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GENETIC RELATIONSHIPS BETWEEN CULTIVARS OF *Cicer arietinum* AND ITS PROGENITOR GROWN IN TURKEY DETERMINED BY USING THE SSR MARKERS

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ABSTRACT

Genetic characterization plays a key role in chickpea breeding programs since genetic base of the cultivated chickpea is narrow. This study aims to determine the genetic relationships among 23 cultivated chickpea (*Cicer arietinum* L.) and 2 genotypes of *Cicer reticulatum* Ladizusing simple sequence repeat (SSR) molecular markers with 10 SSR primer pairs. A total of 58 alleles were detected at 10 loci. The number of alleles per locus ranged from 2 to 11, with an average of 5.8. The polymorphic information (PI) ranged from 0.074 (NCPGR42) to 0.806 (NCPGR28) with an average of 0.42. The genetic similarity among all accessions ranged from 0.10 to 0.90. Genotypes of *C. reticulatum* were considerably different from the cultivated chickpea. The cultivars of chickpea were divided into four groups while Er99 and Dikbas cultivars were genetically the closest each other. ICC 4958, 'microsperma' or desi' chickpea was found genetically the closest cultivar to *Cicer reticulatum*. It was suggested that different groups should be crossed in breeding programs to increase variations in chickpeas.

Key words: Chickpea, *Cicer arietinum*, *Cicer reticulatum*, Genetic relationship, SSR.

INTRODUCTION

Chickpea (*Cicer arietinum*L.) is the only cultivated species belonging to the *Cicer* genus. *Cicer* species are predominantly self-pollinated, and the chromosome number of the species is 2n = 16. It is one of the most important pulse crop in the whole World and Turkey as well (Sepetoglu et al., 2008; Ozalkan et al., 2010; Cagirgan et al., 2011, 2012; Toker et al., 2012; Cevik et al., 2014)and is presently grown in 53 countries. Major producing countries include India, Australia, Pakistan, Turkey, and Myanmar (FAOSTAT 2013). Chickpea is globally produced about 13.1 million t from 13.5 million ha area with an average yield of 968 kg per ha (FAOSTAT 2015). However, the average annual yield world-wide (968 $kgha^{-1}$) is considered to be somewhat lower than its potential yield (Canci and Toker, 2009). Therefore, many chickpea breeding programs are focused on improving the genetic potential to increase yield (Sudupak et al., 2002).

Genetic characterization can be made by different methods. It is generally believed that the use of molecular markers is more reliable and repeatable as compared to other methods. Molecular markers provide highly discriminatory information and, therefore, are frequently used for genetic studies. DNA-based markers such as restriction fragment length poylmorphisms (Serret et al., 1997), amplified fragment length polymorphisms (Nguyen et al., 2004; Singh et al., 2008), randomly amplified polymorphic DNAs (Sudupak et al., 2002; Rao et al., 2007) and inter simple sequence repeats (Sudupak et al., 2004) were used to study genetic diversity and relationships in chickpea. Most of these studies reported narrow genomic variability in chickpea. Genetic variation is very limited in *Cicer arietinum*, and this causes difficulty in breeding efforts aimed at developing new cultivars resistant to stress conditions (Atalay and Babaoglu, 2012).In order to enhance genetic potential, there must be a comprehensive understanding of the amount and pattern of genetic variation that exists within and between the available cultivated and wild *Cicer* accessions (Rao et al., 2007).SSRs, also known as microsatellites, short tandem repeats or sequence-tagged microsatellite sites, are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di-, tri- and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n, are widely distributed throughout the genomes of plants and animals (Jiang, 2013). SSR markers are very useful for the analysis of genetic diversity, genomic mapping and marker-assisted selection in many plant species compared to many other marker systems (Atalay and Babaoglu, 2012).

Turkey has a large number of chickpea germplasm (Sudupak, 2013). There are several investigations about genetic relationships of chickpea germplasm which are grown in Turkey, but most of them have not been characterized at molecular levels. The present study aims to determine the genetic relationships of some chickpea cultivars and the closest wild species (*C. reticulatum* Ladiz.) of the cultivated chickpea in Turkey through SSR molecular markers.

