ANALYSIS OF GENETIC RELATIONSHIPS AMONG PERENNIAL AND ANNUAL *Cicer* SPECIES GROWING IN TURKEY USING ALLOZYME AND DNA POLYMORPHISMS

A DISSERTATION SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE MIDDLE EAST TECHNICAL UNIVERSITY

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
THE DEPARTMENT OF BIOLOGY

JUNE 2001

ABSTRACT

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June 2001, 121 pages

Genetic relationships among annual *Cicer* species have been studied using various approaches. In this study, enzyme and Randomly Amplified Polymorphic DNA (RAPDs) polymorphisms were used to deduce relationships among annual and perennial species with distribution in Turkey.

In allozyme analysis, using seven enzyme systems at 12 putative loci allozyme variation was surveyed in wild and cultivated *Cicer* accessions representing four perennial and six annual species. While variation within species appears to be limited in annuals, relatively high levels observed in some perennials. Generally, allozyme variation was common among species and many of the species could be differentiated on the basis of allozyme they have at the respective loci. Moreover, annuals and perennials as groups had different alleles at many of the loci studied. Genetic distances among accessions were computed from allele frequencies, and by taking averages of pairwise distance coefficients for accessions within each species, pairwise genetic distances for species were estimated. Cluster analysis of distance matrix for species resulted in a dendrogram with two main clusters; one contained three perennial species (*C. montbretii*, *C. isauricum* and *C. anatolicum*) and the other had one perennial and six annual species (*C. incisum, C. pinnatifidum*, *C. judaicum*, *C. bijugum*, *C. echinospermum*, *C. reticulatum* and *C. arietinum*). From this grouping, it was apparent that among studied perennial species *C. incisum* is the closest perennial to the studied annuals, and as in previous studies *C. reticulatum* was found to be the closest annual to the chickpea. Overall, the close clustering of accessions belonging to two closely related species (*C. echinospermum* and *C. reticulatum*) with *C. arietinum* and their distribution in the southeastern

Anatolia is consistent with the idea of the evolution of chickpea from these species, presumably from *C. reticulatum*, and this region is being the center of origin for chickpea.

In RAPD analysis, using seven arbitrary primers selected from the set of 50 depending on their ability to amplify *Cicer* genomic DNA across all species were used to investigate genetic variation and relationships in same *Cicer* collection. Surveying RAPD variation in *Cicer* accessions yielded 95 reproducible RAPD bands (gel positions). The data matrix obtained from RAPD profiles of each accession was used to used to compute genetic distances among accessions according to Nei and Li's formula (1979). Cluster analysis and principle coordinate analysis were carried out to obtain graphical representations of relationships among accessions and species. Generally, the grouping of accessions and species corroborated the grouping obtained in allozyme analysis; *C. reticulatum* is the closest annual to the chickpea, and *C. incisum* is the closest perennial to annuals especially to the group containing *C. pinnatifidum, C. judaicum*, and *C. bijugum*. However, some differences were apparent. Results from RAPD analysis indicate that DNA polymorphisms could be more suitable and quick means to asses relationships and variation in the genus *Cicer*.

Keywords: *Cicer*, Legume, Chickpea, Allozyme, RAPD, Variation, Genetic relationship, Cluster analysis, Principle coordinate analysis.

ÖZ

TÜRKİYE'DE YETİŞEN PERENNİAL VE ANNUAL *Cicer* TÜRLERİ ARASINDAKİ GENETİK İLİŞKİLERİN ALLOZİM VE DNA POLİMORFİZMLERİ İLE ANALİZİ

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Haziran 2001, 121 sayfa

Annual *Cicer* türleri arasındaki genetik ilişkiler bu güne kadar değişik teknikler ile araştırılmıştır. Bu çalışmada ise allozimler ve rasgele çoğaltım (randomly amplified) ile elde edilen polimorfik DNA fragmentleri kullanılarak Türkiye'de yayılış gösteren perennial ve annual *Cicer* türleri arasındaki genetik ilişkiler çalışılmıştır.

