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Genetic Diversity and Population Structure of Ethiopian Chickpea (*Cicer arietinum* L.) Germplasm Accessions from Different Geographical Origins as Revealed by Microsatellite Markers

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Abstract Genetic diversity and population structure were studied in 155 chickpea (*Cicer arietinum* L.) entries using 33 polymorphic microsatellite (SSR) markers. Molecular analysis of variance showed variations of 73% within and 27% among populations. Introduced genotypes exhibited highest polymorphism (70.27%) than the landraces (36–57%). Collections from Shewa, Harerge, W. Gojam and S. Gonder regions also showed higher polymorphism (50–57%) than the rest of the local accessions (36–45%). Analyses of pairwise population Nei genetic distance and PhiPT coefficients, expected heterozygosity (He) and unbiased expected heterozygosity (UHe), Shannon's information index (I) and percent polymorphism (% *P*) showed existence of high genetic variation between geographical regions. Accessions from adjoining geographical regions mostly showed more genetic similarities than those from origins far isolated apart. This could be associated with the ease and likelihood of inter-regional gene flow and seed movement particularly during times of drought. The 155 entries were grouped into five clusters following analysis of population structure. The first cluster (C_1) constituted accessions from Arsi; the second (C_2) from Gojam and Gonder; the third (C_3) from Harerge and E. and N. Shewa; and the fourth (C_4) from W. Shewa, Tigray, and Wello regions. The fifth cluster (C_5) was entirely consisted of improved genotypes. Improved genotypes of both Kabuli and Desi types distinctly fell into cluster five (C_5) regardless of their difference in seed types. The result has firmly established that introduction of genetic materials from exotic sources has broadened the genetic base of the national chickpea breeding program. Further implications of the findings as regards to chickpea germplasm management and its utilization in breeding program are also discussed.

Keywords Chickpea · *Cicer arietinum* · Genetic diversity · Microsatellite markers · Molecular analysis of variance · Population structure

Introduction

The region in the present day South-eastern Turkey and the adjoining areas of Syria has been proposed to be the Vavilovian center of origin for the cultivated chickpea (*Cicer arietinum* L.; van der Maesen 1987). Archeological evidences also suggest that chickpea was first domesticated in Turkey before the late Neolithic period (as early as 3,500 BC; Tanno and Willcox 2006). By the Bronze Age (3,300–1,200 BC), it is believed that chickpea was already grown in proximities of its origin in the Middle East

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including in Egypt and in some places outside the Middle East (van der Maesen 1987). The crop was perhaps diffused by the Spanish and Portuguese travelers (van der Maesen 1987). As early as 1520 BC, chickpea was known to be grown in Ethiopia (Joshi et al. 2001) and Ethiopia is now considered as the Vavilovian secondary center of genetic diversity for chickpea (van der Maesen 1987). Wild relatives, particularly *Cicer cuneatum*, are known to exist in numerous regions in Ethiopia (Taddesse et al. 1994).

Based on differences in seed types, the cultivated chickpeas are distinguished as *Desi* and *Kabuli* types. The *Desi* types have small darker multicolored seeds with a rough seed coat while the *Kabuli* types have larger beige to white colored seeds with smoother seed coat. Existence of a pea-shaped third type characterized by medium to small seed size and creamy color has also been recognized (Upadhyaya et al. 2008), which may be the result of intercrossing between *Desi* and *Kabuli* types that has resulted in a sort of intermediate group (Muehlbauer and Tullu 1997). Currently, about 75% of the area all over the world is covered by the *Desi* and the remaining 25% by the *Kabuli* types (Kassie et al. 2009). The main producers of the *Desi* types are India, Pakistan, and Ethiopia, while Mexico, Iran, Afghanistan, Spain, and Chile are of the *Kabuli* types (Upadhyaya et al. 2008; Kassie et al. 2009).

Ethiopia, as the secondary center of genetic diversity for many food legumes in general (Hagedorn 1984; Mekibeb et al. 1991) and chickpea in particular (van der Maesen 1987), possesses a large number of chickpea germplasm collections from different geographical regions (Tanto and Tefera 2006). For effective utilization of these germplasm collections in breeding programs, genetic characterization in terms of measure of the extent and pattern of genetic diversity within and between populations (Rubenstein et al. 2005), is essential (Carvalho 2004) not only to unveil the magnitude of genetic diversity available in the germplasm for conservation purposes but also to determine genes useful for possible progress in future breeding programs. Screening and selection would be more likely result in better and promising genotypes if germplasm sources were genetically diverse. Crossing is also likely to produce higher heterosis, desirable genetic recombination and segregation in progenies when it is made between genetically diverse parents.

Genetic characterization can be made by different methods ranging from the conventional methods like the use of descriptor lists of morphological characters and biochemical and molecular methods (Carvalho 2004; de Vicente et al. 2005), all with their own comparative advantages and disadvantages. However, it is generally believed that the use of molecular markers is more reliable and repeatable as compared to characterization based on morphological characters (Carvalho 2004). Markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) have been in use to study the genetic diversity and relationships in chickpea (Upadhyaya et al. 2008). A number of studies reported high morphological diversity but limited genomic variability (Millan et al. 2006; Upadhyaya et al. 2008), the reason at least in part could be attributed to the "founder effect" of the monophyletic descendence of chickpea from its wild progenitor, *Cicer reticulatum* (Abbo et al. 2003). High level of genetic diversity has recently been revealed with the advent of SSR markers (Winter et al. 1999; Millan et al. 2006; Upadhyaya et al. 2006; Upadhyaya et al. 2008) suitable for studies of genetic diversity and cultivar identification in crops like chickpea (Imtiaz et al. 2008; Saeed et al. 2011).