MATERIALS AND METHODS

Plant materials

A total of 25 chickpea consisting of 23 cultivars of *Cicer arietinum* and 2 genotypes of *Cicer reticulatum* Ladiz. (Table 1) were used for SSR analyses. *Cicer arietinum* cultivars were developed in Turkey, and most of them were grown by farmers. The seeds of the genetic material were obtained from Akdeniz University Faculty of Agriculture and the Ministry of Agriculture, Field Crops Central Research Institute, Ankara. The plants were grown at 26/22 °C (day/night) temperature and 65±5% relative humidity in a growth chamber with 480 $\mu molm^{-2}s^{-2}$ light (day/ night 16/8 h) for 21 days.

SSR analysis

Young and healthy leaves of genotypes were used for DNA extraction (Table 1). DNA was extracted using the procedure described by Lefort et al. (2001). Concentration and purity of the extracted DNA were analyzed by using a NanoDrop®ND-1000 spectrophotometer and 1% agarose gel. PCR reactions were performed in 15 µL volume containing 15 ng of template DNA, 5 pmol each of forward and reverse primers, 0.5 mM of each of dNTPs, and 0.5 U of *Taq* polymerase. The PCR program was run on a Bio-Rad thermocycler. The PCR conditions consisted of an initial cycle of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 58-66°C and 2 min at 72°C, with a final extension at 72°C for 10 min. To examine the amplification of PCR fragments, PCR products were resolved on ethidium bromide stained with 2% agarose gel in 1 X TBE buffer at 80 V.

Table 1. The Chickpea cultivars/genotypes used as a plant material in SSR analyses.

Cultivars	Kabuli/Desi/Wild	Donor Institutes or Universities
Canitez 87	Kabuli	Anatolian Agricultural Research Institute
Yasa 05	Kabuli	Anatolian Agricultural Research Institute
Isik 05	Kabuli	Anatolian Agricultural Research Institute
Sari 98	Kabuli	Aegean Agricultural Research Institute
Cevdetbey 98	Kabuli	Aegean Agricultural Research Institute
İzmir 92	Kabuli	Aegean Agricultural Research Institute
Menemen 92	Kabuli	Aegean Agricultural Research Institute
Aydin 92	Kabuli	Aegean Agricultural Research Institute
Gokce	Kabuli	Central Research Institute For Field Crops
Akcin 91	Kabuli	Central Research Institute For Field Crops
Dikbas	Kabuli	Central Research Institute For Field Crops
Kusmen 99	Kabuli	Central Research Institute For Field Crops
Uzunlu 99	Kabuli	Central Research Institute For Field Crops
Er 99	Kabuli	Central Research Institute For Field Crops
Cagatay	Kabuli	Black Sea Agricultural Research Institute
Damla 89	Kabuli	Black Sea Agricultural Research Institute
Inci	Kabuli	East Mediterranean Sea Agricul. Research Institute
Aziziye	Kabuli	Eastern Anatolian Agricultural Research Institute
ILC482	Kabuli	GAP Int. Agricultural Research and Training Center
Diyar 95	Kabuli	GAP Int. Agricultural Research and Training Center
TAEK Sagel	Kabuli	Turkish Atomic Energy Authory
ILC 8617	Kabuli	Faculty of Agriculture, Akdeniz University
ICC 4958	Desi	Faculty of Agriculture, Akdeniz University
AWC 611	Wild	Faculty of Agriculture, Akdeniz University
AWC 612	Wild	Faculty of Agriculture, Akdeniz University