Dört perennial ve altı annual *Cicer* türünü temsil eden yabani ve kültür nohutu aksesyonunda yedi enzim sistemi kullanılarak muhtemel 12 lokusta allozim varyasyonu taranmıştır. Bazı perennial türlerde tür içi varyabilite dikkate değer oranda yüksek olduğu tespit edilmesine rağmen çalışılan annual türlerde allozim varyasyonu nispeten düşük bulunmuştur. Diğer taraftan türler arası allozim varyasyonunun oldukça yaygın olduğu ve hatta *Cicer* türlerinin belirli lokuslardaki alleler ile ayırt edilmelerinin mümkün olduğu tespit edilmiştir. Bunun yanında tek ve çok yıllık türlerin bir çok lokusta grup olarak farklı allellere sahip oldukları gözlenmiştir. Aksesyonlar arası genetik mesafe katsayıları kullanılarak yapılan öbeklendirme analizleri (cluster analizi) *Cicer* türlerinin iki guruba ayrıldıklarını göstermiştir. Bu guruplardan birinde üç perennial tür (*C. montbretii*, *C. isauricum* ve *C. anatolicum*) birlikte gruplanırken, bir perennial tür (*C. incisum*) ve altı annual tür (*C. pinnatifidum*, *C. judaicum*, *C. bijugum*, *C. echinospermum*, *C. reticulatum* ve *C. arietinum*) diğer grubu oluşturmuştur. Elde edilen bu gruplama, çalışılan perennial türlerden annual türlere en yakın türün *C. incisum* olduğunu ve daha önceki çalışmalar ile uyumlu olarakta kültür nohutuna en yakın annual türün *C. reticulatum* olduğu göstermektedir. Genel olarak değerlendirildiğinde, kültür nohutu aksesyonları ile *C. reticulatum* ve *C. echinospermum* aksesyonlarının birlikte gruplanması ve bunların Türkiye'nin

güney doğusunda yayılış göstermeleri; kültür nohutunun bu yabani türlerden evrimi ve bu bölgenin nohutun orijin merkezi olduğu görüşü ile uyum içindedir

Aynı *Cicer* koleksiyonundan seçilen 43 aksesyon kullanılarak aksesyonlar ve türler arası ilişkiler RAPD markırları ile çalışıldı. *Cicer* türlerinde genomik DNA yı yinelenebilir olarak çoğaltan yedi 10 mer oligonükleotit primeri ile *Cicer* aksesyonlarında RAPD varyasyonu tarandı. Tespit edilen 95 jel pozisyonundaki çoğaltım ürünlerinin değerlendirilmesi ile hazırlanan data matrisi kullanılarak aksesyonlar için öbeklendirme analizi yapıldı. Genel olarak, RAPD ler ile yapılan öbeklendirmenin allozimle ile yapılan öbeklendirmeye paralel olduğu gözlenmiştir. RAPD analizide allozim çalışması gibi *C. incisum*'un annuallara en yakın perennial tür olduğunu ve *C. reticulatumun* kültür nohutuna en yakın annual tür olduğunu göstermiştir. Genel olarak değerlendirildiğinde, RAPD markırlarının *Cicer* genusunda varyasyon ve türler arası ilişkileri belirlemede informatif bir teknik olduğu tespit edilmiştir.

Anahtar Kelimeler: *Cicer*, Baklagil, Nohut, Yabani nohut, Allozim, RAPD, Varyasyon, Genetik ilişki, Öbeklendirme analizi, Temel koordinatlar analizi.

ACKNOWLEDGMENTS

I am grateful to my major Professor, Aykut KENCE, for his guidance, encouragement and support throughout this study, and Prof. Dr. Musa DOĞAN for his help and guidance in evaluation of newly collected field samples.

I would like to thank Committee members; Prof. Dr. Nihat BOZCUK, Prof. Dr. Semra KOCABIYIK, Assoc. Prof. Dr. Mahinur S. AKKAYA, Assoc. Prof. Dr. Can BİLGİN.

Final thanks go to Mount Ali. I believe she deserves tons of it. She has been the most delightful item of my everyday scenery and given me endless solid support and encouragement to make this study possible.

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INTRODUCTION

1.1. Crop genetic resources and wild relatives

Wild relatives of crops have had an important role in improving agricultural productivity and a number of other characteristics. They are good sources of resistance to biotic stresses such as diseases and pests and tolerance to abiotic stresses such as cold, drought and salinity. Since wild forms live in their natural habitats with a constant exposure to pathogens, pests, severe climates and unfavorable soils, various strategies evolved in their genomes to cope with such harsh growing conditions. These strategies are the products of millions years of evolution and very valuable to agriculture. Thus, wild relatives constitute an arsenal to the modern cultivars as potential sources of variation harboring economically important traits. On the other hand, crops have been under human protection against weeds, pests and pathogens. In addition, a more uniform environment for their

growth in terms of soil conditions, fertilization and irrigation provided by human (Plucknet *et. al.* 1987). Moreover, intensive breeding of crop varieties for decades has narrowed the gene pool (reduction in genetic variation) in many crops and resulted in genetic uniformity. This is especially apparent in self-pollinated crops in which the level of genetic variation in cultivated varieties is often a small fraction of the variation found in nature. The limited genetic variation is undesirable because it generally makes the crop varieties vulnerable to biotic and abiotic stresses. Reduced genetic variability is likely to have another effect; slower rate of crop improvement. The lower the genetic variation in breeding material, the less likely the breeder can identify new and useful genes and gene combinations (Tanksley and Nelson, 1996; Xiao *et. al.* 1996; Hoisington *et. al.,* 1999).

