J. Glob. Innov. Agric. Soc. Sci., 2018, 6(3):75-79.
ISSN (Online): 2311-3839; ISSN (Print): 2312-5225
http://www.jgiass.com

INVESTIGATION OF GENETIC DIVERSITY BY USING MOLECULAR MARKERS IN LOCAL CHICKPEA POPULATIONS COLLECTED FROM KIRSEHIR PROVINCE

Nuri Ercan[1], Ufuk Karadavut[2], Omer Sozen[3,*] and Fahriye ERCAN[4]

[1]Faculty of Agriculture, University of Krşehir Ahi Evran, Kirsehir, Turkey; [2]Biometry and Genetic Unit, Faculty of Agriculture, University of Kırşehir Ahi Evran, Kirsehir, Turkey; [3]Department of Field Crops, Faculty of Agriculture, University of Kırşehir Ahi Evran, Kirsehir, Turkey; [4]Department of Plant Protection, Faculty of Agriculture, University of Kırşehir Ahi Evran, Kirsehir, Turkey.
[*]Corresponding author's e-mail: eekim_55@hotmail.com

Chickpea is very important in Turkey as food source. Turkey is origin of legumes, especially lentils and chickpea. Local genetic resources are continuously being utilized in chickpea breeding programs. The current study was carried out in districts Kaman, Mucur, Boztepe, Akpinar, Akcakent, Cicekdagi and Central of City of Kirsehir province. A total of 30 varieties were evaluated, including 24 local chickpea genotypes and 6 standard varieties (Uzunlu 99, Inci, Gokce, Azkan, Yasa-05 and Aksu). Ten RAPD (Random Amplified Polymorphic DNA) and ten ISSR (Inter Simple Sequence Repeat) markers were used to determine the genetic relationship between the samples. The polymorphisms in samples determined genetic diversity. RAPD primers showed genetic distance ranging from 2.00 to 67.90%. ISSR divided samples into two main groups; however, one group only represented N8. The genetic diversity of chickpea genetic resources belonging to the Kirsehir region has been determined for the first time.

Keywords: Chickpea, RAPD, ISSR, molecular markers, genotype, germplasm.

INTRODUCTION

The agricultural production can be enhanced by providing the necessary inputs to achieve the yield potential. It is also necessary to develop new varieties that meet the needs of the rapidly increasing world population. Plant genetic resources support breeders to breed new varieties. In this context, there is a need for basic genetic stocks that provides broad selection of important traits (Sehirali and Ozgen, 1987). Climatic diversity in Turkey allows both genetic diversity and utilization. Plant-derived proteins are imperative in human nutrition and legumes are one of the most important source. Chickpea (*Cicer arietinum* L.) is an annual crop from the family Leguminosae and is an important food crop with high nutritional value.

In chickpea production, as it is in many crops, high productivity and quality is desired. To achieve this, high yielding varieties are cultivated by applying optimal growing techniques in existing ecological conditions. The preference of the chickpea producers in Turkey is disease resistance and high yield (Bolat *et al.,* 2017). In crop production, genetic structure and environmental factors are the two main determinants. Some of the environmental factors may be overruled by crop improvement. Genetic structure can only be influenced by breeding new varieties (Akdag and Sehirali, 992). The varieties of chickpea grown in different ecological regions since years are important country's genetic resources, which are most influenced by environmental factors. This will enable the development of high-yield and high quality chickpea varieties by conservation of germplasm and identifying genetic structures.

Molecular markers are referred to as a specific gene region within the genome and / or DNA fragments associated with this region. The most frequently used molecular markers in genetic studies are DNA-based marker systems. Molecular markers are often preferred in genetic mapping, phylogenetic studies, analysis of agronomically important genes and selection studies (Joshi *et al.,* 2000). Several molecular markers have been used by many researchers in identifying genetic relationships in chickpea species (Backman and Sollers, 1993; Galvàn *et al.,* 2003; Sethy *et al.,* 2006). Ahmad (1999) reported that RAPD molecular markers could be used to detect the genetic relationship between single-yearly chickpea species. Huttel *et al.* (1999) reported that SSR primers could be used successfully to determine the genetic diversity in chickpea.

This study is aimed to determine the genetic relationship between the local chickpea genotypes collected from Kırsehir province and to elucidate the genotypes using RAPD and ISSR molecular markers.

MATERIALS AND METHODS

***Germplasm*:** A total of 30 varieties including 24 local chickpea genotypes and 6 standard varieties were collected from Kirsehir region.

