Renewable Agriculture and Food Systems: 28(4); 338–349 doi:10.1017/S1742170512000221

Evaluation of Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions for symbio-agronomic performance

Gemechu Keneni[1,2]*, Endashaw Bekele[2], Fassil Assefa[2], Muhammad Imtiaz[3], Tolessa Debele[4], Kifle Dagne[2], and Emana Getu[2]

[1]Holetta Agricultural Research Center, P.O. Box 2003, Addis Ababa, Ethiopia.
[2]College of Natural Sciences, 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.
[4]Ethiopian Institute of Agricultural Research, P.O. Box 2003, Addis Ababa, Ethiopia.
*Corresponding author: gemechukeneni@yahoo.com

Accepted 11 May 2012; First published online 30 July 2012 **Research Paper**

Abstract

Chickpea (*Cicer arietinum* L.) is an economically and ecologically important food legume crop. Ethiopia has a large collection of chickpea germplasm accessions; but, it has not been extensively characterized for desirable sources of agronomic and symbiotic significance for use in breeding programs. A study was conducted at two locations (Ambo and Ginchi) in 2009/2010 to characterize and evaluate Ethiopian chickpea germplasm accessions for symbiotic and agronomic performance. One hundred and thirty-nine germplasm accessions were evaluated with 16 other genotypes including non-nodulating reference checks. Differences among genotypes, locations and genotype by location interaction effects were significant for a number of characters. A number of accessions better performing over the improved genotypes were identified 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 per 5 plants and seed size ranged from 82 to 288 g per 1000 seeds. For both symbiotic and agronomic characters, landraces were found to be overwhelmingly superior to introduced genotypes, except for seed size, where the best genotypes were all from exotic sources. The result indicated that Ethiopian chickpea landraces have better genetic potential for improving a number of symbiotic and agronomic characters over the varieties currently in use. Selection of best individuals within and among the accessions would be expected to be effective.

Key words: characterization, chickpea (*Cicer arietinum*), germplasm accession, symbiotic nitrogen fixation

Introduction

Nitrogen is the most essential nutrient required by plants in large quantities. Its deficiency is the major problem of crop production in many tropical and subtropical areas[1,2], particularly in East Africa[3] including Ethiopia[4,5]. Resource-poor farmers have striven to overcome the nitrogen deficiency problem since antiquity by integration of legumes in crop rotation systems[6], and by manuring and fallowing. Different studies in many parts of the world[7–11] including Ethiopia[12] have shown the importance of symbiotic nitrogen fixation not only to the legumes themselves but also to the cereals grown in association (as a mixed cropping) or rotation with them.

The amount of nitrogen fixed annually with symbiotic association is far more than the amount industrially produced[13,14]. Some estimates show that hundreds of millions of metric tons of nitrogen are symbiotically fixed each year[14,15]. At the farm level, nitrogen fixation with annual legumes may contribute 20–120 kg of nitrogen ha^{-1} in a season in the temperate region[16] and 15–120 kg ha^{-1} in tropical Africa[17].

Among the food legume crops, chickpea (*Cicer arietinum* L.) is a crop of manifold economic and ecological merit at the global level. Evidence indicates that chickpea fixes atmospheric nitrogen to the tune of 100 kg ha^{-1} [18,19] and contributes over 500,000 million tons of nitrogen every year to developing countries[20]. Ethiopia, being the first producer of chickpea in Africa in terms of area coverage, gets a significant amount of nitrogen fixation from the crop[21]. As in other legume crops, however, chickpea's effectiveness and efficiency to

© Cambridge University Press 2012

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

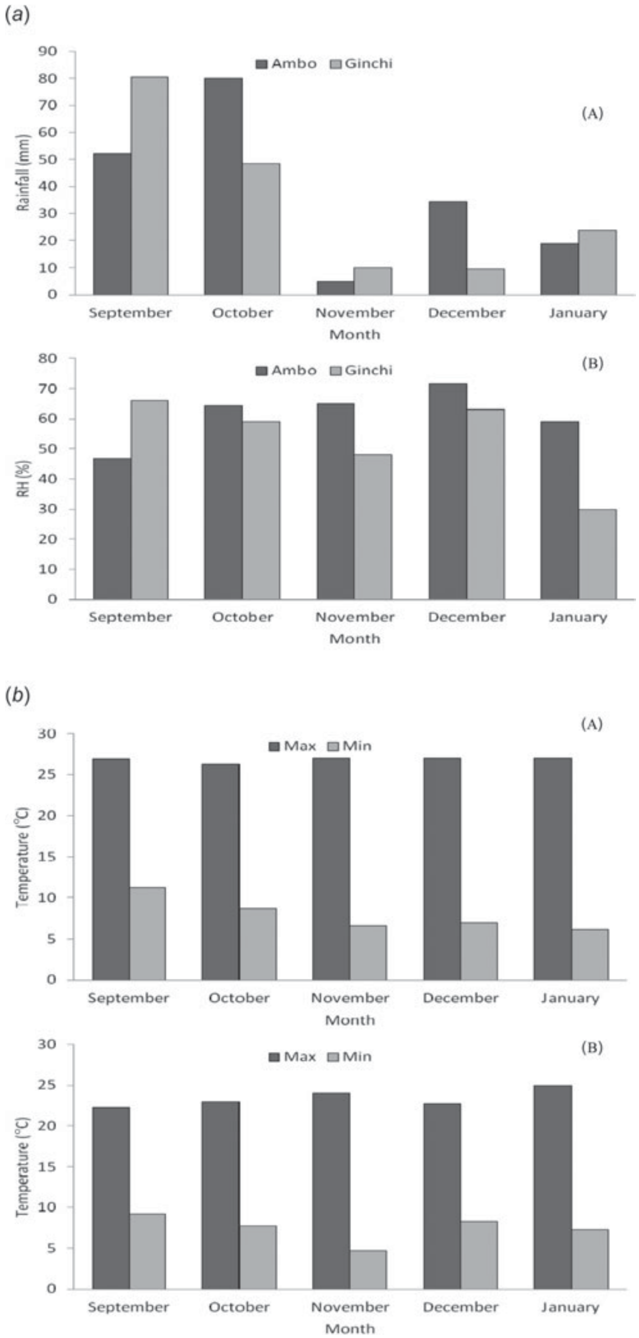

Figure 1. (a) Rainfall (mm) (A) and relative humidity (%) (B) at Ambo and Ginchi during the growing season. (b) Maximum and minimum temperatures (°C) at (A) Ambo and (B) Ginchi during the growing season.

fully realize its potential depends on the type of genotype, compatibility of the micro-symbiont and several environmental factors[22–24].

In most cases, farmers in Ethiopia do not use chemical fertilizers in chickpea production[25]. With the increasing price of nitrogen fertilizer and with growing environmental concern, selection of nutrient-efficient host genotypes with effective endosymbionts is a good alternative to overcome the problem of nitrogen deficiency. Efforts made to enhance nitrogen fixation through selection of better strains of *rhizobacteria* in Ethiopia have resulted in identification of many effective and competitive strains[26]. Nevertheless, development of better strains alone may not provide the required gains in productivity without compatible host genotypes[13,18,27,28]. Some reports indicate that host plants play rather a dominant role as compared to bacterial strain in enhancing symbiotic nitrogen fixation[20,29]. Even though genotypic variation for attributes of host symbiotic and agronomic significance have been reported in many legume crops including chickpea[1,30,31], for a variety of reasons, the role of host plants attracted little attention in Ethiopia and elsewhere[20,32].

