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Full Length Research Paper

Phenotypic diversity for symbio-agronomic characters in Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions

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Breeding chickpea (*Cicer arietinum* L.) cultivars combining desirable symbiotic and agronomic characters has both economic and ecological significance. An experiment was conducted at Ambo and Ginchi, Ethiopia, in 2009/10 to characterize and evaluate 155 genotypes of chickpea for symbiotic and agronomic performance. A randomized complete block design with four replications and the difference technique, with a genetically non-nodulating chickpea genotype as a reference crop were employed to estimate the amount of symbiotic nitrogen fixation. Data analysis of 32 agronomic and symbiotic characters showed significant differences among the genotypes for all traits under study. Trait-based cluster analysis grouped the genotypes into six different classes. Standardized Mahalanobis D^2 statistics showed significant genetic distances between all clusters constituted local landraces and introduced genotypes. This indicated that there were distinct multivariate differences between landraces and introduced genotypes. No clear interrelationship was observed between the geographic origins of the landraces and the pattern of genetic diversity, as there were accessions from the same source of origin that fell into different clusters and vice versa. Different symbiotic and agronomic characters had different contribution to the total differences among the populations. Those characters that contributed more to the total differentiation of the populations and genotypes into the different clusters should be exploited in future breeding.

Key words: Chickpea (*Cicer arietinum*), cluster analysis, genetic diversity, germplasm, symbiotic nitrogen fixation.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important food legumes grown in Ethiopia. The country is considered as one of the centers of secondary diversity for chickpea (Van der Maesen, 1987) with some wild species like *C. cuneatum*, known to grow in numerous regions (Taddesse et al., 1994; Bejiga and Daba, 2006). The production of chickpea is important for human and animal nutrition and for ecological sustainability due to the associated benefits from symbiotic nitrogen fixation.

The genus *Rhizobium* was first assumed as the only fixer of atmospheric nitrogen (Lim and Burton, 1982) until further investigations revealed additional genera of soil bacteria, namely *Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium* as fixers of atmospheric nitrogen symbiotically with legumes (Rincón et al., 2008). Chickpea fixes a substantial amount of atmospheric nitrogen (Kassie et al., 2009) in

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symbiotic association with two species of *rhizobacterium*, namely *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* (Rivas et al., 2006; Willems, 2006).

Efforts made in the past to enhance nitrogen fixation through selection of effective and competitive strains of *Rhizobium* have resulted in the identification of many promising strains in Ethiopia (Hailemariam and Tsigie, 2006). Some strains, including the chickpea isolates, are ready for production and commercial use (Bejiga, 2004). Despite thorough efforts of screening for effective microsymbionts for artificial inoculation (Shantharam and Mattoo, 1997; Hardarson, 2004), the development of efficient host plants attracted little attention in Ethiopia (Bejiga, 2004) and elsewhere (Hardarson, 2004; Winter et al., 2004). The success of symbiotic nitrogen fixation also depends on the genetic potential of the host and strain, and on how they interact with each other and with other components of the growing environment (Abaidoo et al., 1990; Bohlool et al., 1992; Pearson et al., 1995).

Knowledge of the nature of genetic diversity and of the amount of genetic variability in a given gene pool and the subsequent identification of promising accessions is the basis for the development of modern and more productive crop varieties in order to ensure food security. To this effect, attempts to classify, characterize and evaluate Ethiopian chickpea germplasm accessions with regard to their symbiotic characteristics processed with multivariate analysis is very important, so as to facilitate the effective use of chickpea germplasm as inputs to plant breeding (Hayward and Breese, 1993; Singh, 2002; De Vicente et al., 2005).

There are over 1150 chickpea germplasm accessions, collected and preserved by the Ethiopian Institute of Biodiversity Conservation (Tanto and Tefra, 2006). Therefore, in order to best utilize these germplasm accessions for variety development, the first step is to exhaustively characterize and evaluate them for specific traits of breeders' interest. A few accessions have been characterized for attributes of morphological and agronomic significance (Tanto and Tefra, 2006) and have been utilized in breeding programs (Bejiga and Daba, 2006). Previous studies showed existence of high genetic diversity at a morphological, and limited diversity at a molecular level within a subset of Ethiopian collections (Workeye, 2002; Dadi, 2004). Recently, we reported the existence of magnificent level of molecular diversity revealed by SSR markers (Keneni et al., 2011). Nevertheless, much remains, for the subject of investigation aimed at genetic diversity and the breeding potential of Ethiopian chickpea germplasm accessions. While most of the hitherto breeding efforts in chickpea focused on improved seed yield and some related morphological characters (Bejiga and Daba, 2006; Bejiga and Van der Maesen, 2006), efforts made to improve the symbiotic nitrogen fixation, along with physio-agronomic performance have been very limited.

The present work was designed to characterize and evaluate genetic diversity among the Ethiopian chickpea germplasm accessions for multiple traits of symbiotic and agronomic significance, in order to facilitate their use in chickpea breeding programs.

MATERIALS AND METHODS

Plant materials

In this study, a total of 155 chickpea genotypes were evaluated. They included 139 accessions from different regions of Ethiopia kindly provided by the Ethiopian Institute of Biodiversity Conservation (IBC), five improved genotypes from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), eight originally introduced commercial cultivars released in Ethiopia and three genetically non-nodulating reference lines, received from ICRISAT and the International Center for Agricultural Research in the Dry Areas (ICARDA). The passport description of the genotypes and the map of the areas of collection for the Ethiopian accessions are presented in Table 1 and Figure 1. All genotypes were rejuvenated during 2008/9 (September to January) under the same conditions in Ginchi to reasonably minimize initial variation, due to the differences in seed age and indigenous seed nitrogen content (Liao et al., 2008).

The test environment

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. Climatic data of the two locations during the growing period were taken from Ambo and Holetta Research Centers as presented in Figure 2a and b. Soil samples from both locations were collected from the rhizosphere (top 20 cm) for physico-chemical characterization (Table 2).

Rhizobium inoculants and inoculation

An effective strain of *Rhizobium* for chickpea, CP EAL 004, originally isolated by the National Soil Test Centre from a collection of Ada'a District of East Shewa Zone, Ethiopia, was used for the study. 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 Research Center immediately before planting. Seeds of all genotypes were coated with the inoculants, at the rate of approximately 2 g of inoculum for 80 seeds, using 40% gum Arabic as an adhesive.

Experimental design and layout of field trials

A randomized complete block design with four replications was used. A blanket basal application of phosphorus was made to all plots in the form of triple supper phosphate (TSP) at the recommended rate (20 gm for a single row of 4 m). Sowing rate was 5 cm between plants and 40 cm between adjacent rows. Experimental plot was represented by one row 4 m long. The genotypes were assigned to plots at random within each block. Nitrogenous fertilizer

Table 1. Passport description of the test genotypes.