Despite the large number of chickpea germplasm collections held in Ethiopia, most of them have not been characterized at either morphological or molecular levels (Tanto and Tefera 2006). From a few morphological, biochemical (isozyme) and RAPD marker-based studies in Ethiopia, it has been reported that there exists high morphological but low biochemical and molecular diversity (Workeye 2002; Dadi 2004). Another study was also conducted based on SSR markers but was limited to a few varieties released in Ethiopia and additional varieties released in eight other countries (Sefera et al. 2011). However, the results could hardly be inferred for Ethiopian germplasm accessions because of less representation in terms of both number and geographical coverage of accessions. This study was, therefore, designed to assess genetic diversity and population structure of Ethiopian chickpea germplasm accessions at the molecular level using SSR markers and, thereby, establish if there is any definite relationship between genetic diversity and geographical sources of origin.

Materials and Methods

Plant Materials

One-hundred fifty five chickpea entries were considered for the study. While 139 of these are Ethiopian germplasm accessions collected from the major chickpea production areas (Kassie et al. 2009) ranging in altitude from 1,220 to 3,120 m above sea level, eight are nationally released varieties and the other eight are breeding lines introduced, for some agronomic merits, from the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). The local accessions thus represent over 12% of the 1,150 (Tanto and Tefera 2006) chickpea

germplasm holding of the Ethiopian Institute of Biodiversity Conservation. Each germplasm collection, even though considered a single population, represents a bulk of different individuals or populations which are genetic pools treated hereafter as genotypes only for experimental or practical purposes. Descriptions of the test entries are given in Table 1 along with the map of the geographical regions of origins of the Ethiopian material in Fig. 1. The genotypes from specific origins (Arsi, East Gojam, West Gojam, North Gonder, South Gonder, West Harerge, East Shewa, North Shewa, West Shewa, Tigray, Wello and introductions) were considered as belonging to 12 initial groups for reclassification into genetic populations.

DNA Extraction

About 20 seeds of each of the entries were planted in pots on sterile sand in the growth chamber under regulated conditions at ICARDA. Two weeks after planting, approximately equal amount of bulk leave samples were collected from five to ten plants of each entry as suggested by Gilbert et al. (1999). About 100 mg of fresh leaves were placed in 2 ml autoclaved and labeled Eppendorf tubes, covered by paraffin paper with a small slot at one side for air circulation and freeze dried for 3 days at −80°C. The samples were ground using a mixer mill (Retsch MM® 200) and genomic DNA was extracted using the cetyltriethylammonium bromide (CTAB) method (Doyle and Doyle 1990) with some minor modifications. Then the DNA pellet was air dried and dissolved in 100 µl of 1× TE by slightly shaking until the pellet gets away from the bottom of the Eppendorf tube.

Procedure of Polymerase Chain Reaction

The amount of DNA in each sample was quantified on agarose gel (1%) and optimized for polymerase chain reactions (PCR) using a lambda DNA standard, pUC 19 (50 µg). About 50 simple sequence repeat (SSR) markers distributed throughout the chickpea genome and previously reported to be polymorphic with other chickpea genetic materials including world collections were selected from published sequences and genetic maps (Hüttel et al. 1999; Winter et al. 1999; Winter et al. 2000; Millan et al. 2006; Sethy et al. 2003; Radhika et al. 2007; Upadhyaya et al. 2008; Nayak et al. 2010) as described in Table 2. PCR reaction was performed with a thermal cycler (GeneAmp®PCR System 9700) in a total volume of 10 µl containing 50 ng of DNA, 1 µl of dNTPs, 1 µl of buffer 10×, 1 µl of forward and reverse primer (unlabelled) and

Table 1 Passport description of the test genotypes

Region	Zone	No of genotypes (serial No.)	Name of genotypes	Altitude (masl)
Oromiya	Arsi	13 (1–13)	Acc. nos. 231327, 231328, 209093, 208829, 209094, 209092, 209096, 209097, 209098, 41002, 207761, 207763, 207764	1540–2080
Amahara	East Gojam	13 (14–26)	Acc. nos. 41268, 41026, 41074, 41075, 41073, 41076, 41021, 41027, 41222, 207734, 41103, 41320, 41029	1770–2880
Amahara	West Gojam	13 (27–39)	Acc. nos. 41015, 41271, 41272, 41276, 207745, 41275, 41277, 207743, 207744, 41273, 41274, 207741, 207742	1880–2360
Amahara	North Gonder	13 (40–52)	Acc. nos. 41316, 41298, 41311, 41313, 41280, 41312, 41315, 41308, 41299, 41046, 41047, 41304, 41303	1900–2710
Amahara	South Gonder	12 (53–64)	Acc. nos. 41295, 41296, 41289, 41290, 41284, 41291, 41297, 41293, 41019, 41048, 41049, 41053	1820–3120
Oromiya	West Harerge	11 (65–75)	Acc. Nos. 41054, 41052, 209082, 209083, 209084, 209091, 209087, 209088, 209089, 209090, 209081	1500–2130
Oromiya	East Shewa	13 (76–88)	Acc. nos. 41159, 41160, 41161, 207661, 207667, 207666, 41141, 207665, 41134, 41128, 41168, 41129, 41130	1850–2190
Amara/Oromiya	North Shewa	13 (89–101)	Acc. nos. 41110, 207657, 41111, 41106, 207658, 41142, 41207, 41215, 41216, 41066, 41011, 41007, 41008	1220–2700
Oromiya	West Shewa	13 (102–114)	Acc. nos. 41186, 209035, 41176, 41175, 41174, 209027, 41170, 41171, 41185, 209036, 41190, 41195, 41197	1960–2230
Tigray	South, Central, East and West Tigray	12 (115–126)	Acc. nos. 207150, 207151, 207563, 207564, 207894, 207895, 213224, 219797, 219799, 219800, 219803, 221696	1880–2600
Amahara	South Wello	13 (127–139)	Acc. nos. 41114, 212589, 41113, 207659, 207660, 41115, 225878, 225873, 225874, 225877, 207645, 207646, 225876	1560–2540
Improved	India, ICRISAT and ICARDA	16 (140–155)	ICC 5003, ICC 4918, ICC 4948, ICC 4973, ICC 15996, Shasho (ICCV 93512), Arerti (FLIP 89–84 C), Worku (DZ-10-16-2), Akaki (DZ-10-9-2), Ejere (FLIP-97–263 C), Teji (FLI 97–266 C), Habru (FLIP 88-42c), Natoli (ICCX-910112-6), ICC 19180, ICC 19181, PM 233 (155)	–