Ethiopia has a large collection of chickpea germplasm but, apart from some observations in specific trials by microbiologists, mostly with a few improved varieties, no systematic assessment was made to exploit these gene pools especially for improving host symbiotic nitrogen fixation combined with agronomic performance. This study was, therefore, conducted to characterize and evaluate Ethiopian chickpea germplasm accessions and some varieties, and to identify desirable genotypes for symbiotic and agronomic performance.

Materials and Methods

Plant materials

In this study, a total of 155 chickpea genotypes were evaluated. They include 139 accessions collected from different regions of Ethiopia kindly provided by the Ethiopian Institute of Biodiversity Conservation (IBC), five improved genotypes from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), eight originally introduced commercial cultivars released in Ethiopia and three genetically non-nodulating reference checks received from ICRISAT and the International Center for Agricultural Research in the Dry Areas (ICARDA). These chickpeas will be called 'genotypes' hereafter for experimental purpose. The passport description of the genotypes and the map of the areas of collection for the Ethiopian accessions are presented as online supplementary materials in Annexes A and B (at http://journals.cambridge.org/). All genotypes were rejuvenated during 2008/2009 under the same conditions at Ginchi to reasonably minimize initial variation due to differences in seed age and indigenous seed nitrogen contents[33].

The test environment

The experiment was conducted under field conditions at two locations (Ginchi and Ambo) in the central part of Ethiopia for 1 year during the main cropping season of 2009/2010 (September–January). The two locations are assumed to represent the major chickpea production areas

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

Table 1. Description of the test locations for geographical position and physico-chemical properties of the soils.

	Source of soil	
Parameter	**Ambo**	**Ginchi**
Latitude	09°00′N	09°00′N
Longitude	37°22′E	38°10′E
Altitude (m asl)	2225	2200
% 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)[1]	18.07 (high)	4.49 (low)
K (Meq/100 g 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_4 S (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)[2]	650.00 (high)	547.33 (high)

[1]ppm, parts per million; [2]μS, micro Siemens. EC, electrical conductivity.

of Ethiopia. Climatic data of the two locations during the growing period were taken from Ambo and Holetta Research Centers (Fig. 1a and b). Soil samples from both locations were collected from the rhizosphere (top 20 cm) for physico-chemical characterization (Table 1).

Rhizobium *inoculant and inoculation*

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, was used for the study. The isolate was found to be efficient in nodulation and symbiotic nitrogen fixation in previous studies[26]. The inoculum was received at the concentration of approximately 10^9 cells g^{-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 inoculant at the rate of approximately 2 g of inoculum for 80 seeds using 40% gum Arabic as an adhesive.

Experimental design and layout

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 superphosphate (TSP) at the recommended rate (20 g for a single row of 4 m). Seed rate was 5 cm between plants and 40 cm between adjacent rows. The plot size was 1 row 4 m long. The genotypes were assigned to plots at random within each block. Nitrogenous fertilizer 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 nodules. Representative shoot and grain samples collected at 90% physiological maturity from each plot were oven-dried to constant moisture at 70°C for 18 h. The dry samples were ground to pass through a 1 mm mesh sieve to determine nitrogen using the Kjeldahl technique[34] 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. The N-difference method using a non-nodulating line as a reference plant was found to be both reliable and economical[35]. Protein contents of shoot and grain were determined by multiplying nitrogen percentages by the standard conversion factor of 6.25[34]. 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 is the amount of nitrogen in the shoot of the fixing genotype and Nsnfg is the amount of nitrogen in the shoot of the non-fixing genotype.

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

where Ngfg is the amount of nitrogen in the grain of the fixing genotype and Ngnfg is the amount of nitrogen in the grain of the 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 of symbiotic and agronomic characters

Data were collected either on a plot basis or from randomly selected five plants[36].

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

Table 2. Combined analysis of variance (over locations) for attributes of symbiotic and agronomic performance in 155 chickpea genotypes tested at two locations.

Character	Mean square			CV (%)
	L	G	G×L	
Symbiotic characters				
No. of nodules (NN, per 5 plants)	**	**	**	24.38
Nodule dry weight (NDW, mg per 5 plants)	**	**	**	14.69
Nodulation index (NI, mg g^{-1})	**	**	**	21.65
Shoot nitrogen content (SNC,%)	NS	**	NS	19.00
Shoot protein content (SPC,%)	NS	**	NS	19.00
Shoot nitrogen fixation (SNF,%)	**	**	NS	19.25
Grain nitrogen content (GNC,%)	NS	**	NS	9.53
Grain protein content (GPC,%)	NS	**	NS	9.53
Grain nitrogen fixation (GNF,%)	NS	**	NS	10.73
Above-ground biomass nitrogen content (BNC,%)	NS	**	NS	9.66
Biomass nitrogen fixation (BMNF,%)	**	**	NS	9.35
Fixed nitrogen assimilation efficiency (FNAE,%)	**	**	NS	10.38
Grain nitrogen yield (GNY, g per 5 plants)	NS	**	**	26.70
Shoot nitrogen yield (SNY, g per 5 plants)	**	**	**	19.55
Biomass nitrogen yield (BNY, g per 5 plants)	**	**	**	27.13
Nitrogen harvest index (NHI)	**	**	**	10.91
Agronomic characters				
Early vigor (SDWF, g per 5 plants)	**	**	**	25.61
Shoot dry weight ratio before flowering (SDWRF)	**	**	**	25.61
Days to 50% flowering (DTF)	**	**	NS	3.33
Days to 90% maturity (DTM)	**	**	NS	2.42
Grain filling period (GFP)	**	**	NS	5.71
No. of pods (NP, per 5 plants)	**	**	**	26.54
No. of seeds (NS, per 5 plants)	NS	**	**	28.30
Shoot dry weight at maturity (SDWM, g per 5 plants)	**	**	*	29.64
Shoot dry weight ratio at maturity (SDWRM)	**	**	*	29.55
Biomass weight (BMWT, g per 5 plants)	*	**	*	29.73
Harvest index (HI)	**	**	**	15.01
Grain production efficiency (GPE, g per 5 plants)	**	**	**	26.53
Biomass production rate (BPR,%)	**	**	**	19.69
Economic growth rate (EGR,%)	*	**	**	25.00
Thousand seed weight (TSW, g)	NS	**	**	18.39
Grain yield (YLD, g per 5 plants)	NS	**	**	24.57

**Highly significant ($P \leqslant 0.01$), *significant ($P \leqslant 0.05$), and NS = non-significant ($P > 0.05$).

Records of symbiotic characters were taken as follows: (1) number of nodules, (2) nodule dry weight, (3) nodulation index (nodule weight to shoot 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, and (12) NHI.