In conclusion, all crop species were originally domesticated from wild plants with a significant reduction in genetic variation, and crop genetic resources include gene pools of both wild and cultivated forms. Wild forms generally harbor extensive genetic variation containing many economically important traits, thus, constitute an arsenal to fuel the crop improvement. This is a major reason why conserving and studying the close relatives of crops represent an invaluable investment.

Utilization of wild relatives in crop improvement requires exploration, collection, documentation and evaluations of wild germplasm material. Last few decades have witnessed the global awareness for the erosion of crop genetic resources and use of wild relatives to improve crop production to compensate demand for food. As a result, a number of organizations and network systems (e.g., International Plant Genetic Resources Institute, IPGR; System-wide Information Network for Genetic Resources, SINGER) have been set up to coordinate and to develop strategies for *in situ* and *ex situ* conservations of crop genetic resources. They provide instant access to information regarding the conservation activities and germplasm deposited in international gene banks.

In conservation plant genetic resources, the main purpose is to conserve as broad as the genetic diversity of the species or taxonomic unit in consideration. In efficient conservation of crop genetic resources, understanding the structure of germplasm collections and sampling the wild germplasm accessions systematically are important aspects. Molecular markers have been used extensively to quantify the genetic diversity and determine relationships. Cluster and ordination analysis (principle coordinate analysis) are useful for studying the relationships among wild germplasm. They can be used to obtain core collections representing the most of the

diversity for each species avoiding the redundant and inefficient maintenance of large number of accessions (Karp et. al. 1997; Kumar *et. al.* 1999).

1.2. Plant genetic resources in Turkey

Turkey resides on the intersection of three phytogeographic regions; Mediterranean, Irano-Turanian and Euro-Siberian. It is rich in terms of plant genetic resources, especially the wild relatives of major crop species such as wheat, barley and chickpea. Several well known scientists including Vavilov, Zhoray and Harlan have recognized this potential between 1920 and 1970. South East Anatolia has been proposed to be the site of domestication for several crop species such as wheat, barley, and chickpea. This potential makes Anatolia a strategic phyto-geographic area for the improvement of major crop species.

1.3. Chickpea improvement and production

Chickpea, *C. arietinum* L, is one of the most important pulse crop on the global basis. It is grown in South Asia, West Asia, North Africa, East Africa, Australia, South and North America, and southern Europe. Chickpea cultivation constitutes the nearly 15 % (10.2 million hectares) of the area and

accounts for 14 % (7.9 million tons) of the production of pulses in the world (Singh 1997a). It is also widely cultivated throughout Turkey (630 000 hectares with an annual production of 600 000 metric tons, in 1999). Turkey is the largest chickpea exporter after Australia providing the 31 % of the World's exported chickpea (FAO 2000, Figure 1).

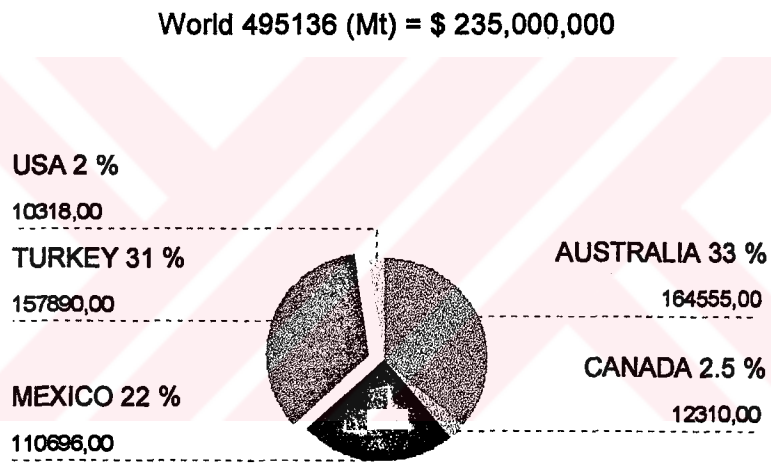

Figure 1. Amount of chickpea exported by some major producer states.