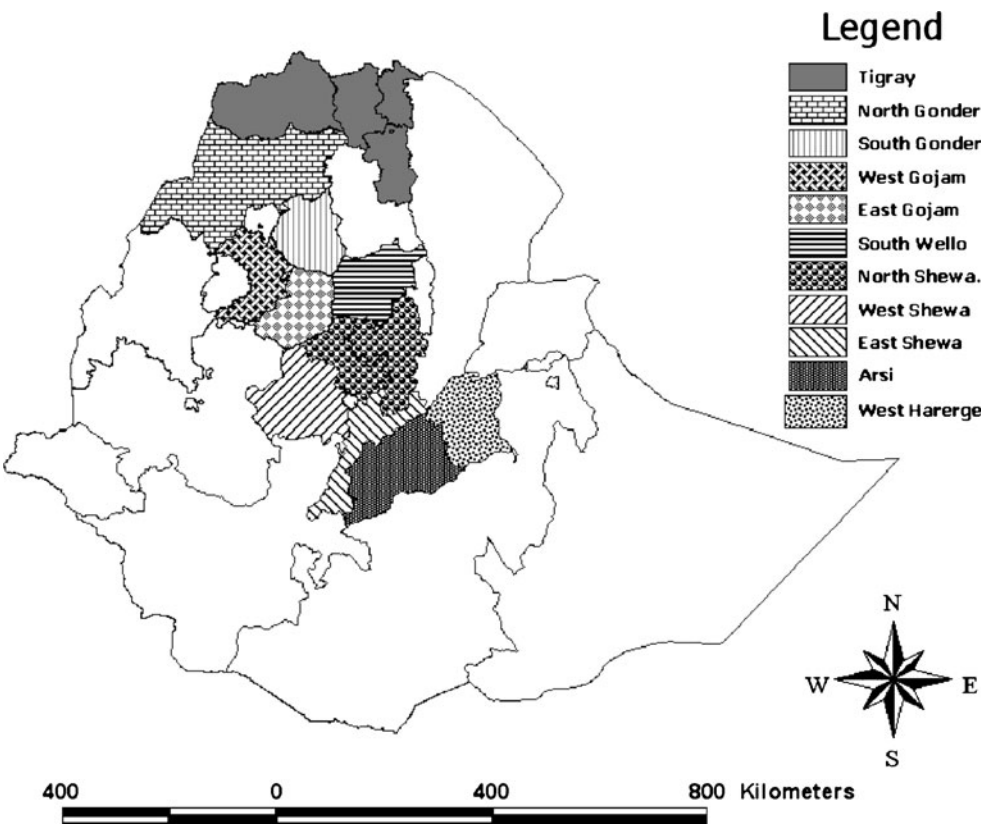

Fig. 1 Map of Ethiopia showing the approximate areas of origins (*shaded region*) of the 139 germplasm accessions (NB: all boundaries are approximate and nothing to do with political borders)

0.1 µl of Taq DNA polymerase in 4.9 µl of dH_2O. The PCR was programmed at an initial denaturation step of 2 min at 94°C followed by 35 cycles of 20 s denaturation at 94°C, annealing at 55 or 60°C (depending on the primer) for 50 s, initial extension at 72°C for 50 s and final extension at 72°C for 7 min. PCR amplification for primers NCPGR 21, NCPGR 33, NCPGR45, NCPGR 53, NCPGR 94, NCPGR 98, NCPGR 99, and NCPGR 100 was carried out using "touchdown" methodology.

Samples were loaded on 8% polyacrylamide gel with a 6× DNA loading dye. Electrophoresis was carried out on a vertical electrophoresis set up (EPS-300 IIV) using a standard DNA ladder (GeneRuler ™ 50 bp) with known reference bands. The gel was shaken in Ethidium bromide (on SK-71 shaker) for 20 min and the amplification was visualized under UV illumination X-ray (Alphalmager® HP). Primers with unclear and missing bands were sorted and repeated. Non-polymorphic, missing, faint and distorted gels (in this specific case) were disregarded at scoring and only records of 33 primers with clear polymorphic bands were considered for statistical analysis (Table 2).

Data Collection and Analysis

The amplified products were visually scored using binary numbers (1 for presence of band and 0 for absence) from the gel photographed under UV illumination (Warburton and Crossa 2002; Saeed et al. 2011). The data from all entries was combined for statistical analysis. The software GeneAlex version 6 (Peakall and Smouse 2006) was used for analysis of molecular variance (AMOVA) to determine the following genetic parameters.

$$\text{Average PhiPT} = \frac{V_{AP}}{V_{WP}+V_{AP}} = \frac{V_{AP}}{V_T}$$

where PhiPT coefficient denotes the proportion of the variance among populations relative to the total variance, V_{AP} is the variance among populations, V_{WP} the variance within populations and V_T is the total variance or the sum of $V_{AP} + V_{WP}$.

Pairwise between populations $\text{PhiPT} = \frac{V_{AP}+V_{AR}}{V_{WP}+V_{AP}+V_{AR}} = \frac{V_{AP}+V_{AR}}{V_T}$, where PhiPT, V_{AP} and V_{WP} are as mentioned above; V_{AR} stands for variance between geographical regions and V_T is the total variance or the sum of $V_{AP} + V_{WP} + V_{AR}$.

