam	13	7	5	-	1	-	-
North Gonder	13	3	8	-	-	2	-
South Gonder	12	6	6	-	-	-	-
West Haragie	11	4	7	-	-	-	-
East Shewa	13	6	7	-	-	-	-
North Shewa	13	6	7	-	-	-	-
West Shewa	13	3	10	-	-	-	-
Tigray	12	4	8	-	-	-	-
South Wello	13	5	8	-	-	-	-
Introduction (ICARDA and ICRISAT)	16	1	5	6	-	2	2
Total	155	54	88	6	1	4	2

Principal component analysis

Principal component analysis indicated that the first vectors were more important than the second and all the other vectors both in the absence and presence of phosphorus fertilizer. The first principal components accounted for 58% of the total multi-trait standardized variations when the genotypes were grown without phosphorus and 52% with phosphorus. The corresponding values for the second principal components were 21% and 23% in that order. The first two principal components of the parameters of phosphorus use efficiency accounted for 42% and 31%, respectively. Totally, the first five principal components accounted for 97% of the total variation without phosphorus, 94% with phosphorus and 100% for parameters of phosphorus use efficiency (Tables 6).

Under no-phosphorus condition, the five top important characters responsible for genetic divergence in the major axis include biomass dry weight (+ 0.305), biomass production rate (+ 0.298), grain phosphorus content (+ 0.296), grain phosphorus yield (+0.296) and grain yield (+ 0.295). Economic growth rate, biomass phosphorus content and yield, shoot dry matter weight and grain production efficiency, with vector weights ranging from + 0.290-0.268, had almost equal contributions. The least contributors were seed size and phosphorus and grain harvest indices.

Similarly, under phosphorus fertilized condition, the five top important characters responsible for genetic divergence in the major axis include grain yield (- 0.314), grain phosphorus yield (-0.303), grain phosphorus content (-0.302), economic growth rate (-0.299) and biomass phosphorus yield (- 0.296). Biomass production rate, biomass dry weight, grain production efficiency, shoot dry matter weight and biomass phosphorus content were also important. The least contributors were again seed size and phosphorus and grain harvest indices.

Among the parameters of phosphorus use efficiency, phosphorus yield efficiency contributed the largest magnitude (+ 0.611) to the differentiation of the population into clusters followed by apparent use of phosphorus from fertilizer and soil (+ 0. 594) and apparent use of phosphorus from fertilizer (+ 0.475). Apparent use of phosphorus from soil (+ 0.132) and phosphorus physiological efficiency (+ 0.180) contributed the least amount. All parameters of phosphorus use efficiency significantly contributed to the differentiation of the population through the second principal component except phosphorus yield efficiency.

It is normally assumed that characters with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). Accordingly, many characters contributed to the total variation and, therefore, the differentiation of the genotypes into different clusters was rather dictated by the cumulative effects of a number of characters.

To examine the contribution of the traits to the total genetic divergence among the genotypes, an ordination was conducted between the first two principal components both in the absence and presence of phosphorus. The length of lines (i.e. vectors from the origin) indicates the importance of a given character to the total variation by the two principal components.

Based on their relative contribution to the first two principal components (PC_1 and PC_2) as reflected by the absolute values of vector weights, the characters may be stratified into four distinct groups. The first group included biomass phosphorus content and phosphorus yield, shoot and biomass dry matter weight, biomass production and economic growth rates, grain phosphorus content and phosphorus yield, grain production efficiency and yield. The second contributors included number of pod and seed. The third group included shoot protein content and phosphorus yield. The last group included seed size and phosphorus and grain harvest indices.

The important characters mentioned above had the same pattern of contribution both in the presence and absence of phosphorus except the change in direction. It is interesting to note that phosphorus yield efficiency had also maintained its best position in terms of contribution to the total genetic divergence by the attributes of phosphorus use efficiency showing the potential to improve this character through selection among the Ethiopian chickpea gene pool (Figure 2).