S/N	Accession/genotype	Region	Zone	District	Altitude (masl)
1	Acc. No. 231327	Oromiya	Arsi	Merti	1540
2	Acc. No. 231328	Oromiya	Arsi	Jeju	1600
3	Acc. No. 209093	Oromiya	Arsi	Dodota Sire	1710
4	Acc. No. 208829	Oromiya	Arsi	Dodota Sire	1740
5	Acc. No. 209094	Oromiya	Arsi	Dodota Sire	1750
6	Acc. No. 209092	Oromiya	Arsi	Dodota Sire	1770
7	Acc. No. 209096	Oromiya	Arsi	Dodota Sire	1850
8	Acc. No. 209097	Oromiya	Arsi	Dodota Sire	1860
9	Acc. No. 209098	Oromiya	Arsi	Dodota Sire	1860
10	Acc. No. 41002	Oromiya	Arsi	Tena	2080
11	Acc. No. 207761	Oromiya	Arsi	Tena	2080
12	Acc. No. 207763	Oromiya	Arsi	Tena	2080
13	Acc. No. 207764	Oromiya	Arsi	Tena	2080
14	Acc. No. 41268	Amahara	E. Gojam	H. Ej Enese	1770
15	Acc. No. 41026	Amahara	E. Gojam	Hulet Ej Enese	2280
16	Acc. No. 41074	Amahara	E. Gojam	Hulet Ej Enese	2450
17	Acc. No. 41075	Amahara	E. Gojam	Hulet Ej Enese	2410
18	Acc. No. 41073	Amahara	E. Gojam	Hulet Ejenese	2400
19	Acc. No. 41076	Amahara	E. Gojam	Hulet Ej Enese	2470
20	Acc. No. 41021	Amahara	E. Gojam	Enarj Enawga	2510
21	Acc. No. 41027	Amahara	E. Gojam	Shebel Berenta	2450
22	Acc. No. 41222	Amahara	E. Gojam	Dejen	2460
23	Acc. No. 207734	Amahara	E. Gojam	Goncha Siso Enese	2560
24	Acc. No. 41103	Amahara	E. Gojam	Enemay	2570
25	Acc. No. 41320	Amahara	E. Gojam	Debay Telatgen	2400
26	Acc. No. 41029	Amahara	E. Gojam	Enarj Enawga	2880
27	Acc. No. 41015	Amahara	W. Gojam	Jabi Tehnan	2020
28	Acc. No. 41271	Amahara	W. Gojam	Adet	1880
29	Acc. No. 41272	Amahara	W. Gojam	Adet	1960
30	Acc. No. 41276	Amahara	W. Gojam	Adet	2230
31	Acc. No. 207745	Amahara	W. Gojam	Adet	2230
32	Acc. No. 41275	Amahara	W. Gojam	Adet	2240
33	Acc. No. 41277	Amahara	W. Gojam	Adet	2240
34	Acc. No. 207743	Amahara	W. Gojam	Adet	2240
35	Acc. No. 207744	Amahara	W. Gojam	Adet	2240
36	Acc. No. 41273	Amahara	W. Gojam	Adet	2300
37	Acc. No. 41274	Amahara	W. Gojam	Adet	2360
38	Acc. No. 207741	Amahara	W. Gojam	Adet	2360
39	Acc. No. 207742	Amahara	W. Gojam	Adet	2360
40	Acc. No. 41316	Amahara	N. Gonder	Gonder Zuria	1900
41	Acc. No. 41298	Amahara	N. Gonder	Gonder Zuria	1920
42	Acc. No. 41311	Amahara	N. Gonder	Dembia	1925
43	Acc. No. 41313	Amahara	N. Gonder	Gonder Zuria	1925
44	Acc. No. 41280	Amahara	N. Gonder	Gonder Zuria	1940
45	Acc. No. 41312	Amahara	N. Gonder	Gonder Zuria	1950
46	Acc. No. 41315	Amahara	N. Gonder	Gonder Zuria	1900
47	Acc. No. 41308	Amahara	N. Gonder	Dembia	2010
48	Acc. No. 41299	Amahara	N. Gonder	Gonder Zuria	1920
49	Acc. No. 41046	Amahara	N. Gonder	Chilga	2160
50	Acc. No. 41047	Amahara	N. Gonder	Chilga	2160
51	Acc. No. 41304	Amahara	N. Gonder	Dabat	2610

Table 1. Contd

52	Acc. No. 41303	Amahara	N. Gonder	Wegera	2710
53	Acc. No. 41295	Amahara	S. Gonder	Fogera	1820
54	Acc. No. 41296	Amahara	S. Gonder	Kemekem	1850
55	Acc. No. 41289	Amahara	S. Gonder	Kemekem	1855
56	Acc. No. 41290	Amahara	S. Gonder	Kemekem	1880
57	Acc. No. 41284	Amahara	S. Gonder	Dera	1900
58	Acc. No. 41291	Amahara	S. Gonder	Kemekem	1900
59	Acc. No. 41297	Amahara	S. Gonder	Kemekem	1950
60	Acc. No. 41293	Amahara	S. Gonder	Kemekem	2040
61	Acc. No. 41019	Amahara	S. Gonder	Este	2500
62	Acc. No. 41048	Amahara	S. Gonder	Farta	2640
63	Acc. No. 41049	Amahara	S. Gonder	Farta	2640
64	Acc. No. 41053	Amahara	S. Gonder	Lay Gayint	3120
65	Acc. No. 41054	Oromiya	W. Harargie	Chiro	1500
66	Acc. No. 41052	Oromiya	W. Harargie	Mieso	1510
67	Acc. No. 209082	Oromiya	W. Harargie	Kuni	1680
68	Acc. No. 209083	Oromiya	W. Harargie	Kuni	1700
69	Acc. No. 209084	Oromiya	W. Harargie	Kuni	1700
70	Acc. No. 209091	Oromiya	W. Harargie	Habro	1730
71	Acc. No. 209087	Oromiya	W. Harargie	Kuni	1740
72	Acc. No. 209088	Oromiya	W. Harargie	Habro	1740
73	Acc. No. 209089	Oromiya	W. Harargie	Habro	1740
74	Acc. No. 209090	Oromiya	W. Harargie	Habro	1740
75	Acc. No. 209081	Oromiya	W. Harargie	Girawa	2130
76	Acc. No. 41159	Oromiya	E. Shewa	Ada'a Chukala	1910
77	Acc. No. 41160	Oromiya	E. Shewa	Ada'a Chukala	1910
78	Acc. No. 41161	Oromiya	E. Shewa	Ada'a Chukala	1940
79	Acc. No. 207661	Oromiya	E. Shewa	Ada'a Chukala	1850
80	Acc. No. 207667	Oromiya	E. Shewa	Akaki	2180
81	Acc. No. 207666	Oromiya	E. Shewa	Akaki	2060
82	Acc. No. 41141	Oromiya	E. Shewa	Lome	2040
83	Acc. No. 207665	Oromiya	E. Shewa	Akaki	2060
84	Acc. No. 41134	Oromiya	E. Shewa	Akaki	2080
85	Acc. No. 41128	Oromiya	E. Shewa	Akaki	2130
86	Acc. No. 41168	Oromiya	E. Shewa	Ada'a Chukala	2150
87	Acc. No. 41129	Oromiya	E. Shewa	Akaki	2170
88	Acc. No. 41130	Oromiya	E. Shewa	Akaki	2190
89	Acc. No. 41110	Amara	N. Shewa	Kewet	1220
90	Acc. No. 207657	Amara	N. Shewa	Efratana Gidim	1400
91	Acc. No. 41111	Amahara	N. Shewa	Efratana Gidim	1400
92	Acc. No. 41106	Amahara	N. Shewa	Mafudmezezo Mojana	1820
93	Acc. No. 207658	Amahara	N. Shewa	Efratana Gidim	1400
94	Acc. No. 41142	Amahara	N. Shewa	Minjarna Shenkora	2290
95	Acc. No. 41207	Amahara	N. Shewa	Siyadebrina Wayuense	2580
96	Acc. No. 41215	Amahara	N. Shewa	Moretena Jiru	2640
97	Acc. No. 41216	Amahara	N. Shewa	Moretena Jiru	2640
98	Acc. No. 41066	Oromiya	N. Shewa	Wara Jarso	2550
99	Acc. No. 41011	Oromiya	N. Shewa	Gerar Jarso	2558
100	Acc. No. 41007	Oromiya	N. Shewa	Yaya Gulale	2670
101	Acc. No. 41008	Oromiya	N. Shewa	Yaya Gulale	2700

Table 1. Contd

102	Acc. No. 41186	Oromiya	W. Shewa	Waliso Goro	1960
103	Acc. No. 209035	Oromiya	W. Shewa	Alem Gena	2010
104	Acc. No. 41176	Oromiya	W. Shewa	Ambo	2020
105	Acc. No. 41175	Oromiya	W. Shewa	Ambo	1970
106	Acc. No. 41174	Oromiya	W. Shewa	Ambo	2120
107	Acc. No. 209027	Oromiya	W. Shewa	Kersa Kondaltiti	2060
108	Acc. No. 41170	Oromiya	W. Shewa	Dendi	2160
109	Acc. No. 41171	Oromiya	W. Shewa	Dendi	2230
110	Acc. No. 41185	Oromiya	W. Shewa	Woliso Goro	2000
111	Acc. No. 209036	Oromiya	W. Shewa	Alem Gena	2220
112	Acc. No. 41190	Oromiya	W. Shewa	Woliso Goro	2080
113	Acc. No. 41195	Oromiya	W. Shewa	Becho	2160
114	Acc. No. 41197	Oromiya	W. Shewa	Becho	2120
115	Acc. No. 207150	Tigray	S. Tigray	Enderta	-
116	Acc. No. 207151	Tigray	S. Tigray	Enderta	-
117	Acc. No. 207563	Tigray	S. Tigray	Hintalo Wajirat	1960
118	Acc. No. 207564	Tigray	C. Tigray	Laelay Maychew	2150
119	Acc. No. 207894	Tigray	S. Tigray	Endamehoni	2600
120	Acc. No. 207895	Tigray	S. Tigray	Alaje	-
121	Acc. No. 213224	Tigray	E. Tigray	Wukro	2100
122	Acc. No. 219797	Tigray	C. Tigray	Laelay Maychew	2150
123	Acc. No. 219799	Tigray	C. Tigray	Laelay Maychew	1970
124	Acc. No. 219800	Tigray	C. Tigray	Adwa	2400
125	Acc. No. 219803	Tigray	W. Tigray	Tahtay Koraro	1880
126	Acc. No. 221696	Tigray	S. Tigray	Enderta	-
127	Acc. No. 41114	Amahara	S. Wello	Werebabu	1560
128	Acc. No. 212589	Amahara	S. Wello	Kalu	1600
129	Acc. No. 41113	Amahara	S. Wello	Kalu	1650
130	Acc. No. 207659	Amahara	S. Wello	Dessie Zuria	1950
131	Acc. No. 207660	Amahara	S. Wello	Dessie Zuria	1950
132	Acc. No. 41115	Amahara	S. Wello	Kutaber	2290
133	Acc. No. 225878	Amahara	S. Wello	Debresina	2420
134	Acc. No. 225873	Amahara	S. Wello	Debresina	2445
135	Acc. No. 225874	Amahara	S. Wello	Debresina	2450
136	Acc. No. 225877	Amahara	S. Wello	Kelala	2450
137	Acc. No. 207645	Amahara	S. Wello	Debresina	2510
138	Acc. No. 207646	Amahara	S. Wello	Debresina	2510
139	Acc. No. 225876	Amahara	S. Wello	Kelala	2540
140	ICC 5003*	India	-	-	-
141	ICC 4918	India	-	-	-
142	ICC 4948	India	-	-	-
143	ICC 4973	India	-	-	-
144	ICC 15996	ICRISAT	-	-	-
145	Shasho (ICCV 93512)	ICRISAT	-	-	-
146	Arerti (FLIP 89-84C)	ICARDA	-	-	-
147	Worku (DZ-10-16-2)	ICRISAT	-	-	-
148	Akaki (DZ-10-9-2)	ICRISAT	-	-	-
149	Ejere (FLIP-97-263C)	ICARDA	-	-	-
150	Teji (FLI 97-266C)	ICARDA	-	-	-
151	Habru (FLIP 88-42c)	ICARDA	-	-	-
152	Natoli (ICCX-910112-6)	ICRISAT	-	-	-
153	ICC 19180	ICRISAT	-	-	-
154	ICC 19181	ICRISAT	-	-	-
155	PM 233 (155)	ICARDA	-	-	-