Agronomic characters recorded include: (1) early vigor (as shoot dry weight before flowering), (2) shoot dry weight at maturity, (3) shoot dry weight ratios (of nodulating to non-nodulating genotypes) before flowering and 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) number of pods and seeds, (7) total above-ground biomass weight, (8) harvest index (proportion of grain to total above-ground biomass), (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 and (13) grain yield.

Statistical analysis

Data based on nodules (number, weight and nodulation index) were log transformed to offset heterogeneity[37,38] for statistical analysis as suggested by Doughton[39]. Pooled analysis of variance was conducted to quantify

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

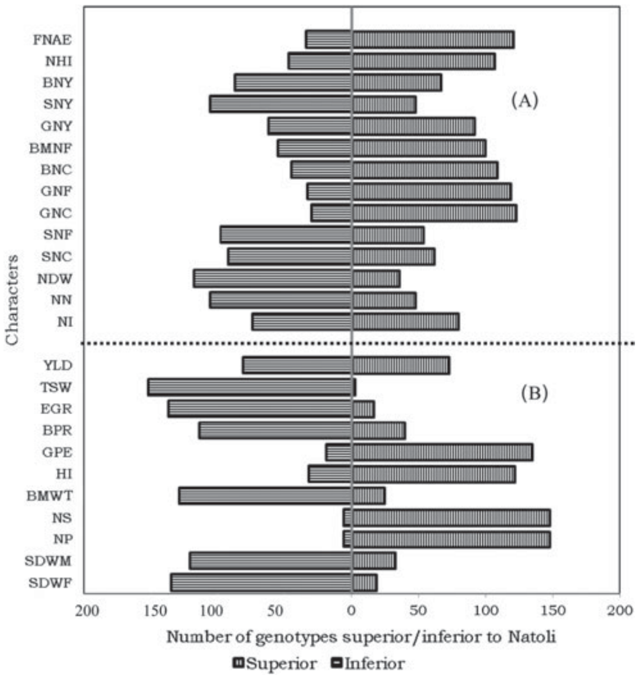

Figure 2. Proportion by number of the 155 chickpea genotypes superior and inferior to the recently released check, Natoli, for (A) symbiotic and (B) agronomic characters showing superiority of a number of landraces to the standard check (see Table 2 above for abbreviations of the characters).

the total variation among the genotypes using the following model of analysis of variance:

$$P_{ijk} = \mu + (b/l)i_k + g_j + l_k + (gl)_{jk} + e_{ijk}$$

where P_{ijk} is the phenotypic observation on genotype j in block i (at location k) ($i = 1, \ldots, B$, $j = 1, \ldots, G$, and $k = 1, \ldots, L$) and G, L and B are the number of genotypes, location and block, respectively, μ is the grand mean, $(b/l)_{ik}$ is the effect of block i (within location k), g_j is the effect of genotype j, l_k is the effect of location k, $(gl)_{jk}$ is the interaction effect between genotype j and location k and e_{ijk} is the residual or effects of random error.

Existence of significant differences among the genotypes, locations and genotype × location interaction effects were determined using the F-test. Mean separation was done using Duncan's multiple range test (DMRT) at 5% probability levels[38].

Results and Discussion

Description of the test locations

The two locations received more or less similar amounts of rainfall with different patterns of distribution, but Ambo was more humid than Ginchi (Fig. 1a). It was witnessed that the weather variables recorded did not deviate much from the long-term trends at both locations[40], indicating that the present findings will be reproducible over seasons.

The physico-chemical properties of the soils from the two test locations, Ambo and Ginchi, showed equally low levels of nitrogen contents (0.103%) but high levels of K, Ca, Mg, Na and Fe[41] with variable amounts. The levels of exchangeable cations were also high with pH values close to neutral. The level of soil phosphorus was high at Ambo and low at Ginchi (Table 1). Similar results were reported from previous analysis of soils at the same locations[42].

Symbiotic performances of the genotypes

Differences among the genotypes were significant for symbiotic nitrogen fixation and a number of associated characters (Table 2 and see supplementary material Annex C). The larger coefficients of variation (CV) values (> 20%) in many traits may be related to the production on residual moisture, where the crop was highly stressed, or a sample-based estimation of mean performances from only a few plants grown on small plots or both. Depending on host genotype, the amount of fixed nitrogen varied from 13 to 49% ($\bar{X}$ = 30%) in foliage, 30 to 44% ($\bar{X}$ = 36%) in grain and 28 to 40% ($\bar{X}$ = 34%) in total above ground biomass. The potential of fixation by the genotypes may be generally limited because of a shortage of soil moisture as the crop was grown with residual moisture. In Syria[43], for instance, fixation in winter-sown chickpea reached over 80–81%, where as spring-sown chickpea, where moisture was a limiting factor, fixed only 8–27%. The difference method of determination of the amount of nitrogen fixation may also underestimate the magnitude[42]. Protein contents of the shoot, grain and biomass, being derivative characters of plant tissue nitrogen, obviously followed the same pattern of variation as nitrogen contents. Other associated characters also showed wider ranges of variation.

Comparison of the test genotypes with a recently released variety, Natoli, and with the overall mean performances of eight released varieties (Shasho, Arerti, Worku, Akaki, Ejere, Teji, Habru and Natoli) identified a number of accessions with superior symbiotic performance including the amount of nitrogen fixation in shoot, grain and total above-ground biomass (Figs 2 and 4). The best 5% of the accessions for total (shoot + grain) nitrogen fixation include accession nos. 41222, 41029, 41021, 41074, 41075, 41129, 41320 and 41026.

These landraces could register additional fixation ranging from 18.52 to 23.88% over the standard check, Natoli, and 17.44 to 22.75% over the mean performance of the released varieties. These accessions also had the highest above-ground biomass nitrogen contents, which varied from 5.24 to 5.61%, the content of Natoli being 4.56%. As protein content follows the same trend as nitrogen contents, these accessions also contained the highest amount of protein in their biomasses (see supplementary table Annex C). There were some other genotypes that had better fixation of nitrogen, either in their shoots (e.g. 41103) or grains (e.g. 207734). Two

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

Table 3. Comparison of mean performances of 5% of the accessions selected for best symbiotic performance with Natoli, a recently released variety, and with mean performances of released varieties.

