Geographical patterns of genetic diversity in cultivated chickpea (*Cicer arietinum* L.) characterized by amplified fragment length polymorphism

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ABSTRACT

The objective of this study was to evaluate the genetic relationships of 28 chickpea accessions from diverse origin using AFLP markers. On average, 13 polymorphic bands per primer were observed in AFLP analysis. The average polymorphic information content (PIC) was 0.71, ranging from 0.48 to 0.92. The lowest and the highest PIC value were recorded for primer P-GAG/M-GC and P-AT/M-GC, respectively. The average GD, based on F_{st} values among the 21 accessions was 0.42, ranging from 0.61 to 0.16. From the UPGMA dendrogram, it is discernible that material taken for the analysis can be divided in four clusters. The results indicate that the greatest genetic diversity occurs in Afghanistan, Iran and Lebanon. In many cases, the diversity between individuals of an accession is as great as between individuals of different accessions. Based on DNA markers it is concluded that there are three centers of diversity for chickpea: Pakistan-Afghanistan, Iran-Turkey and Syria-Lebanon. India and Ethiopia, which were previously considered as a secondary center of diversity for chickpea, showed lower diversity than the above regions.

Keywords: chickpea; genetic diversity; AFLP; geographical diversity

Chickpea is an important self-pollinated grain legume crop, grown mainly in West Asia, North Africa and the Indian subcontinent, where it is a basic component of the human diet. Vavilov (1926) was the first to identify areas with similar physiographic characteristics with maximum variability for the major cultivated species. He recognized the Near Eastern, Central Asian, Indian and Mediterranean regions as the probable centers of origin for chickpea. Harlan (1992) stated that chickpea has one definable center of origin, wide dispersal, and one or more secondary centers of diversity. The crop most probably originates from the area of present-day south-eastern Turkey and adjoining Syria (Van der Maesen 1987, Harlan 1992). Indeed, the archaeological record suggests that cultivated chickpea was one of the first grain legumes to be domesticated in the Old World (Van der Maesen 1987). India and Ethiopia have been proposed as secondary centers of diversity of cultivated chickpea (Harlan 1992). However, Zeven and de Wet (1982) suggested that chickpea has different secondary centers of diversity located in at least four regions; the Near Fast Region (comprising the Fertile Crescent), Hindustani Region (basically the current India and East Pakistan), Central Asian Region (with Afghanistan, Western Pakistan, Iran and the south of the former USSR) and the Mediterranean Region (including Lebanon and Palestine). Assessment of the extent of genetic variability within chickpea is fundamental for chickpea breeding and conservation of genetic resources, and is particularly useful as a general guide in the choice of parents for breeding hybrids. Criteria for estimation of the genetic diversity can be different, including morphological traits (Upadhaya et al. 2007) or molecular markers (Rao et al. 2007). The amplified fragment length polymorphism (AFLP) technique has emerged as a new powerful tool for genomic analysis (Vos et al. 1995). This technique has been applied to determine genetic relationships among populations of legume crops such as wild bean (Tohme et al. 1996), Lima bean (Caicedo et al. 1999), Azuki bean (Ru-Qiang et al. 2000) and peanut (Guohao and Channapatna 2001). The high frequency of

identifiable polymorphic AFLP markers, coupled with their reproducibility, make this technique an attractive tool for detecting polymorphism and for determining genetic linkages among individuals (Gupta et al. 1999). Chickpea-breeding aims at developing high yielding cultivars that combine long lasting resistance against Ascochyta blight and Fusarium wilt with tolerance to abiotic stresses such as drought, cold and salt. Use of AFLP for genetic diversity analysis of individual accessions with different origin has not been demonstrated. The present study was conducted to determine the genetic variability between divers geographical origin accessions using AFLP markers and to provide a better understanding of the genetic diversity of the chickpea accessions across all possible centers of origin.