Expected and unbiased expected heterozygosity values were estimated based on Nei (1978) assuming each band to correspond with a single bi-allelic locus but genetic interpretation of individuals as heterozygous/homozygous for a particular locus was not attempted throughout because each sample from an accession constituted of a bulk of leaves from different individual plants. $He = 1 - \Sigma p^2$, where He is the expected heterozygosity, assuming

Table 2 Description (denomination, sequence, repeats, fragment size, linkage group and number of alleles) of the polymorphic microsatellite markers used in the analysis of the chickpea genotypes

Microsatellite	Primer sequences		Repeat	Fragment size (bp)	Linkage Group[a]	No of allele
	Forward	Reverse				
GA 11	GTTGAGCAACAAAGCCACAA	TTCTTGTCTGGTTGTGTGAGC	(CT)21	159	1/2/6	3
GA 24	TTGCCAAAACCAATAACTCTG	TCCCTTTTACACAAGGCCAG	(GA)19	203	1/2/4	2
NCPGR 45	TGTTTTCAAATCAAACAGGC	GATACACACCAAGGCACAGT	(CT)2GTCAT(CT)5CC(CT)2CC(CT)17	223	2	1
NCPGR 53	CCCTCCTTCTTGCTTACAAA	TAATGGTGAACGAATCATGG	(CT)5CA(CT)CA(CT)10CA(CT)4 CA(CT)TA(CT)4 GTCA(CT)12	194	1	2
TA 144	TATTTTAATCCGGTGAATATTACCTTT	GTGGAGTCACTATCAACAATCATACAT	(TAA)27	241	6	5
NCPGR 94	GGTTTGATGTGTTCTTGGCT	CCCTCAATTCCCTCGATTTA	(CT)25	176	5	1
NCPGR 100	CCATTTTCTACAATCTCATGTCT	GTAGAAAGAGCCAAGAGGCA	(CT)15 N42(CT)2CC(CT)5TT(CT)6AT(CT) 7	263	1	2
TA 18	AAAATAATCTCCACTTCACAAATTTTC	ATAAGTGCGTTATTAGTTTGGTCTTGT	(TAA)24	147	5/7	6
CaSTMS 11	GTATCTACTTGTAATATTCTCTTCTCT	ATATCATAAACCCCCCAC	(GA)20	232	4	2
TA 59	ATCTAAAGAGAAATCAAAATTGTCGAA	GCAAATGTGAAGCATGTATAGATAAAG	(TAA)29	258	2/7	4
TA 1	TGAAATATGGAATGATTACTGAGTGAC	TATTGAAATAGGTCAGGCTTATAAAAA	(TAA)32	243	1	5
TA 27	GATAAAATCATTATTGGGTGTCCTTT	TTCAAATAATCTTTCATCAGTCAAATG	(TAA)21	241	2/7	4
TA 37	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTCA	(TTA)20	282	2/3/7	6
TA 76	TCCTCTTCTTCGATATCATCA	CCATTCTATCTTTGGTGCTT	(AAT)7 (AAT)4[ACT(AAT)11]2ACT(AAT)3 TAT(AAT)2(ATT)5	206	3	4
TA 110	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA	(TTA)22	220	2	6
TR 1	CGTATGATTTTGCCGTCTAT	ACCTCAAGTTCTCCGAAAGT	(TAA)31	224	6	5
TR 2	GGCTTAGAGTTCAAAGAGAGAA	AACCAAGATTGGAAGTTGTG	(TTA)36	210	3	3
TR 3	GAAGTATCAGTATCACGTGTAATTCGT	CTTACGGAGAACATGAACATCAA	(TTA)27	244	6/10/13	4
TR 7	GCATTATTCACCATTTGGAT	TGTGATAATTTTCTAAGTGTTTT	(TTA)25	204	6	6
TR 19	TCAGTATCACGTGTAATTCGT	CATGAACATCAAGTTCTCCA	(TAA)27	227	2	5
TR 29	GCCCACTGAAAAATAAAAAG	ATTTGAACCTCAAGTTCTCG	(TAA)8TAGTAATAG(TAA)32	220	1/5	7
TR 43	AGGACGAAACTATTCAAGGTAAGTAGA	AATTGAGATGGTATTAAATGGATAACG	(TAA)24	297	1/2	3
TS 19	TTTCTTTTGTTAGAGTTAAAAAAAATT	TCTCATGTTTTGCTTTTTATTATTATTA	(TAA)27	117	1/3/5	n.a[b]
NCPGR 21	TCTACCTCGTTTTTCGTGCC	TTGCTCCTTCAACAAAACCC	(CT)15	137	2	2
TA 11	CATGCCATAAACTCAATACAATACAAC	TTCATTGAGGACAATGTGTAATTTAAG	(TTA)17	230	n.a	6
NCPGR 33	ACATCTTGAAGTGCCCC	TGCAAGCAGACGGTTACAAG	(GA)20	248	4	1
NCPGR 98	CATCTTATTTTTCATTTTAGAGGAGG	AGGAAGTGTTATGGAGATGCC	(GA)20GG(GA)14	141	2	1
NCPGR 99	ATCATGAAGCAAATCCTCAC	TGAACCCAACATAGCATACA	(GA)18	227	n.a	1
TA 117	GAAAATCCCAAATTTTTCTTCTTCT	AACCTTATTTAAGAATATGAGAAACACA	(ATT)52	248	5	5
TA 5	ATCATTTCAATTTCCTCAACTATGAAT	TCGTTAACACGTAATTTCAAGTAAAGAT	(TTA)29	205	1/3/5	5
TA 64	ATATATCGTAACTCATTAATCATCCGC	AAATTGTTGTCATCAAATGGAAAATA	(TAA)39	239	1/3	8
TA 72	GAAAGATTTAAAAGATTTTCCACGTTA	TTAGAAGCATATTGTTGGGATAAGAGT	(ATT)36	256	2/4	7
TS 35	GGTCAACATGCATAAGTAATAGCAATA	ACTTTCGCGATTCAGCTAAAATA	(TAA)9 T(A)3(TAA)13	247	1/5	2