Accession No.	Mean of selected accession[1]	Comparative advantage (% over)		Accession No.	Mean of selected accession	Comparative advantage (% over)	
		Natoli	MRV[2]			Natoli	MRV
Shoot N fixation (%)				Grain N fixation (%)			
41222	48.67a	53.73	70.59	41021	43.54a	27.09	22.72
41026	47.29ab	49.37	65.76	41029	43.41ab	26.71	22.35
41074	46.05a–c	45.45	61.41	41222	42.79a–c	24.9	20.6
41103	45.63a–d	44.13	59.94	41074	42.48a–d	23.99	19.73
41075	45.04a–e	42.26	57.87	41320	42.07a–e	22.8	18.57
ICC 4973	42.85a–f	35.34	50.19	207734	42.05a–e	22.74	18.52
ICC 5003	42.80a–f	35.19	50.02	41129	42.04a–-e	22.71	18.49
41320	42.11a–g	33.01	47.6	41075	41.75a–f	21.86	17.67
Natoli	31.66b–v	–	10.97	Natoli	34.26e–u	–	– 3.44
MRV	28.53	– 9.89	–	MRV	35.48	3.56	–
Biomass N fixation (%)				NHI			
41222	40.20a	23.88	22.75	41115	0.69a	18.97	21.05
41029	40.11ab	23.61	22.47	207150	0.67ab	15.52	17.54
41021	39.86a–c	22.84	21.71	231328	0.66a–e	13.79	15.79
41074	39.31a–d	21.14	20.03	207741	0.66a–e	13.79	15.79
41075	38.85a–e	19.72	18.63	209036	0.66a–e	13.79	15.79
41129	38.70a–e	19.26	18.17	207895	0.66a–e	13.79	15.79
41320	38.54a–g	18.77	17.68	219799	0.66a–e	13.79	15.79
41026	38.46a–g	18.52	17.44	41113	0.66a–e	13.79	15.79
Natoli	32.45e–y	–	– 0.92	Natoli	0.58d–z	–	1.75
MRV	32.75	0.92	–	MRV	0.57	– 1.72	–
Shoot N yield (g per 5 plants)				Grain N yield (g per 5 plants)			
41275	1.83a	37.59	45.24	41274	2.46a	35.91	44.71
41103	1.82ab	36.84	44.44	41111	2.34ab	29.28	37.65
41026	1.76a–c	32.33	39.68	207763	2.33a–c	28.73	37.06
207734	1.71a–d	28.57	35.71	207734	2.33a–c	28.73	37.06
41289	1.70a–e	27.82	34.92	207742	2.29a–d	26.52	34.71
41185	1.69a–e	27.07	34.13	ICC 19180	2.28a–e	25.97	34.12
41284	1.67a–f	25.56	32.54	41268	2.23a–f	22.65	30.59
41320	1.66a–g	24.81	31.75	41316	2.22a–g	22.65	30.59
Natoli	1.33a–o	–	5.56	Natoli	1.81a–p	–	6.47
MRV	1.26	– 5.26	–	MRV	1.70	– 6.08	–
Biomass N yield (g per 5 plants)				Fixed N assimilation efficiency (%)			
207734	4.05a	28.57	36.82	ICC 19180	90.61a	15.38	10.16
41274	3.87ab	22.86	30.74	41115	90.15ab	14.8	9.6
41275	3.80a–c	20.63	28.38	207659	89.74a–c	14.27	9.11
41185	3.80a–c	20.63	28.38	219799	89.03a–d	13.37	8.24
41111	3.75a–d	19.05	26.69	207150	88.74a–e	13	7.89
41284	3.70a–d	17.46	25	41277	88.10a–f	12.19	7.11
41103	3.69a–d	17.14	24.66	41113	87.79a–g	11.79	6.74
41289	3.68a–e	16.83	24.32	207894	87.10a–h	10.91	5.9
Natoli	3.15a–m	–	6.42	Natoli	78.53d–r	–	– 4.52
MRV	2.96	– 6.03	–	MRV	82.25	4.74	–

[1]Values sharing the same letter(s) or ranges of letters within the same column are non-significantly different; [2]MRV = mean of released varieties. NHI, nitrogen harvest index.

introductions from ICRISAT, namely ICC 5003 and ICC 4973, were also among the best 5% for shoot fixation. The best genotypes for NHI, with superiorities of 13.79–18.97% over Natoli and 15.79–21.05% over the mean performances of the released varieties, were also landraces. This contradicts the tendency that modern cultivars usually show better NHI than landraces[44].

The best assimilators of fixed nitrogen were accession nos. 41115, 207659, 219799, 207150, 41277, 41113 and 207894. A genotype introduced from ICRISAT as a

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

Table 4. Comparison of mean performances of 5% of the accessions selected for best agronomic performance with Natoli, a recently released variety, and with mean performances of released varieties.

		Comparative advantage (% over)				Comparative advantage (% over)	
Accession No.	**Mean of selected accession[1]**	**Natoli**	**MRV[2]**	**Accession No.**	**Mean of selected accession**	**Natoli**	**MRV**
No. of pods (per 5 plants)				No. of seeds (per 5 plants)			
41289	515a	94.34	105.18	41111	595a	144.86	146.89
41274	497ab	87.55	98.01	207658	575a	136.63	138.59
41215	486a–c	83.4	93.63	41185	566a–c	132.92	134.85
41284	485a–c	83.02	93.23	41274	556a–d	128.81	130.71
209091	480a–d	81.13	91.24	41215	556a–d	128.81	130.71
41015	471a–e	77.74	87.65	ICC 4948	541a–e	122.63	124.48
207563	466a–f	75.85	85.66	207764	535a–f	120.16	121.99
41114	464a–g	75.09	84.86	209084	535a–f	120.16	121.99
Natoli	265u–z	–	5.58	Natoli	243z	–	0.83
MRV	251	−5.28	–	MRV	241	−0.82	–
Biomass weight (g per 5 plants)				Harvest index			
41284	224.32a	32.55	45.1	231328	42.88a	29.59	34.21
41274	204.41ab	20.79	32.22	209093	42.58ab	28.68	33.27
207734	198.76a–c	17.45	28.56	209094	42.07a–c	27.14	31.67
41275	194.41a–d	14.88	25.75	41002	40.41a–d	22.12	26.48
Habru	191.06a–e	12.9	23.58	231327	40.27a–e	21.7	26.04
ICC 19180	188.92a–e	11.64	22.2	207764	40.12a–f	21.25	25.57
41185	183.47a–f	8.41	18.67	207741	40.08a–f	21.12	25.45
207563	182.83a–g	8.04	18.26	41115	39.47a–g	19.28	23.54
Natoli	169.23a–m	–	9.46	Natoli	33.09g–w	–	3.57
MRV	154.6	−8.65	–	MRV	31.95	−3.45	–
Grain production efficiency				Biomass production rate (%)			
207763	70.65a	63.69	57.17	41284	198.82a	37.65	47.06
41111	70.35ab	63	56.51	41274	181.47ab	25.64	34.22
41274	69.71a–c	61.52	55.08	ICC 19180	175.17a–c	21.28	29.56
207742	69.14a–d	60.19	53.82	207734	170.96a–d	18.36	26.45
41053	68.21a–e	58.04	51.75	41275	169.91a–e	17.63	25.67
209093	67.70a–f	56.86	50.61	Habru	169.65a–f	17.45	25.48
207658	67.57a–f	56.56	50.32	207743	162.38a–g	12.42	20.1
219800	66.70a–g	54.54	48.39	207563	161.98a–g	12.14	19.81
Natoli	43.16q–z	–	−3.98	Natoli	144.44b–n	–	6.83
MRV	44.95	4.15	–	MRV	135.2	−6.4	–
Economic growth rate (%)				Grain yield (g per 5 plants)			
41274	125.02a	18.6	34.65	41274	70.10a	30.76	42.22
ICC 19180	118.90ab	12.8	28.06	207763	66.05ab	23.2	34
207763	113.75a–c	7.91	22.51	41111	65.16a–c	21.54	32.2
41268	112.48a–d	6.71	21.14	207742	64.22a–d	19.79	30.29
231328	111.83a–e	6.09	20.44	231328	63.62a–e	18.67	29.07
41293	111.58a–e	5.85	20.17	207563	63.10a–f	17.7	28.02
41111	110.44a–f	4.77	18.94	41053	62.59a–g	16.75	26.98
207563	109.29a–g	3.68	17.71	212589	62.52a–g	16.62	26.84
Natoli	105.41a–l	–	13.53	Natoli	53.61a–t	–	8.76
MRV	92.85	−11.92	–	MRV	49.29	−8.06	–