***DNA isolation*:** In the study, young leaf samples of each genotype were ground with a hand homogenizer with liquid nitrogen and DNA isolation was performed according to the protocol (Gene JET Plant Genomic DNA Purification Kit Thermo Scientific) determined by the DNA isolation kit.

***RAPD and ISSR analyses*:** Ten RAPD and ISSR primers were used in the study (Table 1). PCR applications were performed with Cleaver Scientific Multigene PCR instrument. In the reactions, amplification was performed by performing 38 cycles of PCR with 2 µL (40-50 ng) of genomic DNA and 23 µL of Reaction Mix [Transs 2X EasyTaq PCR SuperMix, 1 µM primer, distilled water]. The PCR programs used were optimized in accordance with the Tm values of the primers used in the study (Table 2). PCR products were run on 1.5% agarose (Sigma) gel electrophoresis.

The DNA was stained with ethidium bromide and the bands were photographed under UV light (Bio-Imaging Systems MiniLumi) (Fig. 1-2).The amplicons produced were scored as present (1) or absent (0), data were then converted to a distance matrixa dendogram was constructed with the UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) method (Sneath and Sokal, 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (Rohlf, 1988).

Table 1. RAPD and ISSR primers used in the study.

Primer	Sequence information 5′ → 3′
RAPD primers	
UBC 5	CCT GGG TTC C
UBC 6	CCT GGG CCT A
UBC 23	CCC GCC TTC A
UBC 30	CCG GCC TTA G
UBC 34	CCG GCC CCA A
UBC 38	CCG GGG AAA A
UBC 44	TTA CCC CGG C
OPAB-17	TCG CAT CCA G
OPAC-09	AGA GCG TAC C
OPAF-16	TCC CGG TGA G
ISSR primers	
UBC 807	$(AG)_8T$
UBC 808	$(AG)_8C$
UBC 809	$(AG)_8G$
UBC 810	$(GA)_8T$
UBC 815	$(CT)_8G$
UBC 817	$(CA)_8A$
UBC 821	$(GT)_8T$
UBC 825	$(AC)_8T$
UBC 826	$(AC)_8C$
UBC 829	$(CA)_8G$

Table 2. ISSR and RAPD PCR conditions used in the study

ISSR PCR cycle		RAPD PCR cycle	
94 °C – 3 min.		94 °C – 3 min.	
94 °C – 1 min.		94 °C – 1 min.	
Tm – 1 min.	38 cycle	Tm – 1 min.	38 cycle
72 °C – 2 min.		72 °C – 2 min.	
72 °C – 5 min.		72 °C – 5 min.	

RESULTS AND DISCUSSION

Genetic distances between the samples were calculated according to the Jaccard coefficient by using data information in the form of present (1) or absent (0) of band indexes composed of polymorphic primers and a dendogram was created by UPGMA method in the NTSYS-pc-2.1 packet program showing the phylogenetic relation between the samples (Fig. 3-4). According to RAPD primers, the genetic distance between samples ranged from 2.00 to 67.90%. According to the dendogram, calculated by the UPGMA method, samples were separated under two main groups. The first group known as Azkan that located separately, while Aksu, also known as variant, was in the same group with the sample N8. All other samples were in the same group. It was observed that the varieties Inci and Yasa-05 grouped together and were close to N9 and N29 samples.

According tothe ISSR primers, the genetic distance between the samples was between 2.00 and 27.80%. The dendogram showing the genetic distance relation, calculated by the UPGMA method, divided samples into two main groups; however, one group only represented N8. Hence, the N8 is entirely different from the other 29 samples.

The utility of the RAPD markers in identifying chickpea species has been reported by Ahmad (1999). At the same time it is known that the ISSR markers are suitable marker system for studying genetic diversity (Ratnaparke *et al.*, 1998). Irulea *et al.* (2002) reported that the RAPD markers successfully demonstrate the genetic variation in chickpea. Sudupak (2004) reported that the ISSR-PCR technique is a convenient and rapid method for determining genetic diversity and phylogenetic relationships in chickpea.

***Conclusion*:** In this study, 30 chickpea samples were characterized using RAPD and ISSR molecular markers and the genetic variation between these samples was evaluated. It was noticed that the standard varieties were very close to other local populations. Another important result was that among local populations, the N8 code sample differed from the other known samples. Identification and registration of these samples is important for the general cultivation of chickpea in the region.