[a] Different markers located on different linkage groups as reported by different investigators are separated by *slash*

[b] *n.a* not available

Hardy–Weinberg Equilibrium where an amplified allele (band present) has frequency $p = 1 - q$, a null allele (band absent) has frequency $q = 1 - p$, frequency of genotype with null allele is q^2=frequency of absence=1−frequency of presence and q=√(frequency of absence).

$$\mathrm{UHe} = \frac{2N}{2N-1} \times \mathrm{He}$$

where UHe is unbiased expected heterozygosity and N is number of population.

$$I = -1 \times [p \times \ln(p) + q \times \ln(q)]$$

Where I Shannon's information index and p and q as given above.

$$\%\, P = \frac{\mathrm{NPL}}{\mathrm{TNL}} \times 100$$

where % P is percent polymorphism, NPL is number of polymorphic bands and TNL is total number of bands.

The polymorphic information content (PIC) values were estimated employing the method suggested by Roldan-Ruiz et al. (2000) as:

$$\mathrm{PIC}_i = 2f_i(1 - f_i)$$

where f_i is the frequency of the amplified allele (band present) and $(1-f_i)$ is the frequency of the null allele (band absent) of marker i.

$$\mathrm{GD} = -\ln(\mathrm{GI}), \mathrm{GI} = \frac{Jxy}{\sqrt{(\mathrm{Jx} \times \mathrm{Jy})}},$$

$$\mathrm{J_{xy}} = \sum_{i=1}^{k} p_{\mathrm{ix}} \times p_{\mathrm{iy}}, \quad \mathrm{J_x} = \sum_{i=1}^{k} p^2{}_{\mathrm{ix}} \text{ and } \mathrm{J_y} = \sum_{i=1}^{k} p^2{}_{\mathrm{iy}}$$

where GD is Nei's genetic distance, GI is Nei's genetic identity, P_{ix} and P_{iy} are the frequencies of ith allele (band present) in populations x and y.

The population structure of the genotypes was defined by the Bayesian model-based clustering method of Pritchard et al. (2000) using the Structure 2.2 software assuming population admixture through inferred ancestry. For each of the K=2 to K=12 settings, 20 independent simulations were performed using the admixture model and 5,000 replicates for burn-in and post burning sampling by Markov Chain Monte Carlo of 50,000 runs to estimate the number of subpopulations for each of the K values. The appropriate number of clusters (K) was determined according to Evanno et al. (2005).

Results and Discussion

Magnitude of Genetic Diversity

Molecular analysis of variance showed a 73% within and 27% among populations variations (Table 3). Saeed et al. (2011) evaluated diversified populations of chickpea involving cultigens, landraces, internationally developed improved lines and wild relatives and found relatively lower within population variance of 59% and higher among population variance of 41% as compared to the present results. The relatively higher among population variance they obtained could be attributed to the presence of wild relatives included in their study. Some reports indicate that the level of polymorphism depends on the type of germplasm (He et al. 2011), marker used (Baraket et al. 2011; Sharma et al. 2011), primers selected (Kong et al. 2011; Sharma et al. 2011) and the sampling strategy (Kong et al. 2011).

The degree of polymorphism among the populations varied from 36.04% for the collections from E. Gojam to 70.27% for the improved genotypes, the average being 49.77% (Table 4). Visual observations on the gels of the specific markers also revealed the existence of more polymorphism in the introduced genotypes than in the landraces (Fig. 2). Chickpea, being a diploid (van der Maesen 1987) and strictly self-pollinated plant (Muehlbauer and Tullu 1997), the existence of accessions with more than two bands imply that such accessions constituted of different individuals (Fig. 2).

It appears that the level of polymorphism among the local collections increases as one goes from the southwest (Arsi) and north (Tigray and Wello) towards the center

Table 3 Analysis of Molecular Variance (AMOVA) showing the distribution of genetic diversity within and among populations of chickpea entries from different sources of origins

Source of variation	*df*	SS	MS	Variance		Statistic	Value	*P*
				Estimated	%			
Among populations (AP)	11	602.357	54.76	3.505	27			
Within population (WP)	143	1361.57	9.521	9.521	73	PhiPT	0.269	0.010
Total (TOT)	154	1963.92	–	13.026	100			

df degrees of freedom, *SS* sum of square, *MS* mean of squares

Table 4 Summary of parameters for genetic diversity in chickpea populations from different sources

Geographical region of origin	No of entries	Expected heterozygosity (He)	Unbiased expected heterozygosity (UHe)	Shannon's information index (*I*)	% *P*
Arsi	13	0.118±0.017	0.122±0.018	0.179±0.025	38.74
E. Gojjam	13	0.086±0.014	0.089±0.015	0.137±0.021	36.04
W. Gojjam	13	0.147±0.018	0.153±0.018	0.227±0.025	52.25
N. Gonder	13	0.129±0.017	0.134±0.018	0.199±0.024	45.05
S. Gonder	12	0.163±0.018	0.170±0.019	0.247±0.026	50.45
W. Harerge	11	0.171±0.018	0.179±0.019	0.263±0.025	56.76
E. Shewa	13	0.157±0.017	0.163±0.018	0.244±0.025	56.76
N. Shewa	13	0.166±0.018	0.172±0.019	0.253±0.026	53.15
W. Shewa	13	0.150±0.017	0.156±0.018	0.231±0.025	52.25
Tigray	12	0.117±0.016	0.122±0.017	0.183±0.023	43.24
S. Wello	13	0.113±0.016	0.118±0.017	0.177±0.023	42.34
Improved	16	0.226±0.018	0.233±0.018	0.345±0.025	70.27
Mean ± SE	13	0.145±0.005	0.151±0.005	0.224±0.007	49.77±2.71