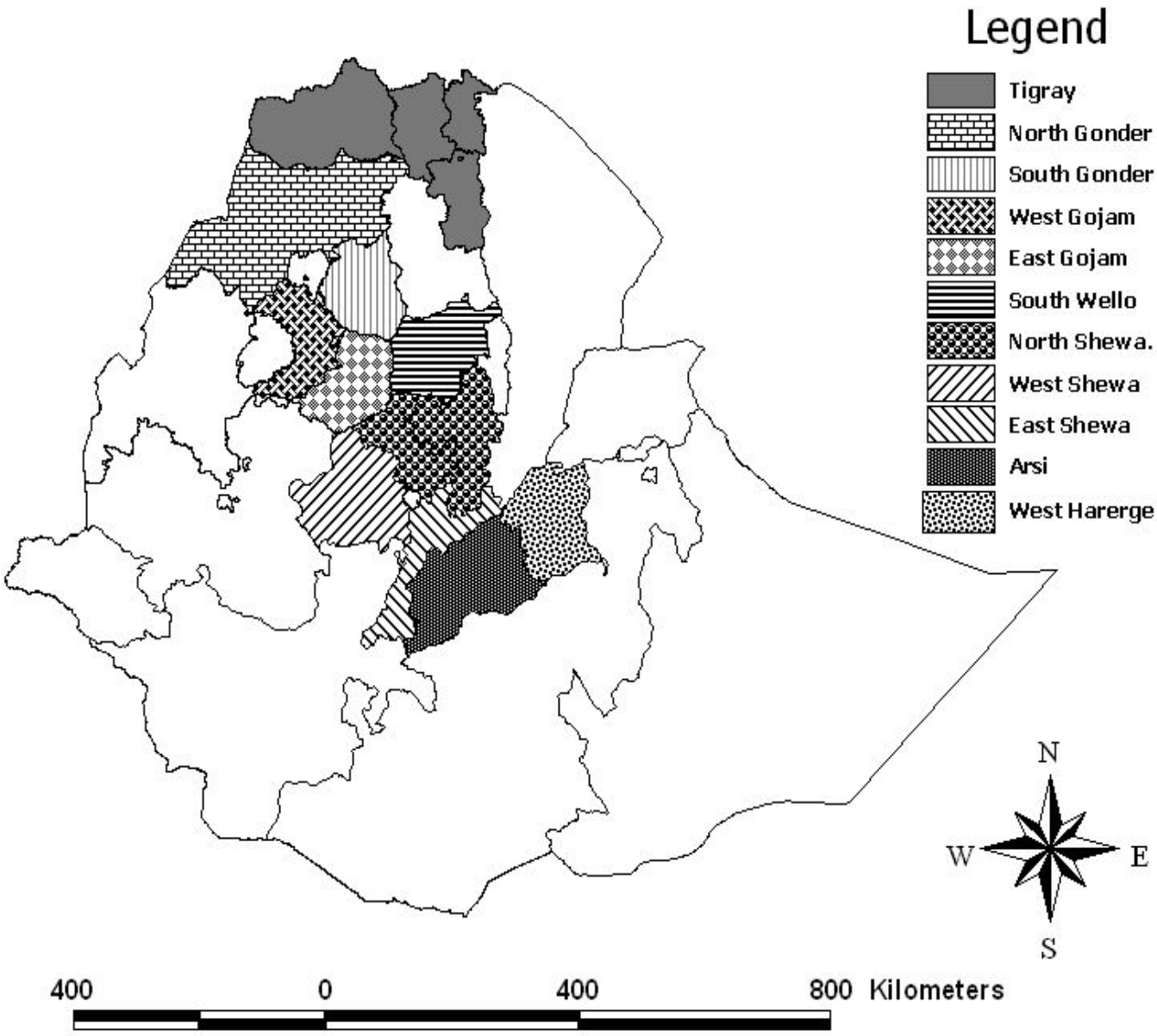

Figure 1. Map of Ethiopia showing the approximate areas of origins (shaded region) of the 139 germplasm accessions (NB: all boundaries are approximate and have nothing to do with political borders).

was totally omitted and all other crop management and protection practices were applied uniformly to all genotypes as required.

Shoot and grain nitrogen analysis

After 45 days of growth (shortly before flowering), five plants from each plot were carefully dug up and their roots washed free from soils with water running over a sieve for collection of released nodules. Representative shoot samples were collected shortly before flowering and again at 90% physiological maturity. The grain samples from each plot were oven-dried to constant moisture at 70°C for 18 h. The dry samples were ground to pass through 1 mm mesh sieve to determine nitrogen, using the Kjeldahl technique (AOAC, 1970) at Holetta and Debre Zeit Soil Science Research Laboratories. The amount of total nitrogen accumulated from fixation in shoots and grains of the test genotypes was estimated by the difference method, using a non-nodulating reference genotype PM 233. Protein contents of shoot and grain were determined by multiplying nitrogen percentages by the standard conversion factor of 6.25 (AOAC, 1970). Based on the nitrogen contents, the following parameters were calculated:

$$\text{N fixed in shoot} = \frac{(\text{Nsfg} - \text{Nsnfg}) \times 100}{\text{Nsfg}}$$

Where, Nsfg = amount of nitrogen in shoot of fixing genotype and Nsnfg = amount of nitrogen in shoot of non-fixing genotype.