Table 6. Eigenvalue, percentage and cumulative variances and eigenvectors on the first five principal components for phosphorus-related and agronomic characters* in hundred fifty five chickpea genotypes grown in the absence and presence of phosphorus (keys to abbreviations are given in materials and methods)

	Principal components (PCs)				
	PC$_1$	PC$_2$	PC$_3$	PC$_4$	PC$_5$
Parameter	Without phosphorus				
Eigenvalue	9.88	3.51	1.69	1.00	0.39
Proportion (%)	58.10	20.60	9.90	5.80	2.30
Cumulative (%)	58.10	78.70	88.60	94.40	96.70
Characters	---------------- Eigenvectors ------------------				
GPC	0.296	-0.081	-0.118	0.163	0.388
SPC	0.164	0.433	0.036	0.249	-0.131
BMPC	0.288	0.188	-0.001	0.093	0.320
SPY	0.164	0.433	0.036	0.249	-0.130
GPY	0.296	-0.080	-0.113	0.170	0.387
BMPY	0.289	0.160	-0.062	0.244	0.207
PHI	0.041	-0.495	-0.146	-0.105	0.311
NP	0.228	-0.134	0.406	-0.015	-0.169
NS	0.206	-0.203	0.457	-0.024	-0.024
SDMW	0.286	0.121	0.050	-0.328	-0.120
BMWT	0.305	0.026	-0.015	-0.240	-0.161
HI	0.043	-0.339	-0.264	0.610	-0.452
GPE	0.268	-0.227	-0.118	0.050	-0.123
BPR	0.298	0.021	-0.016	-0.322	-0.100
EGR	0.290	-0.089	-0.153	-0.123	-0.276
TSW	-0.022	0.180	-0.666	-0.282	-0.053
YLD	0.295	-0.155	-0.131	-0.041	-0.213
Parameter	With phosphorus				
Eigenvalue	8.90	3.82	1.81	0.94	0.52
Proportion (%)	52.30	22.50	10.60	5.50	3.10
Cumulative (%)	52.30	74.80	85.40	91.00	94.10
Characters	---------------- Eigenvectors ------------------				
GPC	-0.302	-0.106	-0.156	0.235	-0.092
SPC	-0.141	0.440	0.032	0.213	-0.203
BMPC	-0.256	0.168	-0.057	0.329	0.601
SPY	-0.141	0.440	0.032	0.214	-0.202
GPY	-0.303	-0.101	-0.156	0.234	-0.087
BMPY	-0.296	0.166	-0.096	0.285	-0.173
PHI	-0.100	-0.464	-0.120	-0.045	-0.024
NP	-0.216	-0.071	0.465	-0.106	0.070
NS	-0.217	-0.145	0.480	0.026	-0.066
SDMW	-0.274	0.182	0.065	-0.407	-0.111
BMWT	-0.286	0.034	0.012	-0.167	0.637
HI	-0.088	-0.414	-0.161	0.328	-0.132
GPE	-0.286	-0.180	-0.019	-0.048	-0.093
BPR	-0.292	0.105	0.034	-0.397	-0.215
EGR	-0.299	-0.080	-0.130	-0.158	-0.057
TSW	-0.020	0.145	-0.649	-0.311	0.048
YLD	-0.314	-0.131	-0.078	-0.112	-0.077
Parameter	Phosphorus use efficiency				
Eigenvalue	2.08	1.56	1.29	0.09	0.01
Proportion (%)	41.50	31.30	25.70	14.00	0.10
Cumulative (%)	41.50	72.80	98.50	99.90	100
Characters	---------------- Eigenvectors ------------------				
APUfs	0.594	-0.373	0.178	0.340	0.601
APUs	0.132	-0.685	-0.419	0.201	-0.545
APUf	0.475	0.265	0.570	0.196	-0.584
PYE	0.611	0.143	-0.354	-0.694	-0.017
PPE	0.180	0.548	-0.586	0.569	0.014