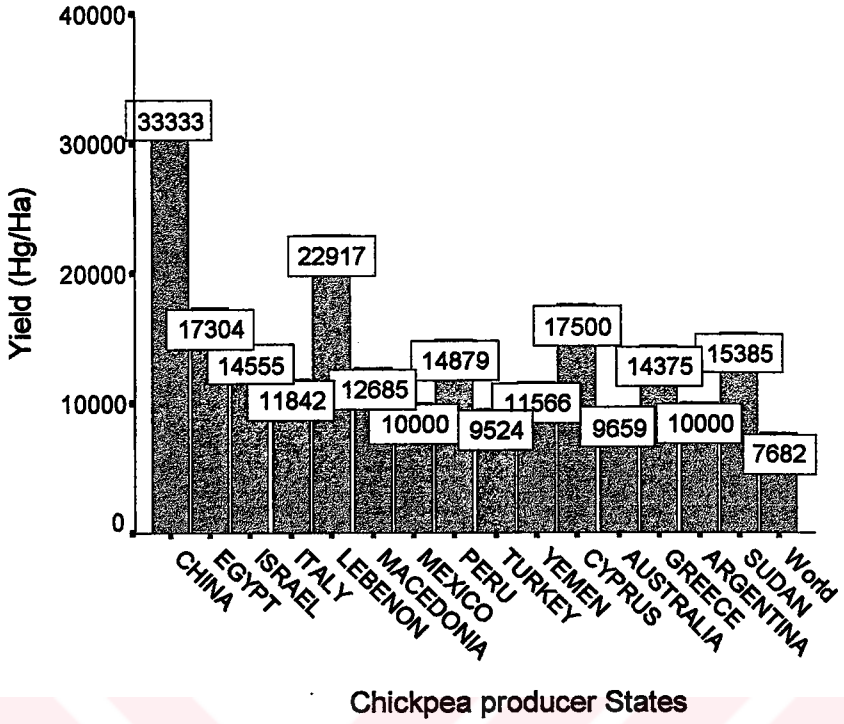

Figure 2. Chickpea yields of some producer states

Although Turkey is one of the major chickpea producer states, the average chickpea yield of it is one of the lowest (Figure 2). Obviously, this is contradictory to the position and the potential of Turkey. It clearly indicates the importance of conservation and utilization of genetic resources to improve yield and other characteristics of chickpea.

Chickpea is mainly cultivated for its edible seeds, which contain high quality protein. For the poor people mainly in under-developed and developing countries and for vegetarians, it is a cheap source of protein.

Other uses of chickpea include feed for livestock and substitute for fallow in cereal rotations in farming. It improves the crop production and reduces the need for N fertilization through fixing the atmospheric nitrogen. Chickpea production appears to fall short of the demand because of the insufficient increase in the area of cultivation or in yield (Muelbauer and Tullu, 1997; Singh, 1997).

As an important grain legume, investigation of relatedness and genetic diversity within and among species of the genus *Cicer* and its genetic resources are an obvious necessity to identify new accessions bearing valuable genes which may be used in breeding, molecular characterization and genetic transformation experiments to generate varieties/transgenic plants with improved qualities and better yield potentials. Use of wild species have yielded significant contributions to the crop improvement mainly in cereals (Stalker, 1980; Xiao *et. al.* 1996; Hoisington *et. al.* 1999). However, in chickpea, use of wild species in chickpea improvement is relatively recent and traditional breeding methods have not produced cultivars with a dramatic increase in production. As a result, the world average yield of the chickpea (700 kg/ha) is believed to be below its presumed potential (Singh *et. al.* 1994). One of the reason for this drawback was speculated to be the lack of traits in the world chickpea germplasm collection needed for effective

improvement (Robertson *et. al.* 1997). However, the screening of *Cicer* germplasms for various traits at the ICARDA and ICRISAT revealed that wild *Cicer* accessions do harbor genes for resistance to a number of biotic and abiotic stresses (Singh *et. al.*1994; Muehlbauer *et. al.* 1994; Akem *et. al.* 2000). *Cicer* species appear to bear genes resistance to ascochyta blight (*Ascochyta rabiei* (Pass) Lab.), Fusarium wilt (*Fusarium oxysporum* Schlecht emend Synd. and Hans. f. sp. *ciceri* (Padwick) Synd. and Hans.), leaf miner (*Liriomyza cicerina* Rondani), seed bettle (*Callosobruscus chinensis* L.), cyst nematode (*Heterodera ciceri* Vovlas, Greco et Di Vito) and cold. These accessions are being utilized in interspecific hybridization experiments to improve seed yield and some other characteristics of chickpea (Singh *et al* 1994; Singh and Ocampo 1997b; Singh 1997).

1.4. Molecular tools to determine genetic variation and relationships

In estimating genetic diversity or relatedness among groups of taxonomic units, genetic markers are commonly used. Genetic markers are alleles residing on specific chromosomal locations (loci) and marking that segment in genome analysis (Kumar *et. al.*1999). They provide direct experimental access to follow the region and its function or traits carried on

that segment. They are also commonly used to determine the amount of genetic variation and genomic closeness in and among taxonomic units.