[1]Values sharing the same letter(s) or ranges of letters within the same column are non-significantly different; [2]MRV, mean of released varieties.

non-nodulating check, ICC 19180, was found to be nodulating with best performance for grain nitrogen yield and assimilation efficiency of fixed nitrogen (Table 3). Whether a change in environment alone can induce nodulation of a genotype that is naturally non-nodulating in another environment needs to be sorted out in the future.

These are accessions with desirable attributes of both symbiotic and agronomic significance. For example, accession no. 41274 possessed both better symbiotic

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

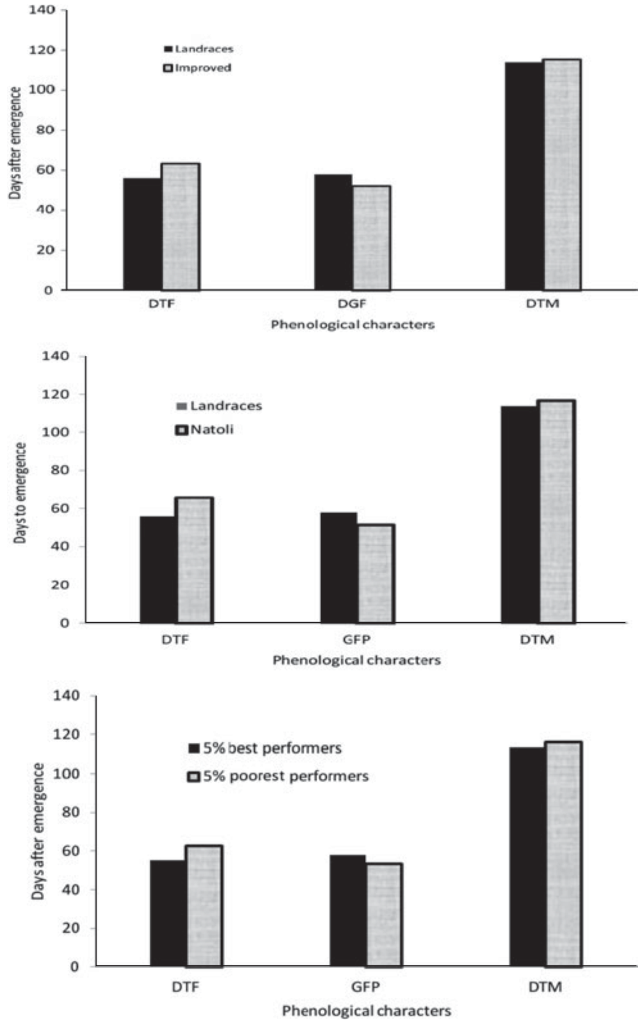

Figure 3. Comparison of phenological characters in 155 genotypes for days to flowering (DTF), grain filling period (GFP) and days to maturity (DTM), showing shorter relative periods of vegetative and longer grain-filling periods in landraces than in improved genotype.

(grain and biomass nitrogen yields) and agronomic (pod and seed setting, biomass weight, production efficiency, biomass production rate, economic growth rate and grain yield) attributes. Among the introductions, ICC 19180 demonstrated relatively better grain nitrogen yield and assimilation of fixed nitrogen with better biomass production and economic growth rates.

Comparison of nitrogen fixation patterns between modern cultivars and landraces does not appear to follow a simple trend as there are conflicting reports. Some found better nitrogen-fixing genotypes from among the old cultivars, landraces or wild relatives than commercial varieties[44,45]. In another study, field pea landraces introduced from Ethiopian to Germany were found to fix more nitrogen than a commercial cultivar[46]. A similar result was also reported in *Phaseolus vulgaris* in Austria[47]. Our experience with primitive forms of *Pisum sativum* contrarily showed superiority of improved cultivars

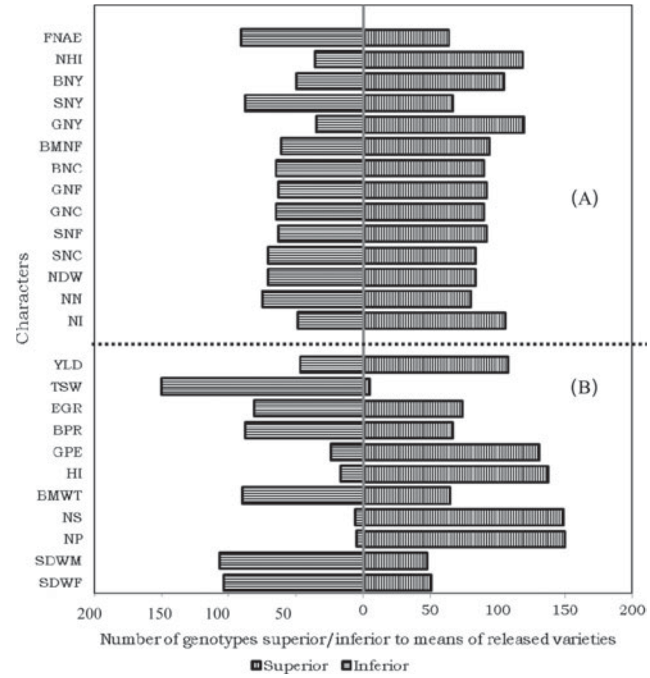

Figure 4. Proportion by number of the 155 chickpea genotypes superior and inferior to mean performances of all the released varieties for (A) symbiotic and (B) agronomic characters, showing superiority of a number of landraces to the standard check (see Table 2 above for abbreviations of the characters).

over the landraces[48]. In chickpea, both nodulation efficiency and grain yield were improved as the result of selection from commercial cultivars[1]. Therefore, specific tests for specific breeding materials, strain and environments may be needed to improve the selection process.

Agronomic performance of the genotypes

Differences among the genotypes were significant for a number of agronomic characters (Table 2). The comparison of the different genotypes with the recently released variety Natoli showed the superiority of a number of landraces for a number of agronomic traits (see supplementary table Annex D). Accordingly, the best 5% of the accessions for yield include accession nos. 41274, 207763, 41111, 207742, 231328, 207563, 41053 and 212589. These accessions recorded yield advantages of 16.62–30.76% over Natoli and 26.84–42.22% over the mean performance of the released varieties. There were many other landraces that were superior for many other traits. The improved cultivars that were originally from exotic sources might be more affected by moisture stress than the primitive landraces that have co-existed with the stress, but the detail will be discussed later. Experience with soybean (*Glycine max*) also showed that exotic of genotypes are usually lower yielding than domestic cultivars when released directly[49]. Nevertheless, no landrace was comparable to the improved genotypes for seed size (Fig. 2), the top 5% of genotypes for this trait being ICC 4918, ICC 5003, ICC 19180, Natoli, Teji, Ejere,

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

Table 5. Mean performances of 155 chickpea genotypes for attributes of symbiotic nitrogen fixation and agronomic performance at two locations in Ethiopia.