(Shewa), and northwestern (Gojam and Gonder) blocks of the country (Fig. 1). Materials from all parts of Shewa, W. Harerge, W. Gojam and S. Gonder showed more polymorphism (50–57%) than those from the rest of the country (36–45%; Table 4). Based on the magnitude of the GD, more differentiations were revealed between the different populations from different geographical regions of Ethiopia and improved genotypes from ICARDA and ICRISAT $(\mathrm{GDrange} = 0.077 - 0.138, \overline{X} = 0.107)$. The second largest inter-regional distance range was observed between accessions from Arsi and those from the rest of the sources $(\mathrm{GD} = 0.081 - 0.134, \overline{X} = 0.106)$. The highest values of GD (0.138 and 0.134) were recorded between accessions from East Gojam and the improved genotypes and those from Arsi and South Wello in that order. The smallest genetic distance (GD=0.016) was observed between accessions from West Gojam and North Gonder (Annex 1). Likewise, germplasm accessions from Arsi exhibited higher pairwise population PhiPT values with all groups of collections from other regions (Table 5), indicating existence of more distinct differences between the two.

Positive and highly significant interrelationship (r= 0.845, $P \leq 0.01$) was observed between the pairwise GD and PhiPT coefficients (Annex 2). This indicates that higher distances between populations of different geographical origins entail higher proportional magnitudes of variation among the populations relative to total variation. Other genetic parameters including expected heterozygosity (He), unbiased expected heterozygosity (UHe) and Shannon's information index (*I*) also showed the existence of high genetic variation within the improved genotypes (Table 4). Differences among the original introductions, the nature and degree of both human and natural selection after introduction and/or specificities of ecological and agricultural conditions as major forces of evolution are normally expected to give rise to a distinct form of genetic diversity (Ford-Lloyd and Jackson 1986; Spagnoletti and Qualset 1987). The differences observed in landrace populations

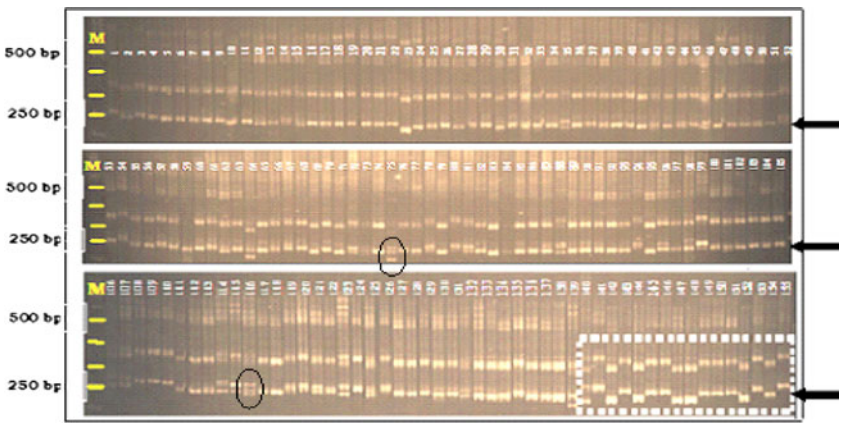

Fig. 2 Autoradiograph of chickpea DNA of 155 genotypes as revealed by a polymorphic SSR marker, TA 144. The lane numbers identify serial No of genotypes as designated in Table 1 above, the *broken rectangle* at the *right angle bottom* shows improved genotypes, *M* stands for DNA ladder GeneRuler ™ 50 bp and arrows indicate polymorphic bands. Some accessions (e.g., *circled*) revealed more than two bands

Table 5 Pairwise population PhiPT values (below diagonal) and probability level based on 99 permutation (above diagonal) showing the proportional magnitudes of variation among population relative to total variation in chickpea populations from different sources

Geographical region of origin	Arsi	E. Gojjam	W. Gojjam	N. Gonder	S. Gonder	W. Harerge	E. Shewa	N. Shewa	W. Shewa	Tigray	S. Wello	Improved
Arsi		**	**	**	**	**	**	**	**	**	**	**
E. Gojjam	0.481		**	**	**	**	**	**	**	**	**	**
W. Gojjam	0.444	0.135		*	**	**	**	**	**	**	**	**
N. Gonder	0.424	0.130	0.047		**	**	**	**	**	**	**	**
S. Gonder	0.382	0.243	0.161	0.091		**	**	**	**	**	**	**
W. Harerge	0.341	0.189	0.097	0.084	0.093		**	*	**	**	**	**
E. Shewa	0.486	0.321	0.203	0.234	0.214	0.104		**	**	**	**	**
N. Shewa	0.382	0.237	0.123	0.135	0.146	0.077	0.196		**	**	**	**
W. Shewa	0.468	0.312	0.189	0.222	0.244	0.173	0.260	0.177		**	**	**
Tigray	0.488	0.306	0.241	0.235	0.224	0.189	0.286	0.214	0.156		**	**
S. Wello	0.530	0.408	0.312	0.327	0.310	0.228	0.222	0.208	0.279	0.246		**
Improved	0.367	0.365	0.284	0.295	0.260	0.239	0.302	0.218	0.228	0.291	0.301	

*$P \leq 0.050$; **$P \leq 0.010$

studied could also result at least partly from combined effects of drift, mutation, migration and selection (Felsenstein 2007). The highest genetic variability observed in the introduced genotypes as compared to landraces could be explained by the broader geographic spectrum from where they were initially acquired and subsequent genetic recombinations undertaken by ICARDA and ICRISAT before their introduction to Ethiopia. It was demonstrated even with these limited numbers of samples (16 genotypes) that introduction of genetic materials from ICARDA and ICRISAT has practically broadened the genetic base of the national breeding programs. Although it is generally believed that plant breeding denudes the genetic base in crops (Witcombe and Hash 2000; Singh 2002; Chung and Singh 2008), a number of recent studies proved rather the contrary in a number of crops (Donini et al. 2000; Bowman et al. 2003; de Wouw et al. 2010) including chickpea (Sefera et al. 2011).