MATERIAL AND METHODS

Plant materials

A total of 28 accessions of *Cicer arietinum* L. from the germplasm collection held at the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, were randomly selected in this study. Those accessions had been collected in 14 different countries of the Near East, Central Asian, India, Mediterranean regions and Africa (Table 1). The accessions were collected by ICARDA in cooperation with the National Agricultural Research Systems (NARS) of the country of origin.

DNA extraction and AFLP analysis

Three seeds of each accession were sown in pods and after 10 days, total genomic DNA was extracted from 2 g of young leaves collected from one of the plants of each accession using the CTAB method with the modification described by Torres et al. (1993). The DNA final concentration was determined by agarose-gel electrophoresis using known concentration of uncut λ DNA as standard. Five hundred nanograms of genomic DNA of each accession were digested simultaneously with 10 units each of MseI and Pst1 (fermentase, Germany) at 37°C for 4 h. Following digestion, Pst1 and MseI adapters (Cinagene, Iran) were ligated to restricted fragments at 20°C for 2 h and digested fragments were preamplified using 20 cycles of 94°C for 30 s, 60°C for 1 min and 72°C for 1 min according to the manufacturer's instructions. Selective amplification was then performed according to the manufacturer's instructions (Cinagene, Iran). 3.75 µl of diluted preselective PCR products were used as DNA templates for selective amplifications. The PCR selective amplifications were carried out in the same manner as PCR preamplifications. For selective amplification, the following cycle profile was used: 2 min at 94°C, 10 cycles: 30 s at 94°C, 30 s at 63°C (touchdown 1°C per cycle to 54°C), 2 min at 72°C

Table 1. The accessions and origin (collection site) of chickpea cultivars taken for AFLP analysis

Number	Accession	Country of origin
1	ILC2628	India
2	ILC3426	Spain
3	ILC4339	Spain
4	ILC2882	Afghanistan
5	ILC1577	Afghanistan
6	ILC2929	Afghanistan
7	ILC153	Greece
8	ILC63	Iraq
9	ILC71	Iraq
10	ILC2553	Ethiopia
11	ILC2537	Ethiopia
12	ILC3843	Morocco
13	ILC3832	Morocco
14	ILC19	Jordan
15	ILC23	Jordan
16	ILC286	Iran
17	ILC1264	Iran
18	ILC317	Iran
19	ILC1279	Palestine
20	ILC4109	Tunis
21	ILC10074	Tunis
22	ILC2625	Lebanon
23	ILC3089	Turkey
24	ILC3210	Turkey
25	ILC3764	Turkey
26	ILC3345	Syria
27	ILC1799	Syria
28	ILC3321	Syria

and 23 cycles: 30 s at 94°C, 30 s at 54°C, 2 min at 72°C. All amplifications were performed in a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA). Amplification reaction products were separated on a 6% denaturing polyacrylamide gel in a 50 cm Sequi-Gen GT Sequencing Cell gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). The amplified fragments were detected by silver staining. The resulting gels were scored manually. The average polymorphic information content (PIC) was calculated according to Botstein et al. (1980) for each primer combination. Cluster analysis based on complete linkage algorithm using Jaccard's coefficient, were performed using the NTSYS-pc version 2.01 software.