Character	Ambo	Ginchi	Mean
Symbiotic characters			
No. of nodules (NN, per 5 plants)	12.33b	14.06a	13.19
Nodule dry weight (NDW, mg per 5 plants)	683.39a	156.99b	420.19
Nodulation index (NI, mg g^{-1})	2.48a	0.38b	1.43
Shoot nitrogen content (SNC,%)	1.19a	1.17a	1.18
Shoot protein content (SPC,%)	7.41a	7.30a	7.36
Shoot nitrogen fixation (SNF,%)	35.77a	24.18b	29.97
Grain nitrogen content (GNC,%)	3.52a	3.51a	3.51
Grain protein content (GPC,%)	21.99a	21.92a	21.95
Grain nitrogen fixation (GNF,%)	35.74a	36.15a	35.95
Total biomass nitrogen content (BNC,%)	4.70a	4.67a	4.69
Total nitrogen fixation (BNF,%)	34.26a	32.22b	33.24
Fixed nitrogen assimilation efficiency (FNAE,%)	77.21b	83.35a	80.28
Grain nitrogen yield (GNY, g per 5 plants)	1.87a	1.83a	1.85
Shoot nitrogen yield (SNY, g per 5 plants)	1.30a	1.20b	1.25
Biomass nitrogen yield (BNY, g per 5 plants)	3.17a	3.03b	3.10
Nitrogen harvest index (NHI)	0.59b	0.61a	0.60
Agronomic characters			
Early vigor (SDWF, g per 5 plants)	30.78b	44.03a	37.4
Shoot dry weight ratio before flowering (SDWRF)	1.02b	1.74a	1.38
Days to 50% flowering (DTF)	55.00b	58.22a	56.61
Days to 90% maturity (DTM)	113.72a	114.41a	114.06
Grain filling period (GFP)	58.72a	56.19b	57.45
No. of pods (NP, per 5 plants)	388.26a	363.20b	375.73
No. of seeds (NS, per 5 plants)	423.72a	419.10a	421.41
Shoot dry weight at maturity (SDWM, g per 5 plants)	109.17a	102.57b	105.87
Shoot dry weight ratio at maturity (SDWRM)	1.55a	1.53b	1.54
Total biomass weight (BMWT, g per 5 plants)	155.16a	148.59b	151.88
Harvest index (HI)	34.63b	35.68a	35.15
Grain production efficiency (GPE, g per 5 plants)	57.1a	51.13b	54.12
Biomass production rate (BPR,%)	136.56a	130.07b	133.32
Economic growth rate (EGR,%)	90.47b	93.20a	91.84
Thousand seed weight (TSW, g)	113.21a	114.38a	113.8
Grain yield (YLD, g per 5 plants)	52.85a	52.20a	52.53

*Values within a row sharing the same letter are statistically non-significant.

Ararti and Habru, which are introductions either from ICRISAT or ICARDA.

The landraces make the majority of the top 5% performers to the improved introductions for many attributes. Some of them, such as accession no. 41274, have displayed superiority for grain yield, pod and seed setting, biomass dry weight and production rate, grain production efficiency and economic growth rate. Another accession, accession no. 207563, was also among the top performers for grain yield, economic growth rate and pod setting. A number of other superior accessions for multiple traits of agronomic significance, including grain yield, were also identified (Table 4).

Detailed physiological reasons for the superiority of the landraces over the improved genotypes may need future study. From our observations, the landraces had relatively shorter period of vegetative growth and longer grain-filling periods. The comparison of the 5% best yielders for phenological characters with the 5% poorest yielders may also show, at least in part, that the genotypes tested here differed in grain yield for a similar reason (Fig. 3). Ideotypes with the faster developmental switch to reproductive growth earlier in the growing season when the soil moisture level is still adequate might have a better comparative advantage to mobilize assimilates and use them more efficiently for reproductive growth. The same mechanism might have provided the landraces with adequately longer period of time for grain filling. A similar observation was made in Ethiopian fenugreek (*Trigonella foenum-graecum*) landraces as compared to a released variety[50]. On the contrary, improved genotypes showed delays in flowering; the consequence may be higher investment in vegetative growth and longer exposure of their reproductive growth to an end-of-season moisture stress. It may be implied, therefore, that landraces may have better structural and functional fitness to survive and reproduce under moisture deficit condition than the improved genotypes.

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

Effect of location

The two locations displayed significant differences for a number of symbiotic and agronomic characters. However, number and size of seeds, shoot and grain nitrogen and protein contents, grain and total nitrogen fixation, grain nitrogen yield and grain yield did not show marked differences between locations (Table 5).

The number of nodules per five plants was higher at Ginchi and lower at Ambo, but nodule weight was heavier at Ambo. As nitrogen fixation was also better at Ambo than it was at Ginchi, this may imply that weight of nodules may be more important (within a limit) for fixation than their mere number. Better fixation at Ambo may also be attributed particularly to the higher level of phosphorus in the soil since phosphorus is of paramount importance in fixation[22].

Genotype by location interaction effects

Genotype by location interaction effects were significant for a number of symbiotic and agronomic characters (Table 2). Significant genotype by location interaction effects were mostly a 'cross-over' type; i.e., interactions were associated with rank order changes among the genotypes (data not shown). This indicated that the two locations were distinctly different for some of the characters and that better genotypes at one location may not also be better performing at another. Even though the inheritance of the process of nodulation was considered to be relatively simple[13], the genetic control of the whole process of symbiotic nitrogen fixation is complicated due to its polygenic nature[51,52]. Plant breeding commonly involves dealing with only a single organism at a time and, while this itself is not simple, breeding for symbiotic nitrogen fixation demands dealing simultaneously with both the host plant and the strain, which interact differently between themselves and with the environment[18]. Most of the traits related to yield and agronomic yield components, nodulation and nitrogen yields showed significant genotype by location interaction. Fortunately, genotype by location interaction effects were non-significant for components related to plant tissue nitrogen contents, including the amount of fixation. This may indicate lesser sensitivity of traits for symbiotic nitrogen fixation to changes in the environment. In our previous study with primitive forms of *P. sativum* native to Ethiopia, we also found similar results[48]. Therefore, genotypes selected at one location for nitrogen (and hence protein) content and fixation levels may perform similarly in other locations.

Conclusions

The present study revealed that the Ethiopian chickpea landraces had considerable significance as sources of genotypes with desirable symbiotic and agronomic characters. Despite the important roles they could have played in chickpea–breeding programs, unfortunately, the potential of the Ethiopian chickpea landraces has not been properly exploited hitherto by the breeding efforts. Almost all of the released varieties currently under production in Ethiopia trace their genetic base to the introductions either from ICARDA or ICRISAT. The accessions selected here could serve as the basis for formulating an efficient scheme of multiple trait selection for both desirable symbiotic and agronomic characters. It can be expected that individual lines developed through selection from among the accessions identified here may result in even better performances than the 'mother' accessions themselves.