Genetic markers are generally two types; morphological and molecular markers. Morphological markers can be detected without a specialized biochemical or molecular technique. They are generally single gene controlled morphological traits. When reproducibly scored over a range of environments and not effected by epistatic or pleitrophic interactions, they can be used as marker. A molecular marker is a protein or a sequence of DNA which reveal polymorphism at molecular level. As in morphological markers, inheritance of these polymorphisms can be followed, but using specialized molecular techniques. Markers that reveal polymorphisms at protein level are named as biochemical markers while markers that reveal polymorphism at DNA level are known as DNA markers. Biochemical markers are the protein molecules whose allelic variation can be detected by electrophoresis. DNA markers can be classified into two groups based on the approach followed in their detection; hybridization-based DNA markers and PCR-based markers (Weising *et. al.* 1995; Kumar *et. al.* 1999).

1.4.1. Enzymes as biochemical markers in studies of genetic relationships

One of the most widely used techniques for revealing genetic variation in enzymes and other proteins is electrophoresis. Enzyme electrophoresis has been used to study population genetics and to describe the patterns of variation since 1960s. Principle of the enzyme electrophoresis is the detection of the variant forms of a specific enzyme. It is relatively easy, fast and inexpensive technique and involves the separation of enzymes contained in an extract prepared from a small sample of tissue in a gel matrix (starch or polyacrylamide) in an electric field. Following electrophoresis, the gel is stained histochemically to visualize electrophoretic variants (Wendel, 1989; Murphy *et. al.* 1990).

Isozymes and allozymes are both structurally and catalytically similar, but different molecular forms of enzymes. Allozymes are encoded by alternative alleles at one locus while isozymes are different molecular forms of an enzyme encoded by the alleles residing at different loci (Murphy *et. al.* 1990). In evaluating banding patterns on a gel, a number of factors have to be considered such as the number of coding genes, their allelic states, quaternary structure of the enzyme (dimeric, tetrameric etc.) and their

subcellular compartmentalization. Inheritance of allozymes are co-dominant, thus, one can distinguish homozygotes from heterozygotes, and depending on the number of polypeptides in an enzyme molecule (quaternary structure) different number of bands are observed in homozygotes and heterozygotes due to the random association of the variant subunits (Murphy *et. al.* 1990). For example, individuals homozygous for an allele encoding a monomer enzyme will have a single band whereas heterozygotes for alternative allozymes will have two bands. Similarly, for the dimer enzymes, heterozygotes will have three bands (two dimer homomers and one heteromer). When the polypeptides of a multimeric enzyme are encoded by different loci, this pattern gets more complicated due to the formation of isozymic heteromers ((Murphy *et. al.* 1990). Allozyme polymorphisms deal with the limited number of enzyme systems (about 40 enzyme systems) and represent a proportion of the variations in the coding region. Thus, they are not very useful markers in studies of variation and genome analysis (Weising *et. al.* 1995).

1.4.2. DNA polymorphisms in studies of genetic relationships

DNA sequence polymorphisms have been widely used to probe the variation in samples of plant populations. Last two decades have witnessed

to the search and development of a number of DNA markers to study complex genomes. Restriction Fragment Length Polymorphisms (RFLP, Botstein *et. al.* 1980) is the fist type of DNA-based markers and used extensively in many crop species to study the various aspects of the plant genomes (Landry and Michelmore 1987; Helentjaris *et. al.* 1985; Bonierbale *et. al.* 1988; Tanksley *et. al.* 1989; Dowling *et. al.* 1990; Heun *et. al.* 1991; Weising *et. al.*1995). RFLP technology is, however, both expensive and time consuming. It also requires large amount of DNA and radioactive or non-radioactive labeled probes for detection. In 90's, with the advances in PCR technology, a number of PCR-based DNA marker techniques have been developed some of which are technically simple and relatively easy to assay. DNA polymorphisms detected via PCR can be random or specific depending on the type of primer used, the stringency of the PCR conditions, and the method of separation and detection. Among these, a subset involves use of a single arbitrary primer in PCR reactions, the result of which is usually the amplification of many discrete DNA fragments. Each product is presumably derived from a region of the genome that contains two short segments, which share sequence similarity to the primer, which are on opposite strands at a distance within amplification range. Techniques of this kind have been named as Multiple Arbitrary Amplicon Profiling (MAAP, Caetano-Anolles, 1994). Random Amplified Polymorphic DNA (RAPD)