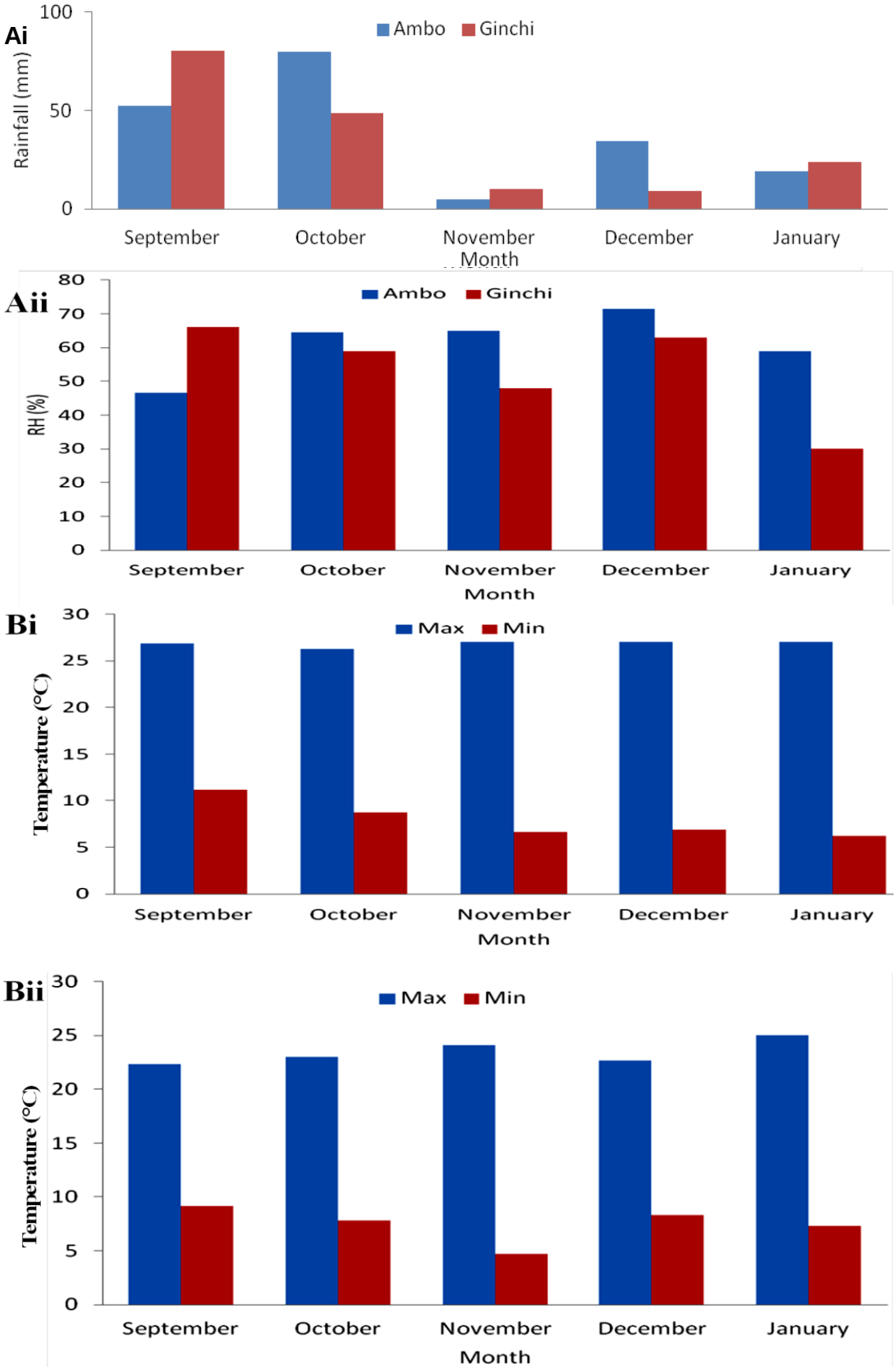

Figure 2. (A) Rainfall (mm) and relative humidity (%) at (i) Ambo and (ii) Ginchi during the growing season; (B) maximum and minimum temperatures (°C) at (i) Ambo and (ii) Ginchi during the growing season.

Table 2. Description of the test locations for geographical position and physico-chemical properties of the soils.

Parameter	Source of soil	
	Ambo	Ginchi
Latitude	09° 00′ N	09° 00′ N
Longitude	37° 22′ E	38° 10′ E
Altitude (masl)	2225	2200
Mean annual rainfall (mm)	1000	1110
% Clay	70.00	65.83
% Silt	15.00	20.42
% Sand	15.00	13.75
% Organic C	1.53 (low)	1.30 (low)
N (%)	0.103 (low)	0.103 (low)
C/N ratio	14.85 (high)	12.62 (high)
P (ppm*)	18.07 (high)	4.49 (low)
K (meq/100 gm soil)	2.438 (high)	2.485 (high)
Ca (meq/100 mg soil)	59.03 (high)	39.62 (high)
Mg (meq/100 mg soil)	11.20 (high)	9.00 (high)
Na (meq/100 mg soil)	0.70 (high)	0.61 (high)
SO_4S (ppm)	5.23 (optimum)	5.62 (optimum)
Fe (ppm)	27.73 (high)	51.50 (high)
pH (1:1 H_2O)	7.23 (optimum)	6.18 (optimum)
EC (µS)**	650.00 (high)	547.33 (high)

*ppm = parts per million; **µS = micro Siemens.

$$\text{N fixed in grain} = \frac{(\text{Ngfg} - \text{Ngnfg}) \times 100}{\text{Ngfg}}$$

Where Ngfg = amount of nitrogen in grain of fixing genotype and Ngnfg = amount of nitrogen in grain of non-fixing genotype.

$$\text{N fixed in biomass} = \frac{(\text{N fixed in shoot} + \text{N fixed in grain}) \times 100}{\text{Nsfg} + \text{Ngfg}}$$

$$\text{N assimilation efficiency} = \frac{(\text{N fixed in grain}) \times 100}{\text{Nsfg} + \text{Ngfg}}$$

Grain N yield = Grain N content × grain yield
Shoot N yield = Shoot N content × shoot yield
Biomass N yield = Grain N yield + shoot N yield

Nitrogen harvest index (NHI) was estimated as:

$$\text{NHI} = \frac{\text{Grain N yield}}{\text{Biomass N yield}}$$

Data collection on symbiotic and agronomic characters

Data were collected either on plot basis or from randomly selected five plants, following the recommendations of international bodies (IBPGR, ICRISAT and ICARDA, 1993).

Records were taken on symbiotic characters as follows: (1) number of nodules (5 plants^{-1}); (2) nodule dry weight (mg 5 plants^{-1}); (3) nodulation index (nodule dry weight to shoot dry weight ratio); (4) shoot nitrogen and protein contents (%); (5) shoot nitrogen fixation; (6) grain nitrogen and protein contents; (7) grain nitrogen fixation (%); (8) above ground biomass nitrogen content (%); (9) above ground biomass nitrogen fixation (%); (10) fixed nitrogen assimilation efficiency (%); (11) shoot, grain and above ground biomass nitrogen yields (g 5 plants^{-1}) and; (12) nitrogen harvest index (NHI; the ratio of the amount of the element in the grain, relative to the amount of the element in the total above-ground biomass).

Agronomic characters recorded included: (1) early vigor as shoot dry weight (g 5 plants^{-1}) before flowering; (2) shoot dry weight at maturity (g 5 plants^{-1}); (3) shoot dry weight ratios of nodulating to non-nodulating genotypes at maturity; (4) days to 50% flowering and 90% maturity; (5) grain filling period (the number of days from 50% flowering to 90% physiological maturity); (6) numbers of pods and seeds; (7) total above ground biomass weight (g 5 plants^{-1}); (8) harvest index (proportion of total above ground biomass that is grain); (9) grain production efficiency (grain filling duration divided by duration of vegetative period and then multiplied by grain yield); (10) above ground biomass production rate (above ground biomass weight divided by days to 90% physiological maturity and then multiplied by 100); (11) economic growth rate (grain weight divided by grain fill duration and then multiplied by 100); (12) thousand seed weight (g) and; (13) grain yield (g 5 plants^{-1}).

Statistical analysis

Data based on nodules (number, weight and nodulation index) were log transformed to offset heterogeneity. Pooled analysis of variance was conducted to quantify the total variation among the genotypes.

Means of 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 Statistical Analysis System

(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 determined, using standardized Mahalanobis 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 (X^2) 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

The crop season and test locations

The two locations received more or less similar amount of rainfall with different pattern of distribution but Ambo was more humid than Ginchi (Figure 2a and b). It was witnessed that more or less, the weather variables recorded did not deviate much from the long-term trends at both locations (data not shown), indicating that the present findings will be reproducible in other seasons. The physicochemical properties of the soils from the two test locations, Ambo and Ginchi, showed equal level of low nitrogen contents (0.103%) but high levels of K, Ca, Mg, Na and Fe (Jones, 2003) with variable amounts. The levels of exchangeable cations were also high, with pH values more or less closer to neutral. The level of soil phosphorus was high at Ambo and low at Ginchi (Table 2). Similar results were reported from previous analysis of soils from the same locations (Dibabe et al., 2001).

Analysis of variance

Differences among genotypes, locations, and genotype by location interaction effects were significant for a number of characters. A number of accessions also better performed over the improved genotypes, for both symbiotic and agronomic characters.

The amount of fixed nitrogen ranged from 13 to 49% in foliage, 30 to 44% in grain and 28 to 40% in total above ground biomass. Grain yield performance varied from 31 to 70 g five plants^{-1} and seed size varied from 82 to 288 g/1000 seeds. The best 5% of the accessions for total (shoot + grain) nitrogen fixation included; accession numbers: 41222, 41029, 41021, 41074, 41075, 41129, 41320 and 41026, and the best assimilators of fixed nitrogen were accession numbers: 41115, 207659, 219799, 207150, 41277, 41113 and 207894. Likewise, the best 5% of the accessions for best yield included accession numbers: 41274, 207763, 41111, 207742, 231328, 207563, 41053 and 212589 (data not shown).