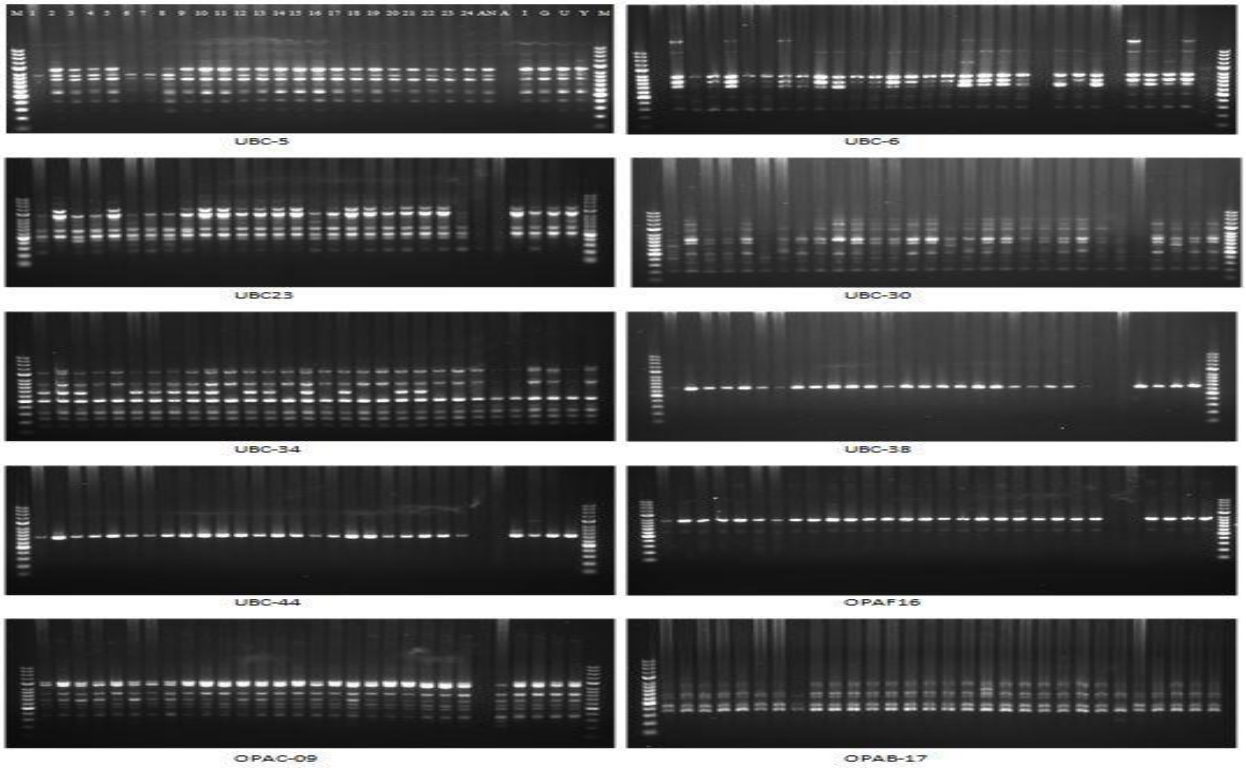

Figure 1. Agarose gel image of PCR products obtained from RAPD primers.