described by Williams *et. al.* (1990), Arbitrary Primed PCR (AP-PCR) described by Welsh and MaClellend (1990), and DNA Amplification Fingerprinting (DAF) described by Caetano-Anolles *et. al.* (1991) generate random PCR markers. RAPD analysis requires use of Polymerase Chain Reaction (PCR) with 10-mer primers to generate random amplified fragments of DNA. Polymorphisms are detected as the presence or absence of same size fragments at a particular gel position, which mainly result from sequence differences in the primer binding sites. Unlike the RFLP analysis, RAPDs do not require DNA probes and time consuming lab work. In addition, other advantages of RAPD analysis include the ease and rapidity of analysis, relatively low coast, the availability of large numbers of primers and the requirement of very small amount of DNA for the analysis (Welsh and MaClellend 1990; Williams et. al. 1990; Whitkus *et. al.* 1994; Weising *et. al.* 1995; Kumar, 1999). Despite these advantages, a number of disadvantages are associated with RAPDs such as problems in reproducibility and dominant inheritance, which have to be taken into consideration when using RAPDs as genetic markers (Devos and Gale 1992; Whitkus *et. al.* 1994). Reproducibility is a major concern for RAPDs, and maintenance of consistent reaction conditions is absolutely critical for the achievement of reproducible profiles. Despite a number of limitations, advantages of RAPDs have made them marker of choice in mapping,

determination of phylogenetic relationships and genetic diversity, identification of cultivars and parents in a number of plant species (Skroch *et. al.* 1993; Weising *et. al.* 1995; Abo-elwafa *et. al.* 1995; Sharma *et. al.* 1995a; Simon and Muehlbauer 1997; Wolff and Richards 1998; Butos *et. al.* 1998).

1.5. Classification of the genus *Cicer*

In the Fabaceae family, the genus *Cicer* is the single genus of its own tribe, *Cicereae*. It contains 43 species, nine of which are annual including chickpea (*Cicer arietinum* L.), and the rest are perennial. *Cicer* species are predominantly self-pollinating, and the chromosome number of annual species were determined to be 2n = 16 (van der Maesen 1987, Ahmad 2000). *C. arietinum* L., is the only cultivated species, which characterizes the genus.

Based on the life cycle, geographic distribution and morphological characteristics, four sections have been recognized within the genus. One of these sections, *Monocicer* contains eight annual species including chickpea (*C. arietinum* L., *C. reticulatum* Ladiz., *C. echinospermum* P.H. Davis, *C. judaicum* Boiss., *C. pinnatifidum* Jaub. & Spp., *C. cuneatum, C. yamashita*

and *C. bijugum* K.H.Rech.). In the section *Chamocicer,* there is one annual (*C. chorassanicum*) and one perennial (*C. incisum*) species. The remaining two sections, *Polycicer* and *Acantocicer*, contain entirely the perennial species. Three of the perennial species from *Polycicer* section were used in this study, *C. montbretii* Jaub., *C. isauricum* Dav. and *C. anatolicum* Alef., (van der Maesen, 1972; 1987).

1.6. Studies of relationships in the genus *Cicer*

Several groups have studied genetic diversity and relatedness among annual *Cicer* species by means of hybridization, electrophoresis of seed storage proteins, isozymes and RAPDs (Ladizinsky and Adler 1976; van der Maesen 1987; Kazan and Muelbauer 1991; Ahmad *et. al.* 1992; Ahmad and Slinkard, 1992; Tayyar and Waines 1996; Labadi *et. al.* 1996; Ahmad 1999). Analysis of crossability among annual species was one of the first strategies used to study genetic relationships. Ladizinsky and Adler (1976) have studied the crossability relationships among seven annual *Cicer* species, *C. arietinum, C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum* and *C. cuneatum,* and they grouped these species into three clusters. Group I species included *C. arietinum, C. reticulatum* and *C. echinospermum.* Group II comprised *C. pinnatifidum, C. judaicum* and *C.*

bijugum. C. cuneatum formed the last group (Group III). In group I species *C. echinospermum* produced sterile hybrids with *C. arietinum* and *C. reticulatum*. However, crossing *C. reticulatum* to one *C. arietinum* variety produced fertile hybrids. In cytogenetic examinations of hybrids involving *C. echinospermum* in both combinations, six bivalents and one quadrivalent were observed indicating that this species carries a reciprocal translocation whereas normal eight bivalents were observed in hybrids between *C. reticulatum* and *C. arietinum*. They also obtained hybrids with relatively high fertility in crosses involving the group II species, and observed mostly bivalents and occasional univalents indicating that these three species are closely related. However, in one of the crosses between *C. judaicum* and *C. pinnatifidum*, a quadrivalent was observed indicating that cytologically *C. pinnatifidum* is closer to the *C. bijugum* in contrast to obvious morphological similarity exist between *C. pinnatifidum* and *C. judaicum* (Ladizinsky and Adler, 1976).