Genetic analysis of SSR

The 10 SSR primers selected in a consequence of a literature review were used for SSR analysis (Table 2). PCR products were diluted in sample loading solution (SLS) and standards from the Genome lab DNA standard Kit-400 were included. The amplified fragments were analyzed using a CEQ 8800 XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA) to ensure reproducibility. Allele sizes were determined for each SSR locus using the Beckman CEQ fragment analysis software. The number of alleles (n), allele frequency, expected (He) and observed (Ho) heterozygosity, estimated frequency of null alleles (r), probability of identity (PI), and presence of identical genotypes were determined for each locus using IDENTITY version 1.0 software. Microsat version 1.5 was used to calculate the proportion of shared alleles with the ps option [i.e. option 1-(ps)] selected to assess genetic dissimilarity. Data were

then converted to a similarity matrix, and a dendrogram was constructed via the unweighted pair-group with arithmetic mean (UPGMA) method, using the Numerical Taxonomy and Multiware Analysis System (NTSYSpc) software, version 2.0.

Table 2. SSR primers information used in this study.

Locus	Primer sequence (5'–3')	Repeat motif	Length (bp)	Tm
NCPGR21F	TCTACCTCGTTTTTCGTGCC	$(CT)_{15}$	137	64
NCPGR21R	TTGCTCCTTCAACAAAACCC			
NCPGR27F	ACCCCATTTTTGGGTTTTCT	$(CA)_{12}$	278	63
NCPGR27R	TGCATCCAACTTGTTGTCTTG			
NCPGR28F	TGATGGAAGGTGATGTGGAA	$(AT)_6(GT)_5gc(GT)_7$	224	64
NCPGR28R	GAGGGGGAAACGTTTTCTTT			
NCPGR33F	ACATCTTGAAGTGCCCCAAC	$(GA)_{20}$	248	64
NCPGR33R	TGCAAGCAGACGGTTACAAG			
NCPGR34F	TGGAAGGTGTTTTAGTGGGTG	$(CT)_{17}$	240	64
NCPGR34R	GACTAACTGGCCCCCAAAA			
NCPGR36F	GTGGAGCCAAAAATCGACAT	$(GT)_{12}$	204	64
NCPGR36R	AACTTTATTTTCATTTGTCCATCAA			
NCPGR40F	TGAACGAATCATGGCAAGAG	$(GA)_{12}gt(GA)ca(GA)_3gtaagt$ $(GA)_4gtgg(GA)_{10}gt(GA)gt(GA)_6$	193	64
NCPGR40R	GCCCTCCTTCTTGCTTACAA			
NCPGR42F	CCCCTAGTAGCAAATATTTTGACC	$(CT)_{27}$	170	62
NCPGR42R	TTTGAATGCATTTCTTCATAGCA			
NCPGR93F	CAAAGTTTGTTGCTAGGATTC	$(CA)_2$ $(CT)_{24}$ $(CA)_{13}$	299	58
NCPGR93R	GAAGATCTCCGACGATGATA			
NCPGR100F	CCATTTTCTACAATCTCATGTCT	$(CT)_{15}N42(CT)_2cc(CT)_5tt$ $(CT)_6at(CT)_7$	263	59
NCPGR100R	GTAGAAAGAGCCAAGAGGCA			

RESULTS AND DISCUSSION

Ten SSR markers were used for 23 chickpea cultivars and 2 genotypes of *C. reticulatum* to determine genetic variation. A total of 58 alleles were detected at 10 loci. The number of alleles per locus ranged from 2 (NCPGR28-the least informative locus, PI: 0,806) to 11(NCPGR42-the most informative locus, PI: 0,074), with an average of 5.8 (Table 3). Some researchers have previously demonstrated genetic variation in chickpea using SSR markers. For instance, Choudhary et al. (2012) used 48 chickpea cultivars and detected 2 to 11 alleles and an average of 4.8 alleles per locus using 100 SSR markers. Saeed et al. (2011) obtained 2 to 13 alleles among 44 chickpea genotypes using 16 SSR markers while Singh et al. (2008) analyzed 21 chickpea cultivars using 18 SSR markers and detected 2 to 5 alleles. Huttel et al. (1999) used 22 SSR markers to determine genetic diversity in 4 chickpea genotypes and obtained 2 to 4 alleles whereas Singh et al. (2003) detected 1 to 4 alleles among 13 chickpea cultivars using 12 SSR markers. Data of allele numbers per locus, obtained during these studies, were either close to or less than our data. On the other hand, Naghavi et al. (2012), Seferaet al. (2011), Atalay and Babaoglu (2012) and Udupa et al. (1999) used SSR markers within chickpea genotypes and found 19.31, 10.5, 13.4 and 14.08 average alleles per locus, respectively. These values were higher than our findings. Utilization of different population sizes and also from different geographical origins and use of different SSR markers might be the reason of these differences. (Naghavi et al., 2012).