Cluster and diversity analysis

Cluster analysis of the 155 genotypes distinguished six different groups of genotypes (Table 3). Hierarchical classification presented in a dendrogram also identified two main branches of six clusters (Figure 3). Members within a single cluster are considered similar (zero distance), while those in clusters with non-significant distance are assumed to have more close relationships with each other, than they are with those in significantly distant clusters. The first cluster (C_1, n = 51) had the largest number of accessions from all over the regions in Ethiopia. The fourth cluster (C_4, n = 35) was the second largest in number of accessions which constituted with accessions from all over the regions in Ethiopia with the exception of Arsi. The second cluster (C_2, n = 29) was the third in terms of number of accessions with most of the accessions collected from Wello and Tigray and an introduction from India through ICRISAT. The third cluster (C_3, n = 25) was the fourth in terms of number of accessions from all over the regions with the exception of East Gojam and Tigray. The fifth cluster (C_5, n = 13) comprised only introduced genotypes that are nodulating. The sixth cluster (C_6, n = 2) constituted only the two non-nodulating genotypes (Table 3 and Figure 3). The sharp distinctness of the non-nodulating genotypes from all the nodulating genotypes (both landraces and introductions), could be due to the small sample size of the former or to the fact that the non-nodulating behaviour is associated with agronomic inferiority as reflected in this study.

There was high genetic diversity for both symbiotic and agronomic characters in the populations studied. The genetic distances as measured by the pairwise generalized D^2 statistics between each cluster as shown in Table 4. The standardized Mahalanobis D^2 statistics showed existence of high genetic distances among clusters. The first exceptionally divergent D^2 values were obtained between clusters, ranging from C_1 to C_5 on the one hand and cluster C_6 on the other hand with D^2 value ranging from 25465 to 25744. The uniquely high distance values in this case may stem from the presence of highly contrasting, non-nodulating references, together with nodulating test genotypes, which resulted in D^2 values disproportionately high among the clusters. The maximum genetic distance was found between clusters C_4 and C_6 with D^2 = 25744. The second most divergent clusters were C_1 and C_6 with D^2 = 25718 and the third were C_3 and C_6 with D^2 = 25649. The fourth and fifth

Table 3. Clustering of 155 chickpea genotypes into six clusters using mean of 32 agronomic and symbiotic characters.

Cluster	Number of genotype	Genotype
C_1	51	Acc. Nos. 231327, 209093, 209096, 207761, 207763, 207764, 41268, 41076, 41027, 207734, 41015, 41272, 41276, 207745, 41275, 207743, 41274, 207742, 41316, 41298, 41313, 41312, 41047, 41295, 41284, 41293, 41019, 41049, 41053, 209084, 209091, 209087, 41160, 41161, 207661, 41141, 207665, 41128, 41110, 41111, 41106, 207658, 41215, 41066, 41176, 41185, 207563, 219803, 225877, 207645, 207646
C_2	29	231328, 208829, 209094, 209092, 207741, 41052, 209082, 207666, 207657, 41186, 209036, 41190, 207150, 207151, 207564, 207894, 207895, 219797, 219799, 219800, 221696, 41114, 212589, 41113, 207659, 41115, 225873, 225876, ICC 4918
C_3	25	209097, 209098, 41002, 41159, 41271, 41277, 207744, 41315, 41299, 41303, 41296, 41297, 209088, 209089, 209081, 41130, 41142, 41007, 41175, 41174, 209027, 41195, 41197, 207660, 225874
C_4	35	41026, 41074, 41075, 41073, 41021, 41222, 41103, 41320, 41029, 41273, 41311, 41280, 41308, 41046, 41304, 41289, 41290, 41291, 41048, 41054, 209083, 209090, 207667, 41134, 41168, 41129, 41207, 41216, 41011, 41008, 209035, 41170, 41171, 213224, 225878
C_5	13	ICC 5003, ICC 4948, ICC 4973, ICC 15996, Shasho (ICCV 93512), Arerti (FLIP 89-84C), Worku (DZ-10-16-2), Akaki (DZ-10-9-2), Ejere (FLIP-97-263C), Teji (FLI 97-266C), Habru (FLIP 88-42c), Natoli (ICCX-910112-6), ICC 19180
C_6	2	ICC 19181, PM 233

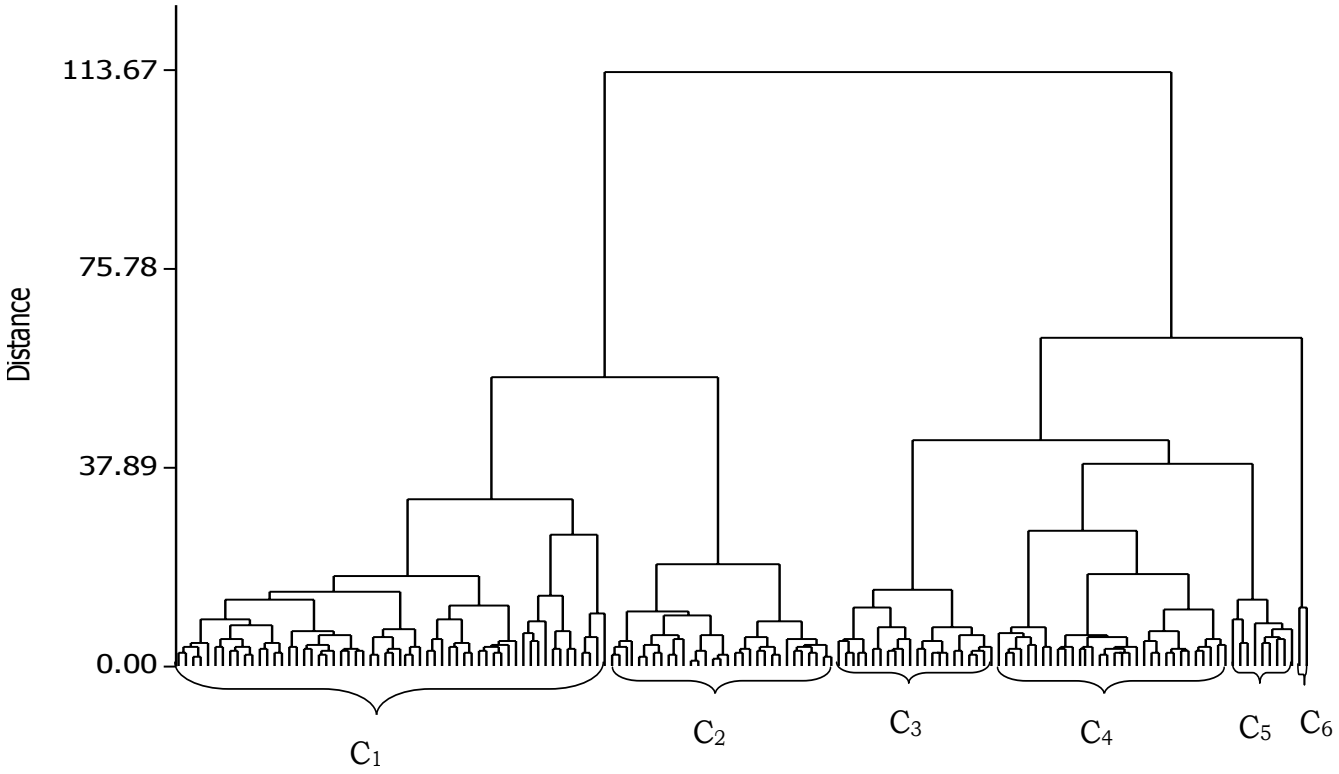

Figure 3. Dendrogram of 155 chickpea genotypes developed by Ward's agglomerative hierarchical classification method based on Euclidian distance, using mean of 32 agronomic and symbiotic characters.

Table 4. Pair-wise generalized squared distances between six clusters constituting 155 chickpea genotypes.

Cluster	C_1	C_2	C_3	C_4	C_5	C_6
C_1	0	12	24	11	84**	25718**
C_2		0	18	20	78**	25612**
C_3			0	15	72**	25649**
C_4				0	81**	25744**
C_5					0	25465**
C_6						0

**$P \leq 0.01$.

most divergent clusters were C_2 and C_6 with $D^2 = 25612$ and C_5 and C_6 with $D^2 = 25465$, respectively. The most divergent classes separated all genotypes (C_1 to C_5) on one side from the non-nodulating references (C_6) on the other side (Table 4). However, it was witnessed that introductions from foreign sources were relatively more closely related to the non-nodulating references ($D^2 = 25465$) than the local landraces ($D^2 = 25612$-25744), disregarding the contribution of a single Indian introduction which was exceptionally grouped with the landraces in cluster C_2 (Table 3).

In the second category, clusters C_1 to C_4 which comprised only local accessions with the exception of a single genotype (ICC 4918 in cluster C_2) showed more divergence with cluster C_5 which constituted all improved genotypes introduced from ICRISAT and ICARDA ($D^2 = 72$-84). Clusters formed by the local accessions, that is, C_1 to C_4, were more closely related with each other. The distances between the clusters, consisting only of landraces, may be in part underestimated because of the existence of extremely unique non-nodulating genotypes, which were inferior for almost all symbiotic and agronomic traits. It also appears that the landraces may lack multivariate diversity while they possess high diversity for specific traits.