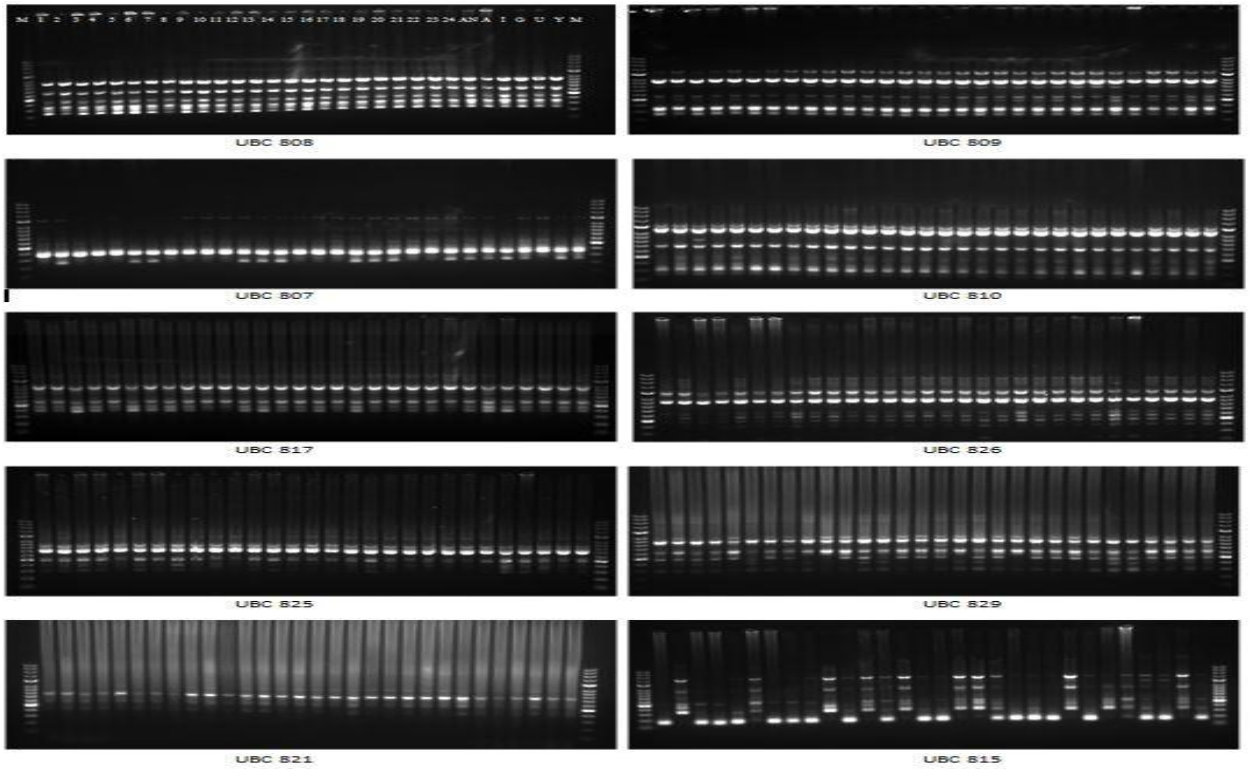

Figure 2. Agarose gel image of PCR products obtained from ISSR primers.

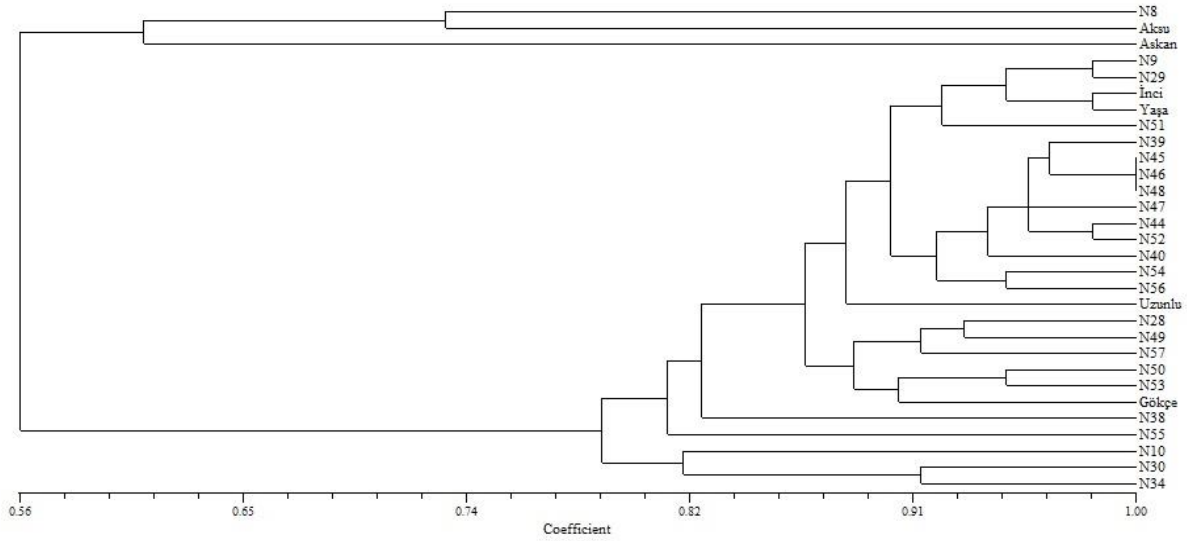

Figure 3. UPGMA dendograms showing genotypic variation from scored bands obtained from 30 chickpea genotypes using RAPD markers with NTSYS-pc2.1