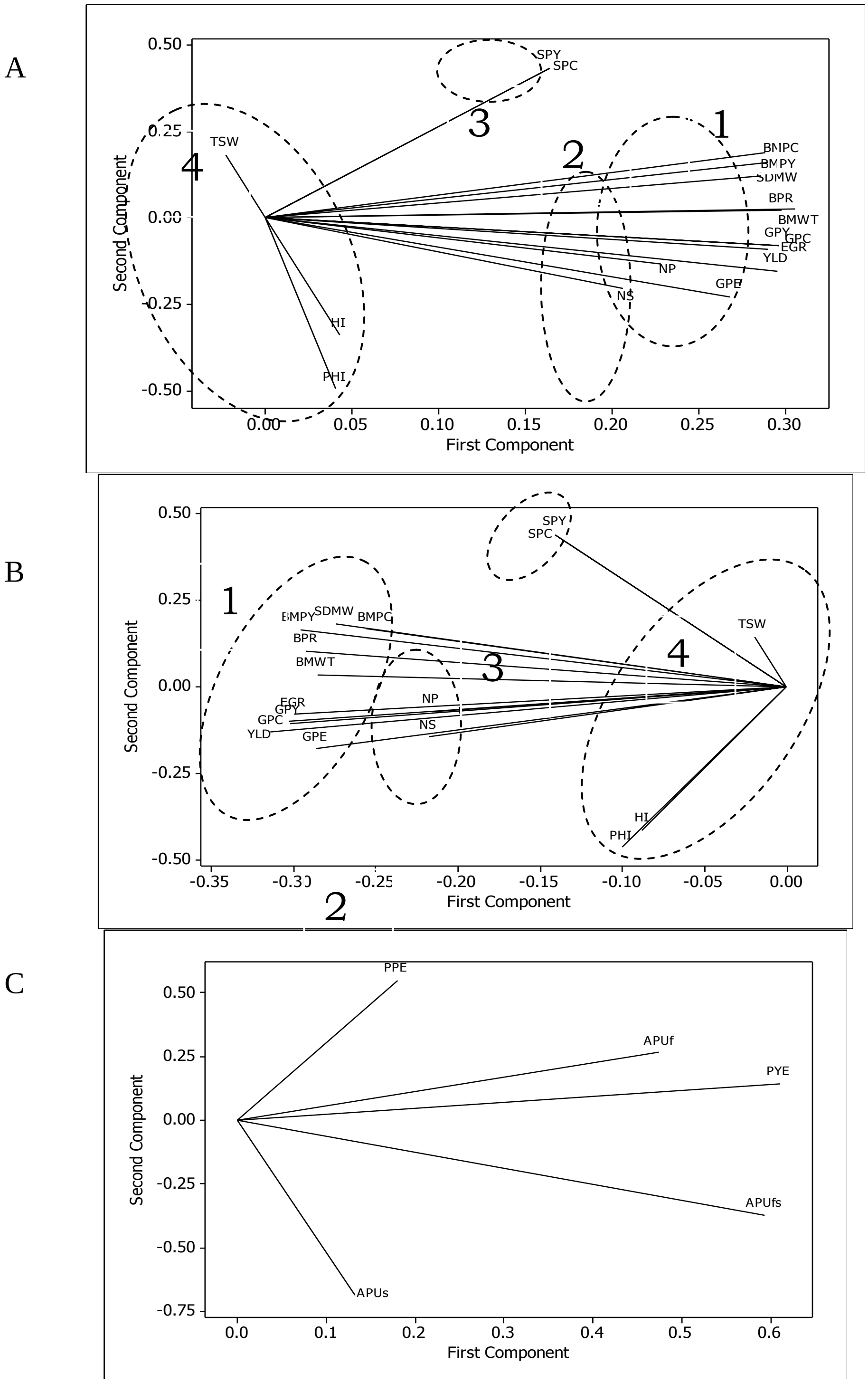

Figure 2. Loading plots of the first two principal components showing the contribution of different phosphorus-related and agronomic characters to the total variation by the two components (A) in the absence of phosphorus, (B) in the presence of phosphorus and (C) for attributes of phosphorus use efficiency in 155 chickpea genotypes. Keys to abbreviations are given in materials and methods.

Conclusions

It was demonstrated that Ethiopian chickpea germplasm accessions were more distinctly diverged from the introductions of ICARDA and ICRISAT than they diverged from each other. This may imply that chickpea had given rise to a new and distinct pattern of variation after its introduction to Ethiopia. The distinct grouping of the introduced genotypes to a separate cluster may also be somehow related to the level of prior breeding to which they had been subjected at ICRISAT and ICARDA before their introduction to Ethiopia (Keneni *et al*., 2012). The relative similarity among Ethiopian collections may also be due to the extensive seed exchange between farmers or to common features of the chickpea original introduction in different regions of Ethiopia. It may also be implicated that the easy access to a wide array of improved cultivars developed by the international institutions supported the broadening of genetic base of chickpea breeding in Ethiopia.

The present study also revealed that the Ethiopian chickpea landraces are still important sources of genotypes with desirable traits including phosphorus uptake and use efficiency. The utilization of these valuable germplasm particularly in the efforts underway to develop efficient genotypes for phosphorus uptake and use efficiency warrants a critical assessment. A series of multiple crossing may be required in order to bring desirable traits distributed among multiple parents into a single genetic background for further selection among the progenies. Introductions from exotic sources should also be included in the parents to be developed from the selected accessions particularly in order to exploit complementary genes, e.g. to improve seed size as an economic trait.

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