It is interesting to note from the hierarchical classification of the genotypes, using a dendrogram, that no matter how many clusters above two are formed, the non-nodulating checks always go together to the individual level (Figure 3). It is difficult to guess what could happen, if the representation of non-nodulating references in the sample is increased. However, the local accessions were also more closely related among themselves than with the introductions from foreign sources, for both symbiotic and agronomic characters.

Principal component analysis showed that the first five principal components accounted for 82.60% of the total variation among 155 genotypes for the 32 characters considered in this study. Of these, the first and the second principal components constituted 30.15 and 23.70% of the variation, respectively (Table 5). Different characters accounted for different levels of differentiation among the populations into different clusters. Some very useful traits like pod and seed setting, dry matter accumulation, nitrogen and grain yields, growth rate and grain production efficiency and nitrogen fixation showed greater absolute values of eigenvectors in the first and/or second principal components, indicating that these traits had higher contributions to the total differentiation of the populations into clusters. Selection efforts based on these traits may be more effective. It was observed that other characters like early vigor, crop phenological characters, number of nodules and grain nitrogen yield with more contribution to the third principal component which had a gross contribution of only 13.04% of the total variation, had lesser contribution to the total differentiation of the populations into clusters.

Performances of genotypes in different clusters

Cluster mean performances showed existence of considerable variation among the different clusters for individual traits (Table 6). The first cluster (C_1) was characterized by the highest biomass accumulation at early (vigour) and for later (maturity) stages, it was good for pod and seed setting, economic growth rate and grain production efficiency, nitrogen (shoot, grain and biomass) yields and grain yield. This cluster was also superior to grand mean of all other traits averaged over all clusters, indicating that this cluster contained desirable genotypes, according to multiple symbiotic and agronomic characters.

The second cluster (C_2) had the lowest nodulation index, nitrogen fixation and protein contents, but the highest nitrogen and grain harvest indices and fixed nitrogen assimilation efficiency.

The third cluster (C_3) showed the highest nodulation index, nodule number and dry weight, and nitrogen harvest index but the least total above ground biomass (grain and shoot) weight, biomass and economic growth rates and nitrogen and grain yields. This indicates that our result deviated from the previous report that the values of nitrogen harvest index were consistently higher in modern crops than in landraces (Sinclair and Vadez, 2002).

The fourth cluster (C_4) was characterized by the best nodulation and nodule dry weight, highest foliar and grain nitrogen and protein contents, highest percentage of foliar

Table 5. Eigenvalue, percentage and cumulative variances and eigenvectors on the first five principal components for 32 agronomic and symbiotic characters in hundred fifty five chickpea genotypes.

Parameter	Principal component (PCs)				
	PC_1	PC_2	PC_3	PC_4	PC_5
Eigenvalue	9.948	7.820	4.303	2.749	2.437
Variance (%)	30.15	23.70	13.04	8.33	7.40
Cumulative Variance (%)	30.15	53.84	66.88	75.21	82.60
Character	**Eigenvectors**				
No of nodules 5 plants^{-1}	0.075	0.158	-0.021	0.295	-0.161
Nodule dry weight (g 5 plants^{-1})	0.108	0.119	-0.077	0.396	-0.323
Nodulation index	0.087	0.154	-0.095	0.268	-0.398
Shoot nitrogen content (%)	0.062	0.309	0.112	-0.091	-0.003
Shoot protein content (%)	0.063	0.309	0.112	-0.090	-0.002
Shoot nitrogen fixation (%)	0.092	0.304	0.067	-0.079	0.031
Grain nitrogen content (%)	0.156	0.255	-0.124	-0.068	0.156
Grain protein content (%)	0.156	0.256	-0.123	-0.068	0.156
Grain nitrogen fixation (%)	0.192	0.223	-0.164	-0.039	0.173
Total biomass nitrogen content (%)	0.135	0.309	-0.038	-0.087	0.106
Total biomass nitrogen fixation (%)	0.190	0.240	-0.142	-0.039	0.163
Fixed nitrogen assimilation efficiency (%)	0.195	0.045	-0.238	0.024	0.192
Grain nitrogen yield (g 5 plants^{-1})	0.288	-0.102	-0.040	-0.051	0.052
Shoot nitrogen yield (g 5 plants^{-1})	0.212	0.134	0.287	-0.032	-0.022
Biomass nitrogen yield (g 5 plants^{-1})	0.299	0.004	0.125	-0.050	0.023
Nitrogen harvest index	0.062	-0.210	-0.346	-0.004	0.035
Early vigor (g 5 plants^{-1})	0.096	-0.072	-0.053	0.317	0.270
Shoot dry weight ratio before flowering	0.098	-0.073	-0.059	0.311	0.254
Days to 50% flowering	-0.150	0.080	0.313	0.037	-0.297
Days to 90% maturity	-0.093	0.034	0.209	-0.139	0.049
Grain filling period	0.123	-0.067	-0.230	-0.130	-0.126
No of pods 5 plants^{-1}	0.208	-0.066	0.009	-0.207	-0.134
No of seeds 5 plants^{-1}	0.204	-0.063	-0.029	-0.256	-0.268
Shoot dry weight at maturity (g 5 plants^{-1})	0.235	-0.086	0.281	0.062	-0.001
Shoot dry weight ratio at maturity	0.235	-0.087	0.279	0.063	-0.005
Above ground biomass weight (g 5 plants^{-1})	0.247	-0.135	0.216	0.076	-0.021
Harvest index	0.069	-0.160	-0.316	-0.104	0.007
Grain production efficiency (g 5 plants^{-1})	0.234	-0.195	-0.111	-0.062	-0.046
Biomass production rate (%)	0.252	-0.139	0.188	0.091	-0.005
Economic growth rate (%)	0.223	-0.187	0.088	0.023	0.071
Thousand seed weight (g)	-0.044	-0.072	0.130	0.310	0.364
Grain yield (g 5 plants^{-1})	0.249	-0.201	-0.002	-0.028	0.100

and grain nitrogen fixation, better nitrogen yield and the least for shoot dry matter accumulation (particularly early in the growing season) and seed size.

Cluster C_5 showed better early vigour, long vegetative and short grain filling period, lower nitrogen harvest index, largest seed size, lower harvest index, and pod and seed setting.

The last cluster (C_6), constituted from the non-nodulating reference lines, was obviously the least for all characters, including attributes of symbiotic and agronomic significance, characterized particularly by the least above ground biomass and shoot dry weight, number of seeds plant^{-1}, harvest index, foliar and seed protein contents, growth rate and production efficiency and low yield (Table 6).

Geographical pattern of genetic diversity

The introduced genotypes were distinctly grouped into

Table 6. Differences among the six clusters of 155 chickpea genotypes for mean performance of 32 agronomic and symbiotic characters.