The past breeding strategy based on direct release of introduced genotypes has produced special cultivars that are substantially different from commonly grown landraces, particularly in terms of seed size and color. As far as a number of symbiotic and other agronomic merits are concerned, the Ethiopian chickpea landraces should constitute the predominant part in the genetic background of the breeding program. When the desired character does not exist in the available gene pool, as we indicated for seed size, screening of exotic germplasm becomes essential to identify good donor parents. Except for a few accessions, the majority of the landraces did not show a better combination of desirable symbiotic and agronomic characters, which may be of rare natural occurrence. In order to achieve varieties that combine many desirable symbiotic and agronomic attributes, therefore, there is a need to concomitantly incorporate many desirable traits from different sources into a single genotype through hybridization.

Acknowledgements. The authors wish to acknowledge the Ethiopian Institute of Agricultural Research, the International Center for Agricultural Research in the Dry Areas (ICARDA), and Addis Ababa University for material, technical and financial support to this study as a component of the PhD thesis of the first author. The test genotypes were received from the Ethiopian Institute of Biodiversity Conservation, the International Crop Research Institute for the Semi-Arid Tropics, ICARDA and the National Chickpea Research Project in Ethiopia. The inoculum used in this study was kindly provided by the National Soil Analysis Laboratory under Ministry of Agriculture and Rural Development. The authors also sincerely thank staff members of Holetta, Debre Zeit and Ambo Agricultural Research Centers who assisted in trial management and data collection.

References

1 Ali, M.Y., Krishnamurthy, L., Saxena, N.P., Rupela, O.P., Kumar, J., and Johansen, C. 2002. Scope for genetic manipulation of mineral acquisition in chickpea. Journal of Agronomy and Crop Science 191:464–472.

2 Beebe, S.E., Rojas-Pierce, M., Yan, X., Blair, M.W., Pedraza, F., Munóz, F., Tohme, J., and Lynch, J.P. 2006. Quantitative trait loci for root architecture traits correlated

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

with phosphorus acquisition in common bean. Crop Science 46:413–423.
3 Sanchez, P.A. 2002. Soil fertility and hunger in Africa. Science 295:2019–2020.
4 Mamo, T., Haque, I., and Kamara, C.S. 1988. Phosphorus status of some Ethiopian vertisols. In S.C. Jutzi, I. Haque, J. McIntire, and J.E.S. Stares (eds). Management of Vertisols in Sub-Saharan Africa. Proceedings of a Conference, August 31–September 4, 1987, ILCA, Addis Ababa, Ethiopia. p. 232–252.
5 Tsegaye, T. 1992. Vertisols of the Central Highlands, Ethiopia – characterization, classification and evaluation of the phosphorus status. MSc Thesis, Alemaya University of Agriculture, Ethiopia.
6 Hirsch, A.M. 2009. Brief history of the discovery of nitrogen-fixing organisms. Available at Web site http://www.mcdb.ucla.edu/Research/Hirsch/imagesb/HistoryDiscoveryN2fixingOrganisms.pdf (accessed November 2010).
7 Adjei-Nsiah, S., Kuyper, T.W., Leeuwis, C., Abekoe, M.K., Cobbinah, J., Sakyi-Dawson, O., and Giller, K.E. 2008. Farmers' agronomic and social evaluation of productivity, yield and N_2-fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. Nutrient Cycle and Agroecosystem 80:199–209.
8 Romdhane, S.B., Aouani, M.E., Trabelsi, M., de Lajudie, P., and Mhamdi, R. 2008. Selection of high nitrogen-fixing rhizobia nodulating chickpea (*Cicer arietinum*) for semi-arid Tunisia. Journal of Agronomy and Crop Science 194:413–420.
9 Sharma, R. and Behera, U.K. 2009. Recycling of legume residues for nitrogen economy and higher productivity in maize (*Zea mays*)–wheat (*Triticum aestivum*) cropping system. Nutrient Cycling in Agroecosystems 83:197–210.
10 Smith, A.P., Chen, D., and Chalk, P.M. 2009. N_2 fixation by faba bean (*Vicia faba* L.) in a gypsum-amended sodic soil. Nutrient Cycling in Agroecosystems 45:329–333.
11 Yusuf, A.A., Iwuafor, E.N.O., Abaidoo, R.C., Olufajo, O.O., and Sanginga, N. 2009. Grain legume rotation benefits to maize in the northern Guinea savannah of Nigeria: fixed-nitrogen versus other rotation effects. Nutrient Cycling in Agroecosystems 84:129–139.
12 Gorfu, A. 1998. The role of fertilizers and faba bean (*Vicia faba*) in sustaining production of cereals in different crop rotations in the South-eastern Ethiopian Highlands. Doctoral Dissertation, Georg-August-Universität Göttingen, Germany.
13 Rengel, Z. 2002. Breeding for better symbiosis. Plant and Soil 245:147–162.
14 Jensen, E.S. and Hauggaard-Nielsen, H. 2003. How can increased use of biological N_2 fixation in agriculture benefit the environment? Plant and Soil 252:177–186.
15 Hardarson, G. and Atkins, C. 2003. Optimising biological N_2 fixation by legumes in farming systems. Plant and Soil 252:41–54.
16 Pearson, C.J., Norman, D.W., and Dixon, J. 1995. Sustainable Dryland Cropping in Relation to Soil Productivity – FAO Soils Bulletin 72. Food and Agriculture Organization of the United Nations (FAO), Rome.
17 Dakora, F.D. and Keya, S.O. 2000. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. Soil Biology and Biochemistry 29:809–817.
18 Crouch, J.H., Buhariwalla, H.K., Blair, M., Mace, E., Jayashree, B., and Serraj, R. 2004. Biotechnology based contributions to enhancing legume productivity in resource poor areas. In R. Serraj (ed.). Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture. Oxford & IBH Publishing, New Delhi. p. 47–65.
19 Shiferaw, B., Bantilan, M.C.S., and Serraj, R. 2004. Harnessing the potentials of BNF for poor farmers: Technological, Policy and Institutional constraints and research needs. In R. Serraj (ed.). Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture. Oxford & IBH Publishing, New Delhi. p. 3–27.
20 Hardarson, G. 2004. Enhancement of symbiotic nitrogen fixation in grain legumes: selected results from the FAO/IAEA program. In R. Serraj (ed.). Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture. Oxford & IBH Publishing, New Delhi. p. 163–171.
21 Kassie, M., Shiferaw, B., Asfaw, S., Abate, T., Muricho, G., Ferede, S., Eshete, M., and Assefa, K. 2009. Current situation and future outlooks of the chickpea sub-sector in Ethiopia. ICRISAT and EIAR. Available at Web site http://www.icrisat.org/tropicallegumesII/pdfs/Current_Situation.pdf (accessed November 2010).
22 Shukla, U.C. and Yadav, O.P. 2000. Effect of phosphorus and zinc on nodulation and nitrogen fixation in chickpea (*Cicer arietinum* L.). Plant and Soil 65:239–248.
23 Broughton, W.J., Zhang, F., Perret, X., and Staehelin, C. 2003. Signals exchanged between legumes and *Rhizobium*: agricultural uses and perspectives. Plant and Soil 252:129–137.
24 Lindemann, W.C. and Glover, C.R. 2003. Nitrogen Fixation by Legumes. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State University, Mexico.
25 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 K. Ali, G. Keneni, S. Ahmed, R. Malhotra, S. Beniwal, K. Makkouk, and M.H. Halila (eds). Food and Forage Legumes of Ethiopia: Progress and prospects. Proceedings of a Workshop on Food and Forage Legumes, September 22–26, 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria. p. 135–145.
26 Hailemariam, A. and Tsige, A. 2006. Biological nitrogen fixation research on food legumes in Ethiopia. In K. Ali, G. Keneni, S. Ahmed, R. Malhotra, S. Beniwal, K. Makkouk, and M.H. Halila (eds.). Food and Forage Legumes of Ethiopia: Progress and Prospects. Proceedings of a Workshop on Food and Forage Legumes, September 22–26, 2003, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria. p. 172–176.
27 Chen, Q., Zhang, X., Terefework, Z., Kaijalainen, S., Li, D., and Lindström, K. 2003. Diversity and compatibility of peanut (*Arachis hypogaea* L.) bradyrhizobia and their host plants. Plant and Soil 255:605–617.
28 Giongo, A., Luciane, M.P., João, P., Freire, R.J., and de Sá, E.L.S. 2007. Genetic diversity and symbiotic efficiency of population of rhizobia of *Phaseolus vulgaris* L. in Brazil. Biology and Fertility of Soils 43:593–598.
29 Shantharam, S. and Mattoo, A.K. 1997. Enhancing biological nitrogen fixation: An appraisal of current and