Analysis of seed storage proteins was another approach used to determine relationships among annual species. Ahmad and Slinkard (1992) fractionated the total seed storage proteins of nine annual species on sodium dodecyl sulphate polyacrylamide gels, and constructed the dendrogram of species based on protein profiles. Their grouping of annual *Cicer* species

was consistent with the results obtained from studies of morphological variation, crossability and isozyme variation on interspecific relationships (Ahmad and Slinkard 1992).

Several groups studied isozyme polymorphisms to determine variation and genetic relationships in annuals and two perennial species (Kazan and Muelbauer 1991; Ahmad *et. al.* 1992; Ahmad and Slinkard, 1992; Tayyar and Waines 1996; Labadi *et.al.* 1996). The level of allozyme variation was low in general, and based on allozyme polymorphisms, annual species were classified into four groups. The first group included the chickpea (*C. arietinum* L.) and its closest relatives (*C. reticulatum* Ladiz. and *C. echinospermum* P.H. Davis). The second group included *C. pinnatifidum* Jaub&Spp., *C. judaicum* Boiss. and *C. bijugum* K.H. Rech. The remaining two annual species (*C. cuneatum* and *C. chorassanicum*) formed the last two groups. *C. reticulatum* was the closest wild relative of the chickpea and regarded as the wild progenitor of chickpea.

Although a number of isozyme study have provided considerable insight into relationships and genetic diversity among annual *Cicer* species, in a few studies, DNA-based procedures have been utilized. Weising *et. al.* (1992) and Sharma *et. al.* (1995b) have assayed simple sequence repeat

polymorphisms in chickpea with hybridization based oligonucleotide fingerprinting and found it very informative. Udupa *et. al.* (1999) have recently utilized PCR-based simple sequence repeat $(TAA)_n$ polymorphism in chickpea and two closely related wild species (*C. reticulatum* and *C. echinospermum*) found high levels of polymorphisms. (Weising *et. al.* 1992 ; Sharma *et. al.* 1995b; Udupa *et. al.* 1999). Recently, Ahmad (1999) has used RAPD markers to assess the genetic relationships among annual species.

1.7. *Cicer* species in Turkey

As in many other taxonomic groups, Turkey has considerable diversity in the genus *Cicer*. A quarter of known wild *Cicer* species is distributed in Turkey (van der Maesen 1987; P. H. Davis 1970). *C. bijugum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, *C. reticulatum* are the five annual species, and *C. montbretii, C. isauricum, C. anatolicum*, *C. floribundum* and *C. incisum* are the perennial species showing distribution in Turkey. Turkey resides on one of the centers of diversity proposed for chickpea by Vavilov. A number of other investigators have recognized southeastern Turkey to be the center of origin for chickpea (Ladizinsky and Adler 1976; van der Maesen, 1987; Singh, 1997b). Moreover, studies of

genetic relationships among annual *Cicer* species have revealed that southeast Turkey is an important region in the evolution of chickpea and other annual species. Therefore, the assessment and efficient conservation of genetic resources in Turkey exhibit a prime importance for chickpea improvement. Available information is generally limited to the annual species, and no study has been conducted to evaluate the genetic variation and relationships among annual and perennial species in Turkey. Regarding the perennial species, two studies, one of which included only *C. anatolicum* and the other one studied two perennial species *C. anatolicum* with *C. songoricum* have revealed inconsistent grouping of species *C. anatolicum* with annuals (Kazan and Muelbauer, 1991, Tayyar and Waines, 1996).

1.8. Purpose of the study

Aim of this thesis was to use allozyme and RAPD markers to determine inter- and intra species genetic variation, relationships among annual and perennial species growing in Turkey, the closest perennial relative(s) of the annual species in Turkey, and determine which wild species could be ancestral to the cultivated species.

CHAPTER 2

MATERIALS AND METHODS

2.1. Plant material

Plant material used to survey allozyme variation in this study included 57 wild and cultivated *Cicer* accessions representing ten species. However, only 43 of them were studied in RAPD analysis due to the difficulty in obtaining plant material for DNA extraction. Seeds of perennial species were collected from their natural habitats (given in the flora of Turkey; P.H. Davis, 1988, and obtained from herbarium records). Seeds of annual species were obtained from ICARDA (International Center for Agricultural Research in the Dry Areas Aleppo, Syria), and five accessions provided by Turkish Ministry of Agriculture, Aegean Agricultural Research Institute (AARI), Menemen, İzmir. In addition, six populations of *C. pinnatifidum* were sampled from their natural populations (Table 1). Accessions of *C. arietinum* were provided by Anatolian Agricultural Research Institute, Eskişehir (AARI*, Table 1).