Table 3. Simple sequence repeat (SSR) loci, number of alleles (n), expected heterozygosity (He), observed heterozygosity (Ho), probability of identity (PI), and the frequency of null alleles (r) of 25 cultivars analyzed at 10 SSR markers.

SSR locus	n	He	Ho	PI	r
NCPGR36	4	0.36	0.36	0.482	0.006
NCPGR40	6	0.77	0.92	0.160	-0.082
NCPGR93	7	0.69	0.12	0.188	0.338
NCPGR28	2	0.11	0.04	0.806	0.065
NCPGR33	5	0.70	0.08	0.265	0.365
NCPGR34	5	0.62	0.04	0.366	0.358
NCPGR21	10	0.69	0.20	0.208	0.290
NCPGR42	11	0.84	0.60	0.074	0.134
NCPGR100	3	0.51	1.00	0.577	-0.316
NCPGR27	5	0.56	0.04	0.297	0.337
Total	58				
Average	5.8	0.58	0.34		

The mean heterozygosity (Ho) and expected heterozygosity (He) were determined to be 0.344 and 0.594, respectively (Table 3). Genetic information is quite similar in chickpea genotypes (Atalay and Babaoglu, 2012) and this may be the cause of low heterozygosity. Saeed et al. (2011), Atalay and Babaoglu (2012), Sethy et al. (2006) reported similar results to our data. Chickpea is a self-pollinated crop with low frequency of outcrossing

(Toker et al. 2006). The heterozygosity in self-pollinated crop is mainly resulted in the low level.

In the dendrogram from SSR analysis, it was observed that genotypes of *C. reticulatum* were found to be clearly different from cultivars of *C. arietinum*, and 23 cultivars of *Cicer arietinum* branched in four main groups (Figure 1). According to the dendogram, most of the genetic similarity values were between 0.55 and 0.85. These results may demonstrate that cultivated chickpea cultivars are closely related among themselves. Most of the cultivars may share the same genetic gene pools. This explains why yield improvement and increased tolerance to various biotic and abiotic stresses have been slow in chickpea. (Choudhardy et al., 2012)