The 33 microsatellite markers used to study genetic diversity revealed a total of 111 bands with a range of 2–5 or on average 3.364 bands marker^{-1} among the studied 155 entries of chickpea. High PIC values of the markers ranging from 0.278 to 0.500 ($\overline{X} = 0.412$) were obtained (Fig. 3), indicating the usefulness of most of the markers in germplasm characterization (Saxena and Chandra 2010; Sharma et al. 2011).

With cluster analysis, it was possible to classify the 155 entries from 12 different sources of origins (considered as 12 initial groups) into five distinct clusters whereby the different members within a cluster are assumed to be more closely related with each other than with those members in different clusters. Populations from Arsi were generally assigned to cluster C_1. The distinctness of Arsi as such could be related to the existence of the Great Rift Valley as a buffer zone of approximately over 100 km in all directions between the other regions. Arsi could also be considered as a relatively self-contained region where the local population is sufficient to satisfy its limited domestic needs for seed and grain.

Pattern of Genetic Differentiation Among Populations

The populations from the different sources were grouped into five clusters of distinct genetic populations (Fig. 4) showing that they had evolved from different lines of ancestry or derived from independent events of evolutionary forces (genetic drift, mutation, migration, selection and in flux/out flux of genes in the form of germplasm exchange) that separated them into different gene pools. The clustering pattern showed the existence of definite pattern of relationships between geographical origins and genetic diversity. High levels of intra-regional similarities were observed within each origin or, in other cases, between adjoining geographical origins. Populations from the same geographical origin were observed to characteristically fall exclusively in a single or two clusters. Some clusters constituted populations mostly from the same geographical origin while others had populations from more than one sources and, hence, the number of entries varied from cluster to cluster. The cross-border similarities between a few adjoining regions may be attributed, at least in part, to seed movements among neighboring regions. He et al. (2011) also observed definite relationship between sources of geographic origin and genetic diversity in *Eriobotrya japonica*.

The first cluster, C_1, constituted accessions mainly from Arsi and, the last cluster, C_5, constituted almost entirely

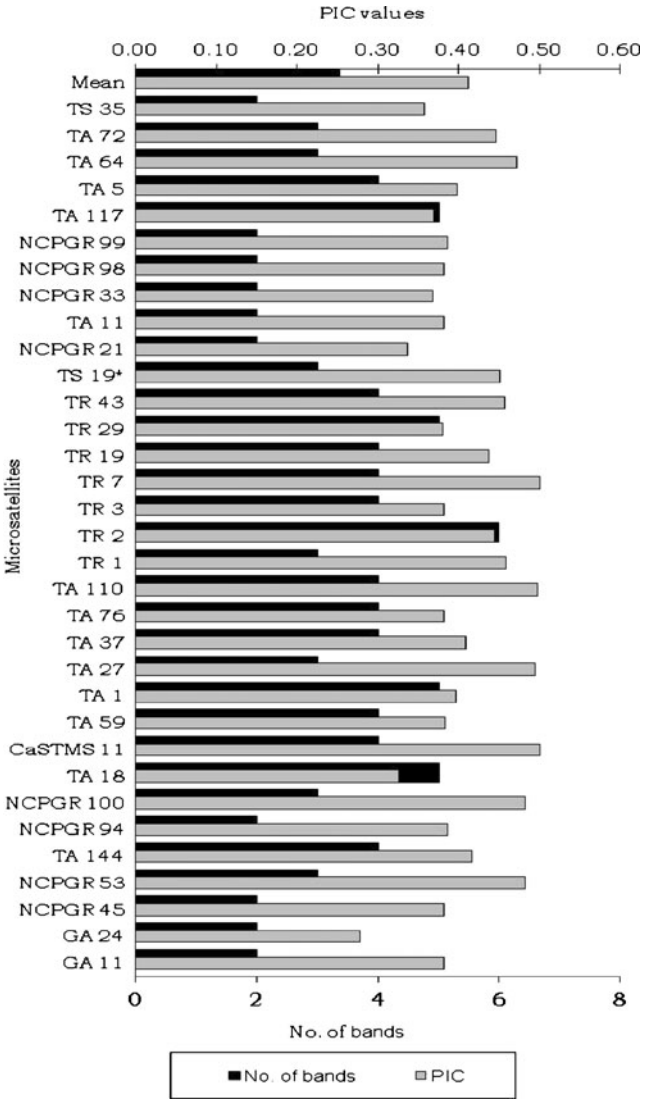

Fig. 3 Comparison of no. of bands amplified and PIC values among 33 polymorphic SSR markers evaluated across 155 chickpea genotypes

improved genotypes regardless of being *Kabuli* and *Desi* types. Likewise, the only *Kabuli* type landrace (Acc. no. 41197) collected from W. Shewa Zone was grouped in the same cluster with the *Desi* types collected from the same origin rather than with the *Kabuli* types in cluster C_5. This indicates that genotypes from different seed types might have similar genetic background for SSR markers provided that they are exposed to similar events of domestication, both natural and artificial selection. The rest of the clusters (i.e., C_2–C_4) comprised accessions from two or more geographical origins grouped together showing closer genetic relationships. The second cluster, C_2, constituted accessions mostly from Gojam and Gonder; the third cluster, C_3, comprised those from Harerge and East and North Shewa while the fourth cluster, C_4, consisted of accessions from West Shewa, Tigray, and South Wello (Annex 3). It can be discerned from Fig. 4 that accessions from North Shewa share a significant portion of their ancestral gene pool with accessions from the adjoining regions of East Shewa on one side and those from Gojam (and Gonder via Gojam) on the other.