RESULTS AND DISCUSSION

Differences between genotypes with regard to agronomic characters, morphological characters, biochemical characters (e.g. storage proteins, isozymes), and molecular characteristics are either indirect or direct representations of differences at the DNA level and are therefore expected to provide information about genetic relationships. The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources. For this purpose 28 cultivars of chickpea from different countries were analyzed by 8 reproducible AFLP markers. AFLP analysis revealed a good polymorphism among chickpea cultivars. Eight AFLP combination primers were taken and on average 13.1 bands per primer and 36.25% polymorphic bands were observed in the total of 288 bands. The P-CA/M-TG primer gave rise to maximum polymorphic band (16) and P-AT/M-GC primer showed the least number of polymorphic band (9) (Table 2). The average polymorphic information content (PIC) was 0.71, ranging from 0.48 to 0.92. The lowest and the highest PIC values were recorded for primer P-GAG/M-GC and P-AT/M-GC, respectively. The average GD, based on F_{st} values among the 21 accessions was 0.42, ranging from 0.61 to 0.16. From the UPGMA dendrogram, it is discernible that material taken for the analysis can be divided in four clusters (Figure 1). In order to determine the ability of AFLP analysis to display genetic relationships among accessions, the principle component analysis (PCo) was carried out, and accessions were plotted in the coordinate system for the first two coordinates which accounted for 68.75 and 9.23% of the variation respectively. PCo provided a better graphical illustration and a clear separation of cultivars (Figure 2). Although the chickpea are predominantly self-pollinating, more variation was observed among them. The reason for this genetic variation could be that the specific accessions were heterozygous at some marker loci. Similar observations were reported in chickpea (Rao et al. 2007, Talebi et al. 2008), in pea and lentil (Simon and Muehlbauer 1997). In this investigation, AFLP markers showed a high level of polymorphism and a high number of clearly amplified bands (Figure 3). Extensive DNA polymorphism has been reported using AFLP markers in several other crops (Tavoletti and Iommarini 2007, Altintas et al. 2008). Harlan (1992) postulated that south-eastern Turkey is a definable center of origin for chickpea and the Indian subcontinent is a diversification centre. Our analysis of diversity for each country suggests that in addition to south-

Table 2. Characteristics of the amplification products obtained with 8 AFLP primer pairs used to analyze the genetic diversity of chickpea accessions

Number	Primer combination	Total number of amplification products	Number of polymorphic bands	Percent of polymorphism	PIC/primer
1	P-CA/M-CG	39	14	36	0.61
2	P-CA/M-TG	40	16	40	0.79
3	P-CA/M-GC	32	10	31	0.86
4	P-AT/M-TG	29	12	41	0.59
5	P-AT/M-GC	34	13	38	0.92
6	P-AT/M-CG	32	9	31	0.89
7	P-GAG/M-GC	40	14	35	0.48
8	P-GAG/M-TG	42	16	38	0.61

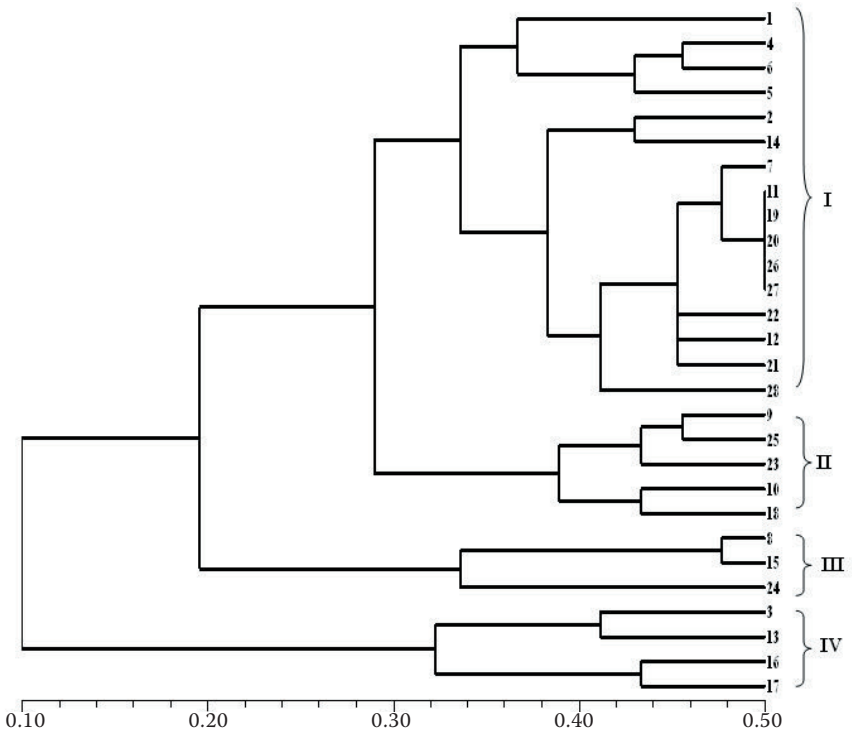

Figure 1. Dendrogram showing clustering pattern of 28 chickpea accessions based on genetic similarity values obtained from the AFLP data