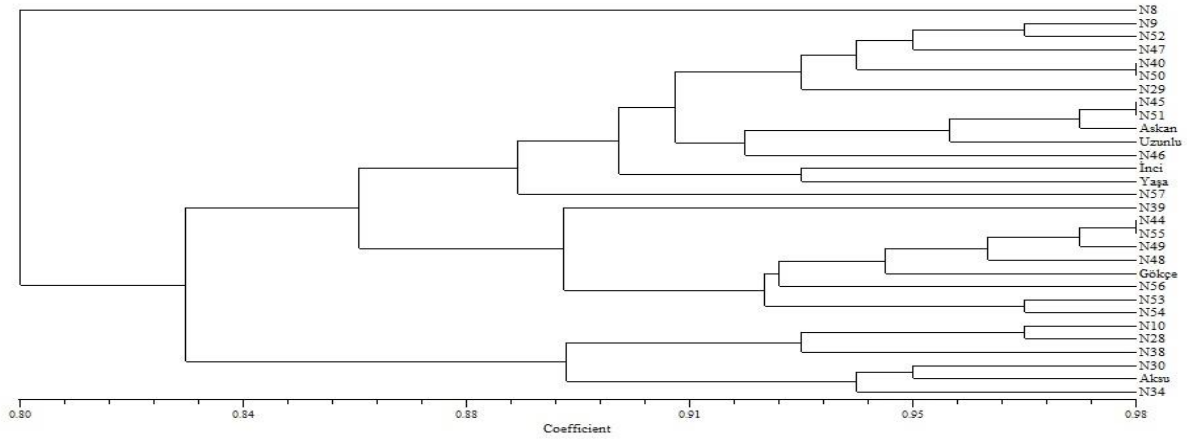

Figure 4. UPGMA dendograms showing genotypic variation from scored bands obtained from 30 chickpea genotypes using ISSR markers with NTSYS-pc2.1.

REFERENCES

Ahmad, F. 1999. Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. Theor. Appl. Genet. 98:657-663.

Akdag, C. and S. Sehirali. 1992. A research on relations among the characters and path coefficient analysis in chickpea. Doga Publications 16:763-772.

Backman, J.S. and M. Sollers. 1983. Restriction fragment length polymorphisms in genetic improvement methodologies mapping and costs. Theor. Appl. Genet. 67:35-43.

Bolat, M., S. Karabak, R. Taşçı, A. Aydoğan, M. Küçükçongar, K. Taşdan, M. Aydoğan, T. Monis, H. Özçelik, S. Yılmaz, G. Başbağcı, M. Kan, M. Önder, A. Çıkman, İ. Dellal and C. Akbay. 2017. Determination of the factors affecting the production and consumption of edible grain legumes decision in Turkey (in Turkish). Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policy, Project Reort, Project No: TAGEM/TEAD/16/A15/P01/007, Ankara, Turkey, pp.104.

Galvan, M.Z., B. Bornet, P.A. Balati and M. Branchard. 2003. Inter simple sequence repeat (ISSR) markers as a tool fort the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132:297-301.

Huttel, B., P. Winter, K. Weising, W. Choumane, F. Weigand and G. Kahl. 1999. Sequence-tagged microsatellite site

markers for chickpea (*Cicer arietinum* L.). Genome 42:210-217.

Iruela, M., J. Rubio, J.I. Cubero, J. Gil and T. Millán. 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. Theor. Appl. Genet. 104:643-651.

Joshi, S.P., V.S. Gupta, R.K. Aggarwal, P.K. Ranjekar and D.S. Brar. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus. Theor. Appl. Genet. 100:1311-1320.

Ratnaparkhe, M.B., D.K. Santra, A. Tullu and F.J. Muehlbauer. 1998. Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theor. Appl. Genet. 96:348-353.

Rohlf, F. 1988. NTSYS-PC numerical taxonomy and multivariate analysis system, version 2.0. Exeter Publishing Ltd., Setoukat, New York.

Sehirali, S. and M. Ozgen. 1988. Plant Breeding, 3rd Ed. University of Ankara, Faculity of Agricultural Publications, Ankara.

Sethy, N.K., S. Choudhary, B. Shokeen and S. Bhatia. 2006. Identification of microsatellite markers from *Cicer reticulatum* moleculer variation and phylogenetic analysis. Theor. Appl. Genet. 112:347-357.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy: The principles and practice of numerical classification, 2nd Ed. WH Freeman and Co., San Francisco, CA., USA; p.573.

Sudupak, M.A. 2004. Inter and intra-species inter simple sequence repeat (ISSR) variations in the genus *Cicer*. Euphytica 135:229-238.