Conversely, accessions from Tigray and Wello, which are frequently experiencing severe drought, showed a significant level of genetic similarity with accessions from the geographically non-adjoining West Shewa. The latter is in fact among the most important producers of chickpea. East Harerge, another drought-prone region, also shared similar ancestral gene pool with the adjoining East Shewa. The probable reason for these impressive genetic similarities between accessions from Tigray and Wello with those from W. Shewa, and those from West Harerge with these from East Shewa and implicitly North Shewa (via East Shewa) could be related to massive seed movements associated with response to recurrent drought. According to McGuire and Sperling (2008), periodic provision of a huge amount of seeds of many crops including chickpea (as a suitable crop even after the failure of the long-season crop) has a long history in drought-prone areas in Ethiopia. The native accessions in these areas may become genetically eroded and significantly replaced with seeds purchased from other regions.

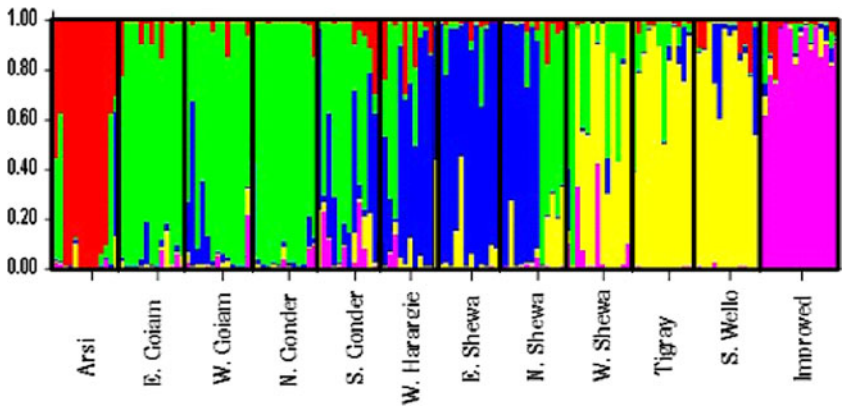

Fig. 4 Structure bar-plot of the tested chickpea entries from different origins showing the pattern of assignment of the entries from the 12 sources of origins into five clusters

Traces of new "bloods" of improved genotypes within the local accessions almost from all over the country (Fig. 4) including in the drought-prone areas like Tigray and Wello, should not be a surprise as improved seeds have been distributed to farmers through both the formal and informal channels including through the "relief seed system" (McGuire and Sperling 2008). In Ethiopia, once improved varieties are introduced, there is no planned "generation control", i.e., replacement of the old seeds with new ones. As a result, the local accessions can harbor genes from the improved varieties through the limited natural hybridization and recombination over a long period of time of togetherness. Similarly, each region showed a few overlapping or admixtures with those from other regions.

Pattern of Genetic Diversity Among Individual Genotypes

Based on a cluster assignment probability of 0.80 (80%) as a cutting-point to assign a given population or individual to a given cluster (Garris et al. 2005), populations or individual members of a population were assigned fully or partially to clusters. The patterns of genetic diversity among individual accessions were also consistent with the pattern of genetic diversity in the populations at large. However, a few individual accessions slightly deviated from the general trend and were identified as admixtures. For instance, out of 13 accessions from Arsi, nine were characteristically grouped into a single cluster C_1. Three remaining accessions (Acc. nos. 231327, 231328, and 207763) partly shared ancestral gene pools with accessions from Gojam and Gonder in cluster C_2 and another accession (Acc. No. 207764) with accessions from Harerge and E. and N. Shewa in cluster C_3. Rather than seed movement per se, the gene transport in this case could be related to the historical influx of people from the other parts to Arsi in search of farmland along with their chickpea seeds. Two accessions from Gojam (Acc. Nos. 41268 and 41222) and two from Gonder (Acc. Nos. 41303 and 41293) shared limited ancestry with accessions from Arsi. Similarly, a number of accessions from Gojam (Acc. Nos. 41015, 41271, and 41276) and Gonder (Acc. Nos. 41316, 41304, 41295, 41289, 41290, 41293, 41049, and 41053) showed limited genetic relations with accessions in clusters C_2 and C_3. Other examples could also be presented from the rest of the sources of origins, showing possibilities for close genetic interrelationships among the accessions regardless of their sources of origins (Annex 4). The interchange of genes somehow through hybridization and interbreeding may have resulted in such sporadic cross-border genetic similarities.

Conclusions

The present study showed existence of high genetic diversity in Ethiopian chickpea germplasm accessions. The magnitude and pattern of genetic variation detected in this study can be useful for more systematic germplasm management and utilization in breeding programs (Tanya et al. 2011). The exploitation of crosses between genetically distant parents (e.g., recombinants parents from the local accessions and the introduced genotypes) and those from diverse local sources (e.g., crosses between parents from accessions of Arsi and other regions) may produce higher heterosis, better genetic recombination and segregation in their progenies and result in varieties with broad genetic base (Chahal and Gosal 2002). Future germplasm collection and utilization strategies should take into consideration the magnitude and pattern of genetic diversity established by the present investigation. The results of this study generally suggest the existence of a large number of duplications of accessions in the Ethiopian chickpea germplasm collections. In order to reduce such a high level of redundancy in the germplasm collections, strategies such as systematic bulking and the formation of "core collections", i.e., a subset of accessions which contains most of the genetic diversity in the whole collections (Brown 1989), may need to be tested and validated. However, it should be noted that this investigation could provide only preliminary information as the existence of genetic diversity alone may not be sufficient in terms of germplasm utilization. Marker-based genetic diversity may not show the expression of the markers in the phenotype (Carvalho 2004). For such information on genetic diversity to be more useful, the markers need to somehow be associated with characters of breeders' interests. A comprehensive study to map the associations of the markers revealed here and agronomic traits of economic importance is required. This investigation also proved the efficiency and effectiveness of SSR markers to unravel methodological limitation in DNA fingerprinting and study of genetic diversity in chickpea as denoted by others (Sefera et al. 2011).

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