east Turkey, the region comprising Afghanistan and Iran, north of Africa (Tunis and Ethiopia) and south-east of Europe (Greece) could be other centres of diversity, with even higher values than the center of origin proposed by Harlan (1992). Our data, showed high genetic diversity between geno-

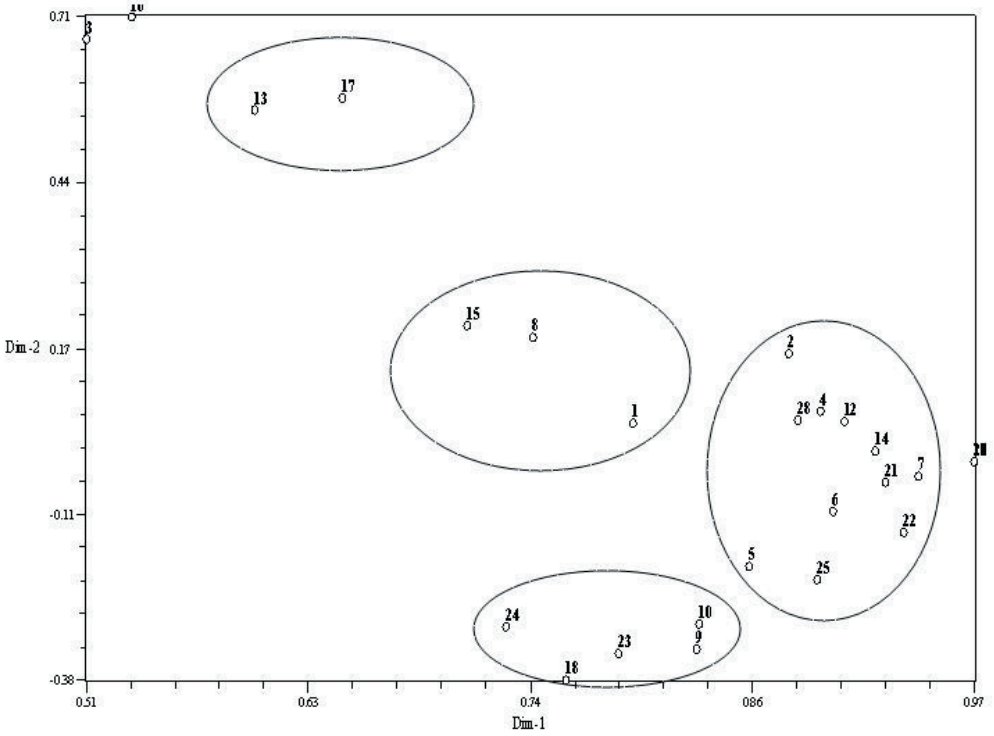

Figure 2. Two-dimensional representation of genetic relations among 28 chickpea genotype from different countries