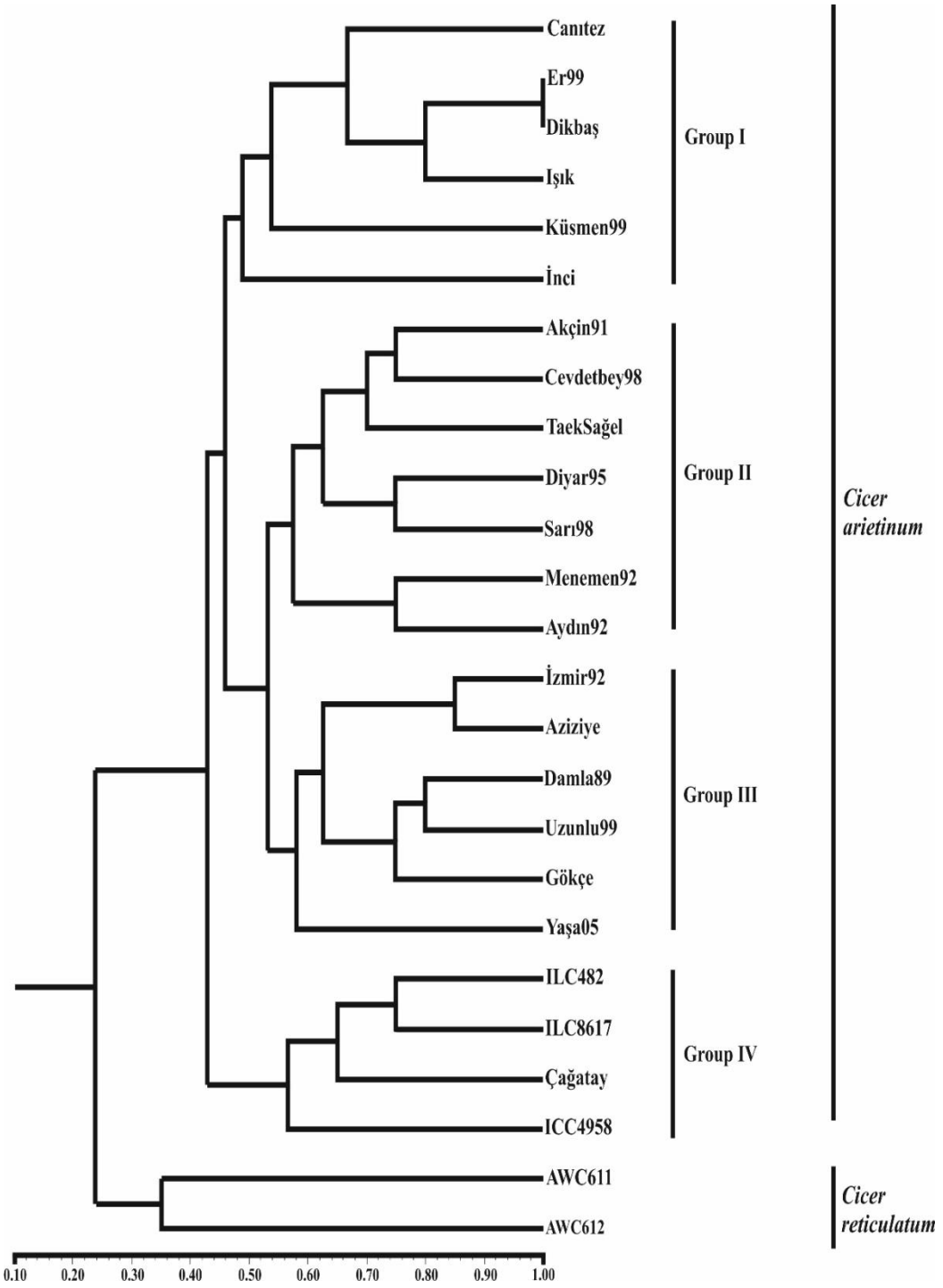

Figure 1. Genetic similarity (%) dendogram of chickpea cultivars used in this study.

For a long time, cultivated species have been selected and hybridized naturally and artificially; the wild species have almost not been used (Weiguo et al., 2007). Resistance to biotic and abiotic stresses has been found in wild *Cicer* species (Singh, 1998); therefore the use of resistant cultivars will help to stabilize chickpea yield (Tar'an et al., 2007). Selection for resistance to biotic and abiotic stresses has become important. The knowledge of genetic relationships between the cultivated chickpea and its wild relatives can be used for introgression of useful traits into the cultigen in plant breeding programs (Singh et al., 2008; Toker et al. 2013). Chickpea improvement programs are underway, but traditional breeding approaches are slow, and possibilities to introgress useful traits from wild germplasm are limited by crossability barriers (Smykal et al. 2014). Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Our result demonstrated that ICC 4958 (*C. arietinum*) which Radhika et al. (2007) indicated it as a drought tolerant cultivar was genetically the closest cultivar to ACW611 and AWC612 (*C. reticulatum*). Toker et al. (2013) reported that the hybrid vigor for yield and yield components in F_1s was higher in interspecific crosses than those of intraspecific crosses. Also, transgressive segregations in $F_{2\text{-}3}$s were higher in interspecific crosses than those of intraspecific ones. Their results have proven that genetic differences among *Cicer* species based on SSR markers have been powerful predictor for hybrid vigor in F_1s and fruitful segregations. Therefore, genetically different cultivars should be undertaken in breeding programs.

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