Character	Cluster						$\overline{X}$ ± Sd
	C_1	C_2	C_3	C_4	C_5	C_6	
No of nodules 5 plants^{-1}	13.18	9.79	15.4	14.94	14.15	0	11.24±3.24
Nodule dry weight (mg 5 plants^{-1})	427.56	270.06	510.47	511.13	372.34	0	348.59±129.90
Nodulation index	1.43	0.84	1.88	1.82	1.06	0	1.17±0.44
Foliar nitrogen content (%)	1.17	1.07	1.16	1.3	1.19	0.83	1.12±0.08
Foliar protein content (%)	7.29	6.72	7.24	8.16	7.44	5.19	7.01±0.49
Foliar nitrogen fixation (%)	29.82	24.34	29.13	36.87	30.78	0	25.16±4.35
Seed nitrogen content (%)	3.53	3.39	3.5	3.66	3.49	2.44	3.34±0.12
Seed protein content (%)	22.07	21.18	21.91	22.89	21.8	15.22	20.85±0.74
Seed nitrogen fixation (%)	36.65	33.76	36.18	38.74	35.62	0	30.16±2.17
Above ground biomass nitrogen content (%)	4.7	4.46	4.66	4.97	4.68	3.27	4.46±0.16
Total nitrogen fixation (%)	33.87	31.04	33.39	35.99	33.09	0	27.90±1.81
Fixed nitrogen assimilation efficiency (%)	81.63	82.9	81.83	79.48	80.67	0	67.75±2.95
Grain nitrogen yield (g 5 plants^{-1})	2.05	1.89	1.63	1.79	1.69	0.78	1.64±0.17
Shoot nitrogen yield (g 5 plants^{-1})	1.37	1.09	1.04	1.38	1.29	0.69	1.14±0.16
Biomass nitrogen yield (g 5 plants^{-1})	3.42	2.98	2.67	3.17	2.99	1.46	2.78±0.30
Nitrogen harvest index	0.61	0.64	0.61	0.57	0.56	0.54	0.59±0.02
Early vigor (g 5 plants^{-1})	38.85	37.59	38.15	34.66	38.84	27.05	35.86±4.79
Shoot dry weight ratio before flowering	1.45	1.39	1.43	1.26	1.41	0.95	1.32±0.18
Days to 50% flowering	55.82	55.28	55.84	56.71	62.69	71.5	59.64±0.66
Days to 90% maturity	114.04	113.83	113.44	114.51	114.38	121	115.20±0.98
Grain filling period	58.28	58.46	57.56	57.85	51.88	49.57	55.60±1.16
No of pods 5 plants^{-1}	417.8	385.55	339.96	379.34	265.62	264	342.05±35.26
No of seeds 5 plants^{-1}	475.04	436.21	378.96	428.23	269.31	245	372.13±42.16
Shoot dry weight at maturity (g 5 plants^{-1})	117.4	101.21	89.57	104.14	110.62	82.54	100.91±11.09
Shoot dry weight ratio at maturity	1.71	1.47	1.31	1.52	1.6	1.21	1.47±0.16
Above ground biomass weight (g 5 plants^{-1})	169.31	148.46	129.94	146.3	153.89	115.75	143.94±15.97
Harvest index	35.3	37.76	35.67	34.03	31.63	29.91	34.05±5.18
Grain production efficiency (g 5 plants^{-1})	60.83	59.12	48.03	50.55	42.75	22.75	47.34±5.08
Biomass production rate (%)	148.77	130.66	114.73	127.92	134.76	95.38	125.37±13.99
Economic growth rate (%)	99.98	95.52	81.08	85.17	94.21	66.4	87.06±8.12
Thousand seed weight (g)	108.32	111.85	108.51	100.54	184.02	123.14	122.73±7.40
Grain yield (g 5 plants^{-1})	57.9	55.63	46.45	49	48.86	32.26	48.35±4.56

nodulating and non-nodulating types as clusters C_5 and C_6, respectively. However, the landraces collected from the regions within Ethiopia were dispersed over clusters C_1 to C_4, indicating that there was no definite association between sources of origin and clustering pattern. The latter may be either related to extensive seed exchange between farmers or similarity of the initial germplasm introduced to different regions.

Not all sources of origins within Ethiopia had equal levels of genetic diversity. Accessions from six zones of origins, namely West Gojam, West Hararge, Shewa and Wello were distributed all over the clusters C_1 to C_4. Accessions from Arsi, Gonder and Tigray were distributed over three of the four clusters, which constituted the landraces. Accessions from East Gojam were distributed over two of the four clusters (Table 7).

The grouping for symbiotic and agronomic characters of the genotypes in this case, showed distinct grouping only between landraces and introduced genotypes. No clear interrelationship was observed between the origins of landraces in Ethiopia and the pattern of genetic diversity, as there were a number of accessions from the same source of origin which fell into different clusters and accessions from different origins overlapped in the same clusters (Table 7). This indicated that geographic diversity should not serve as an index of genetic diversity in selecting suitable parents for hybridization. Jomová et al. (2005) also characterized chickpea accessions from four countries and reported that genetic divergence was not apparently related to geographic diversity for morpho-agronomic traits. Contrary to the present finding, in another study, cluster analysis based on morpho-

Table 7. Clustering pattern of 155 chickpea genotypes from different origins over six clusters based on mean performance of 32 agronomic and symbiotic characters.

Origin	Number of genotype	Number of genotypes in each cluster					
		C_1	C_2	C_3	C_4	C_5	C_6
Arsi	13	6	4	3	-	-	-
East Gojam	13	4	-	-	9	-	-
West Gojam	13	8	1	3	1	-	-
North Gonder	13	5	-	3	5	-	-
South Gonder	12	6	-	2	4	-	-
West Haragie	11	3	2	3	3	-	-
East Shewa	13	6	1	2	4	-	-
North Shewa	13	6	1	2	4	-	-
West Shewa	13	2	3	5	3	-	-
Tigray	12	2	9	-	1	-	-
South Wello	13	3	7	2	1	-	-
Improved genotypes	14	-	1	-	-	13	-
Non-nodulating checks	2	-	-	-	-	-	2
Total	155	51	29	25	35	13	2

agronomic traits separately grouped the Kabuli from the Desi types (Upadhyaya et al., 2007). However, the level of representation of the Kabuli types in our study was not as good.

On the other hand, diversity and cluster analysis of the same genotypes at molecular level, using simple sequence repeats (SSR) markers, distinguished five clusters, but there was more or less clear association between pattern of genetic diversity and geographic sources of origin (Keneni et al., 2011). This may be related to better power and precision of differentiation of the SSR markers over the morphological markers (Carvalho, 2004). It may also be hypothesized that, at least within a limit, the same phenotypic differentiation and clustering pattern may be attained through different paths of combination, among morpho-agronomic characters as can be witnessed from the cluster mean performances (Table 6).

Implications for germplasm utilization

The present study shows that there is no special “hot spot” region for multiple traits of symbiotic and agronomic characters in Ethiopia. Nevertheless, it may not be by coincidence that a few of the best 5% of the accessions selected for specific symbiotic and agronomic characters existed in specific clusters and geographical regions. For attributes of symbiotic nitrogen fixation, cluster C_4 constituted much greater number of best accessions, followed by clusters C_1 and C_2. Cluster C_1 also constituted most of the best accessions for attributes of agronomic significance (Figure 4). In the same type, the best accessions (41222, 41029, 41021, 41074, 41075, 41320 and 41026), selected for symbiotic nitrogen fixation, had their origins in East Gojam, while those for shoot, grain and total above ground biomass nitrogen yields were associated with East and West Gojam zones. Likewise, from among the agronomic traits, six (out of eight) best accessions (231328, 209093, 209094, 41002, 231327 and 207764) selected for harvest index, were collected from Arsi. Even though best performers for agronomic traits were more distributed over the regions than those for symbiotic traits, West Gojam was found to be better source of accessions for agronomic traits (Figure 5). Therefore, specific origins may exhibit best accessions for specific characters, which may facilitate selection of groups of accessions possessing specific usage in breeding programs.

It may be difficult, if at all possible, to find out a single genotype combining all desirable characters to the required levels for direct selection and use as a cultivar. In order to produce demanded segregants, a step-wise combination and recombination of valuable genes should be made, using intercrossing of selected parents from the accessions with desirable characters. Parents with one or more of the desired characters can be chosen from the sources grouped in this study, based not only on group performance but also on performances of individual accessions for specific traits (Singh, 2002). Once parental accessions are identified and purified, multiple crossing schemes of several parental lines and selection of improved varieties may help to recombine desirable symbiotic and agronomic characters into a single genotype. For example, best lines for nitrogen fixation to be developed from accession 41222, in cluster C_4, can be crossed with the best lines for yield to be developed from accession 41274 in cluster C_1. Even though introduced genotypes gave lower yields when directly utilized in field