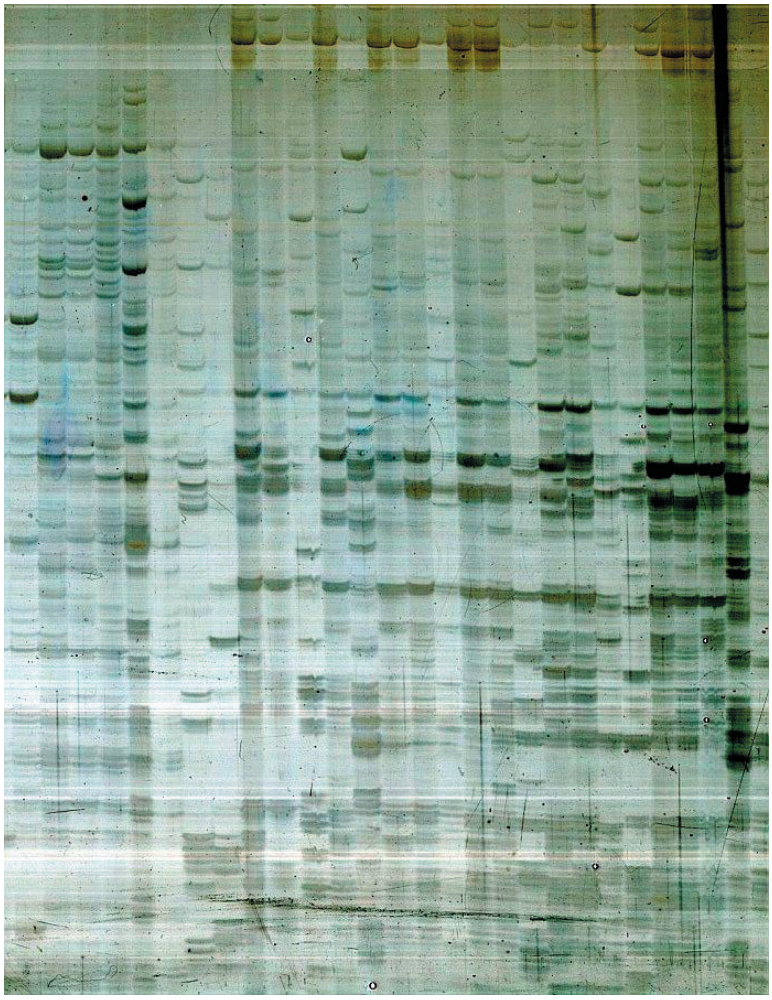

Figure 3. Amplified AFLP electrophoretic pattern on chickpea DNA using primer P-CA/M-TG

types from Jordan, Spain, Ethiopia and Morocco. A recent study based on AFLP markers revealed much greater polymorphism in cultivated chickpea than reported in previous works using isozyme (Labdi et al. 1996), seed storage protein (Ahmad and Slinkard 1992), ISSR (Rao et al. 2007) and morphological traits and RAPD (Talebi et al. 2008). AFLP are useful for studying phylogenetic relationships among different accessions of cultivated plants. Our results revealed that the germplasm originating from Afghanistan, Iran, Lebanon and Turkey had relatively high level of genetic diversity. These areas, previously identified as centers of origin and/or diversity by Vavilov (1926), are important for future collection missions if the estimated genetic diversity is not fully reflected by genetic diversity in the existing collection. In some cases, such as in all accessions from Iran and one accession from Syria (ILC3321), Turkey (ILC3210) and India (ILC2628), the individual plants within an accession differ greatly with respect to AFLP profiles. The observed heterogeneity and diversity in those accessions could be related to the mode of collection of those accessions. Most were collected either from farmers' holds or from the local markets. In many cases, the diversity between individuals of an accession is as great as between individuals of different accessions. This could happen because accessions are frequently collected by the NARS in their own countries. Hence, the different accessions may be collected in geographically close areas, even when these areas belong to different countries. In this context it is worth noting accessions collected in Iran and Turkey or in Iraq and Jordan (e.g. ILC317 and ILC3089 or ILC63 and ILC23). This study has implications not just for the origin of this crop, but also for the management of genetic resources and their uses in applied breeding programmes, particularly for the development of a core collection. If entries were to be chosen only on the basis of geographical origin, genetic migration in this species may not be adequately represented. The use of these genetic resources in breeding pro-

grammes needs to be based on intensive screening at both the within- and between-accession level, particularly for entries from areas of high diversity. Further, great genetic variation that exists between chickpea cultivars can be used efficiently in gene tagging and genome mapping of cultivar crosses for introgression of the disease and insect resistance into high yield or favorable genotypes.

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