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press

alternative technologies for N input into plants. Plant and Soil 194:205–216.

30 Krasilnikoff, G., Gahoonia, T., and Nielsen, N.E. 2003. Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere processes. Plant and Soil 251:83–91.

31 Walley, F.L., Kyei-Boahen, S., Hnatowich, G., and Stevenson, C. 2005. Nitrogen and phosphorus fertility management for desi and kabuli chickpea. Canadian Journal of Plant Science 85:73–79.

32 Bejiga, G. 2004. Current status of food legume production and use of biological nitrogen fixation in Ethiopia. In R. Serraj (ed.). Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture. Oxford & IBH Publishing, New Delhi. p. 263–266.

33 Liao, M., Hocking, P.J., Dong, B., Delhaize, E., Richardson, A.E., and Ryan, P.R. 2008. Variation in early phosphorus-uptake efficiency among wheat genotypes grown on two contrasting Australian soils. Australian Journal of Agricultural Research 59:57–166.

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

35 Pimratch, S., Jogloy, S., Vorasoot, N., Toomsan, B., Patanothai, A., and Holbrook, C.C. 2008. Relationship between biomass production and nitrogen fixation under drought-stress conditions in peanut genotypes with different levels of drought resistance. Journal of Agronomy and Crop Science 194:15–25.

36 IBPGR, ICRISAT, and ICARDA. 1993. Descriptor for Chickpea (*Cicer arietinum* L.). IBPGR, Rome, Italy/ ICRISAT, Patancheru, India/ICARDA, Aleppo, Syria.

37 Little, T.M. and Hills, F.J. 1978. Agricultural Experimentation: Design and Analysis. John Wiley and Sons, New York.

38 Gomez, K.A. and Gomez, A. 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons, New York.

39 Doughton, J.A., Saffigna, P.G., Vallis, I., and Mayer, R.J. 1995. Nitrogen fixation in chickpea: II. Comparison of ^{15}N enrichment and ^{15}N natural abundance methods for estimating nitrogen fixation. Australian Journal of Agricultural Research 46:225–236.

40 PEDO. 1997. National Atlas of West Shewa. Planning and Economic Development Office (PEDO), West Shewa Administrative Zone, Ministry of Economic Development and Cooperation, Addis Ababa, Ethiopia.

41 Jones, J.B. 2003. Agronomic Handbook: Management of Crops, Soils and their Fertility. Corner Research Center, USA.

42 Dibabe, A., Bekele, T., and Mamo, T. 2001. Nutrient management in highland vertisols of Ethiopia. In P. Dubale, A. Dibabe, A. Zeleke, G. Ayele, and A. Kirub (eds). Advances in Verisols Management in Ethiopian Highlands. Proceedings of the International Symposium on Vertisols Management, November 28–December 1, 2001, Debre Zeit, Ethiopia. Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopa. p. 81–98.

43 Beck, D.P. and Rupela, O.P. 1996. Symbiotic nitrogen fixation in chickpea in WANA and SAT. In N.P. Saxena, M. C. Saxena, C. Johansen and H. Harris (eds). Adaptation of Chickpea in the West Asia and North Africa Region. ICRISAT, Patancheru, India. ICRDA, Aleppo, Syria. p. 207–216.

44 Sinclair, T.R. and Vadez, V. 2002. Physiological traits for crop yield improvement in low N and P environments. Plant and Soil 245:1–15.

45 Provorov, N.A. and Tikhonovich, I.A. 2004. Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. Genetic Resources and Crop Evolution 50:89–99.

46 Adgo, E. and Schulze, J. 2002. Nitrogen fixation and assimilation efficiency in Ethiopian and German pea varieties. Plant and Soil 239:291–299.

47 de Oliveira, W.S., Meinhardt, L.W., Sessitsch, A., and Tsai, S.M. 1998. Analysis of *Phaseolus–Rhizobium* interactions in a subsistence farming System. Plant and Soil 204:107–115.

48 Keneni, G., Assefa, F., Imtiaz, M., Bekele, E., and Regassa, H. 2011. Genetic diversity for attributes of biological nitrogen fixation in Abyssinian field pea (*Pisum sativum* var. Abyssinicum) germplasm accessions. Journal of Genetics and Breeding (under review).

49 Sneller, C.H., Nelson, R.L., Carter, T.E. Jr, and Cui, Z. 2005. Genetic diversity in crop improvement: The soybean experience. In M.S. Kang (ed.). Genetic and Production Innovations in Field Crop Technology: New Developments in Theory and Practice. International Book Distributing Co., Delhi, India. p. 103–144.

50 Fikresilassie, M. 2008. Genetic divergence for morpho-physiological and nodulation traits and their associations in Ethiopian fenugreek (*Trigonella foenum-graecum* L.) landraces. MSc thesis, Haramaya University, Ethiopia.

51 Austin, R.B. 1993. Augmenting yield-based selection. In M.D. Hayward, N.O. Bosemark, and I. Romagosa (eds). Plant Breeding: Principles and prospects, Chapman and Hall, Great Britain. p. 391–405.

52 Tate, R.L. 2000. Soil Microbiology. 2nd ed. John Wiley & Sons, New York.

https://doi.org/10.1017/S1742170512000221 Published online by Cambridge University Press