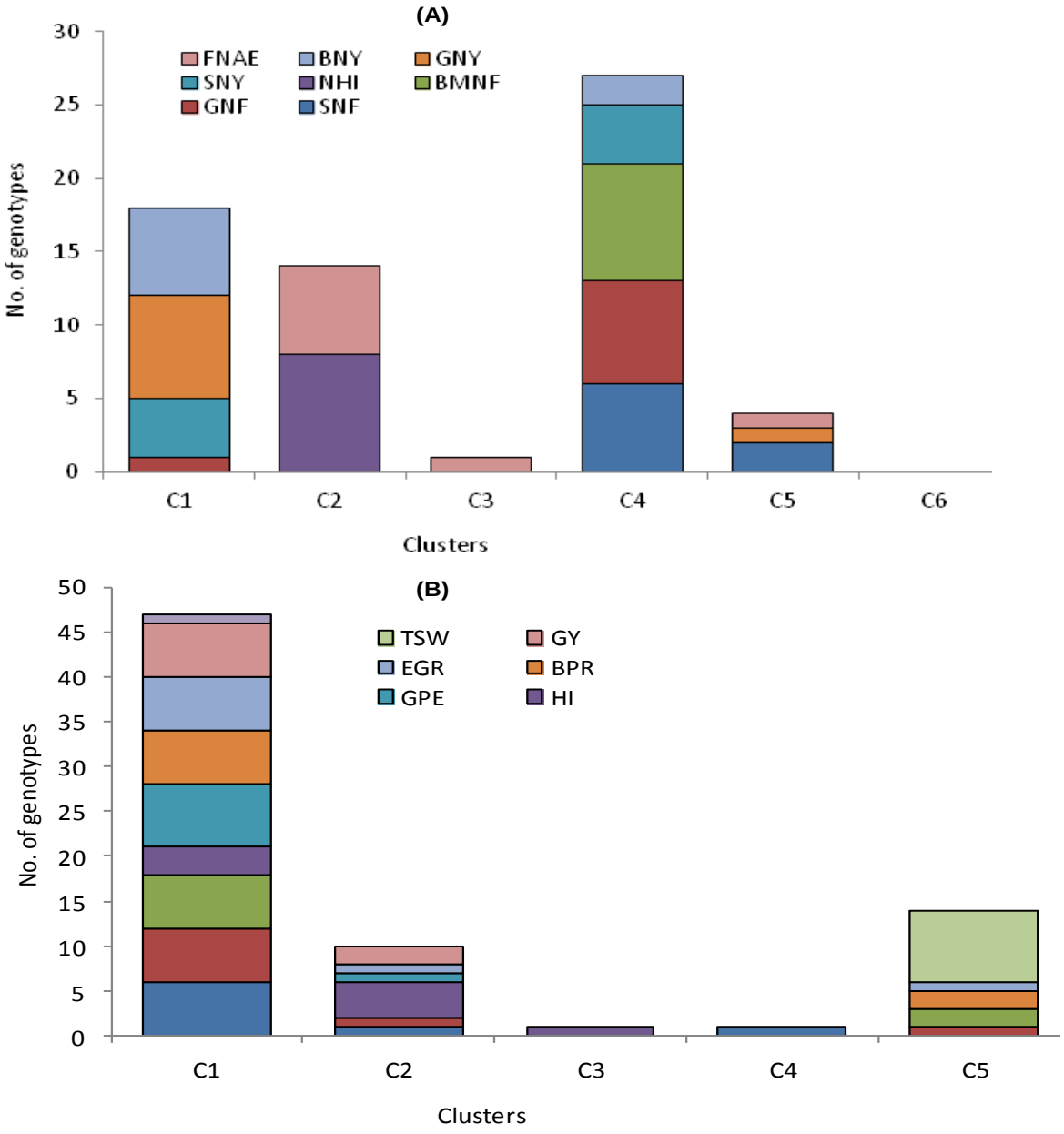

Figure 4. Number of best 5% of chickpea genotypes selected for (A) symbiotic and (B) agronomic characters in each cluster based on evaluation at two locations in Ethiopia. FNAE = fixed nitrogen assimilation efficiency; BNY = biomass nitrogen yield; GNY = grain nitrogen yield; SNY = shoot nitrogen yield; NHI = nitrogen harvest index; BMNF = biomass nitrogen fixation; TSW = thousand seed weight and GY = grain yield; EGR = economic growth rate; BPR = biomass production rate; GPE = grain production efficiency; HI = harvest index.

trials as compared to the selected best landraces, they may also serve as donors with parents chosen from the adapted landraces because wider genetic recombination is expected in progenies of distant parents (Sneller et al., 2005).

For example, best lines combining nitrogen harvest index and fixed nitrogen assimilation efficiency to be developed from accession 41113 in cluster C_2 can be crossed with ICC 19180 in cluster C_5. The latter is the best genotype not for nitrogen fixation but for the assimilation of fixed nitrogen. Further crossing of the single cross progeny to make multiple crosses, followed by selection in segregating generation under inoculation with effective *Rhizobium* strains is expected to result in the recombination of the desirable symbiotic and agronomic traits from all parents into a single genotype. Alternatively, the most widely adapted parent may be selected as seed parent to make the genetic background

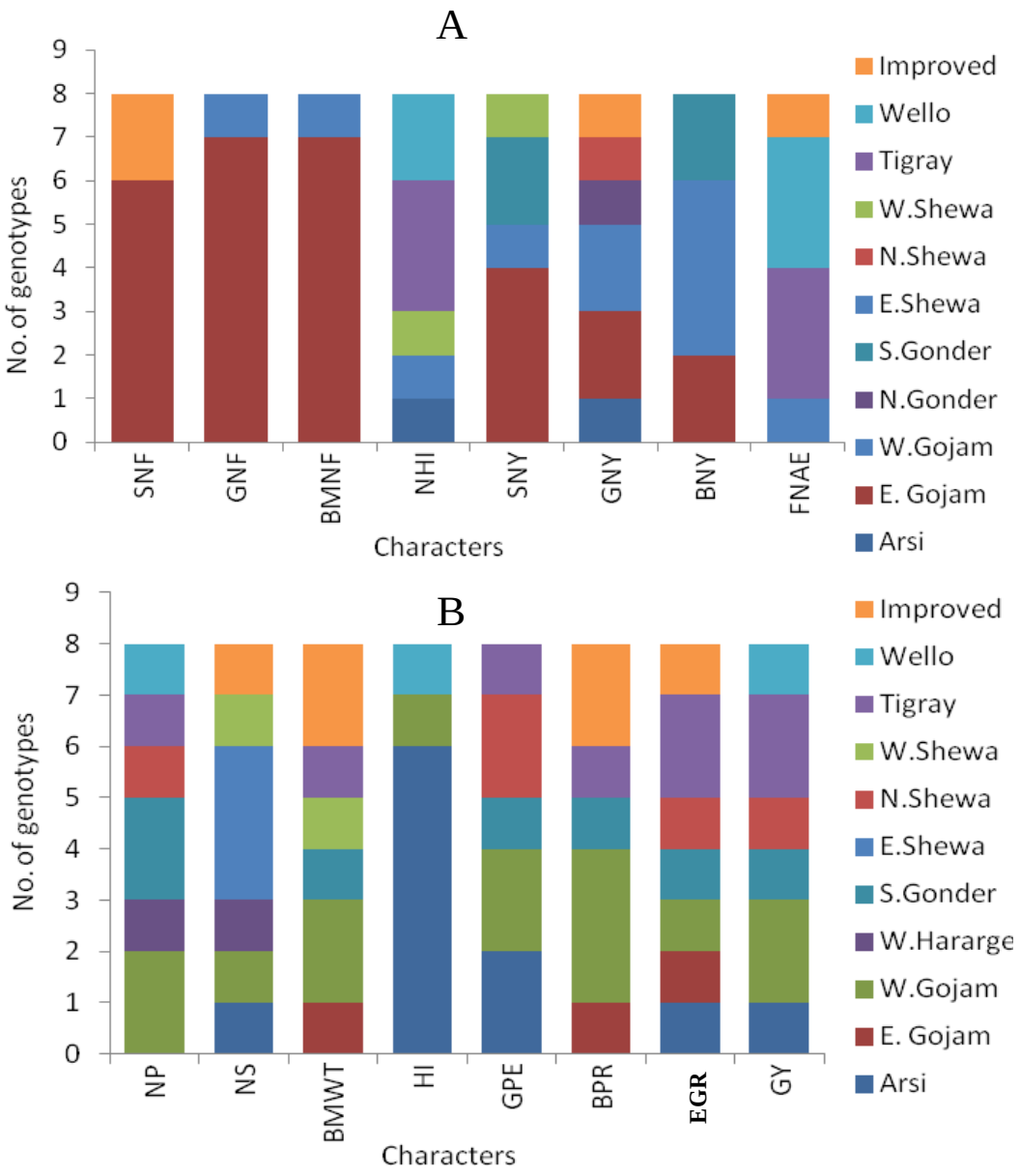

Figure 5. Distribution of the best 5% of chickpea genotypes selected for selected (A) symbiotic and (B) agronomic characters over different sources of origin based on evaluation at two locations in Ethiopia. SNF = shoot nitrogen fixation; GNF = grain nitrogen fixation; BMNF = biomass nitrogen fixation; NHI = nitrogen harvest index; SNY = shoot nitrogen yield; GNY = grain nitrogen yield; BNY = biomass nitrogen yield; FNAE = fixed nitrogen assimilation efficiency; NP = number of pods, NS = number of seeds; BMWT = biomass weight; HI = harvest index; GPE = grain production efficiency; BPR = biomass production rate; EGR = economic growth rate; GY = grain yield.

for crossing all other parents as carriers of specific traits with them.

Conclusion

It was demonstrated that Ethiopian chickpea germplasm accessions are more distinctly diverged from the introductions of ICARDA and ICRISAT than 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 improved genotypes to a separate cluster may be somehow related to the level of prior breeding to which they have been subjected at ICRISAT and ICARDA before their introduction to Ethiopia. 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 potential of Ethiopian chickpea germplasm accessions for improving attributes of both symbiotic and agronomic significance was also revealed.

It was observed that the desirable characters were found to exist, distributed among different accessions, and as such, a single group or a single accession, combining desirable symbiotic and agronomic attributes may be of rare occurrence in this gene pool. The utilization of these valuable germplasm, particularly in the efforts underway to develop efficient genotypes for symbiotic nitrogen fixation and agronomic performance 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, particularly in order to exploit complementary genes, for example to improving seed size as an economic trait.

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