Accessions of annual species were mainly from southeast Turkey concentrating around provinces; Mardin, Diyarbakır, Gaziantep and Urfa. Limited number of samples were collected from central Anatolian region. Accessions of perennial species were from Mediterranean, Western and Central Anatolian regions.

Table 1. *Cicer* species and their accessions used in Allozyme (A) and RAPD (R) analysis.

Cicer Species	No+		Accession	Source§	Origin†
Cicer montbretii Jaub.	1	A, R	Kaz dağı	This study	Çanakkale
	2	A	Bergama	This study	İzmir
	3	A, R	Kurucaova	This study	Konya
Cicer isauricum Dav.	4	A	Ermenek	This study	Konya
	5	A	Akseki	This study	Antalya
	6	A	Kızılcahamam	This study	Konya
Cicer anatolicum Alef.	7	A, R	Kazancı	This study	Kayseri
	8	A, R	Erciyes	This study	Ankara
Cicer incisum	9	A, R	Yahyalı	This study	Kayseri
Cicer judaicum Boiss.	10	A, R	ILWC 148	ICARDA	Gaziantep
	11	A	ILWC 168	ICARDA	Elazığ
	12	A, R	Besni	This study	Adıyaman
	13	A, R	Ereğli	This study	Konya
	14	A, R	Avcılar	This study	K. Maraş
	15	A, R	Erkenek	This study	K. Maraş
	16	A, R	ILWC 33	ICARDA	Adana
	17	A, R	ILWC 236	ICARDA	Urfa
Cicer pinnatifidum Jaub&Spp.	18	A, R	ILWC 9	ICARDA	Elazığ
	19	A, R	ILWC 250	ICARDA	Elazığ
	20	A, R	ILWC 29	ICARDA	Gaziantep
	21	A, R	ILWC 78	ICARDA	Mardin
	22	A, R	ILWC 248	ICARDA	Diyarbakır
	23	A, R	ILWC 251	ICARDA	Diyarbakır
	24	A, R	Kır obası	This study	İçel Mut
	25	A	TR 23039	AARI	Bingöl

Table 1. (continued)

Cicer Species	No+		Accession	Source§	Origin†
	26	A, R	ILWC 239	ICARDA	Urfa
	27	A, R	ILWC 245	ICARDA	Urfa
Cicer	28	A, R	ILWC 246	ICARDA	Urfa
echinospermum P.H. Davis	29	A, R	ILWC 235	AARI	Gaziantep
	30	A, R	ILWC 238	AARI	Gaziantep
	31	A, R	TR 39241	ICARDA	Bingöl
	32	A, R	TR 54962	ICARDA	Diyarbakır
	33	A, R	ILWC 240	ICARDA	Urfa
	34	A, R	ILWC 241	ICARDA	Urfa
	35	A, R	ILWC 243	ICARDA	Urfa
Cicer bijugum K.H. Rech.	36	A, R	ILWC 8	ICARDA	Diyarbakır
	37	A, R	ILWC 177	ICARDA	Diyarbakır
	38	A, R	ILWC 62	ICARDA	Mardin
	39	A, R	ILWC 32	ICARDA	Gaziantep
	40	A, R	ILWC 36	ICARDA	Mardin
	41	A, R	ILWC 182	ICARDA	Mardin
	42	A, R	ILWC 216	ICARDA	Mardin
Cicer reticulatum Ladz.	43	A, R	ILWC 218	ICARDA	Mardin
	44	A, R	ILWC 81	ICARDA	Diyarbakır
	45	A, R	ILWC 237	ICARDA	Urfa
	46	A, R	ILWC 247	ICARDA	Adıyaman
	47	A, R	TR 54961	AARI	Siirt
	48	A, R	TR 54963	AARI	Siirt
	49	A	ILC195	AARI*	
	50	A	ILC482	AARI*	
	51	A, R	Diyar 95	AARI*	
Cicer arietinum L.	52	A	Kırmızı nohut	AARI*	
	53	A	Akcin 91	AARI*	
	54	A, R	Canitez 89	AARI*	
	55	A, R	Eser 87	AARI*	
	56	R	Aydin 92	AARI*	
	57	A	Akn 51	AARI*	
	58	A	Menemen 91	AARI*	

§ ICARDA: International Center for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria. AARI: Aegean Agricultural Research Institute, Menemen, İzmir. AARI*: Anatolian Agricultural Research Institute, Eskişehir.

† Detailed collection addresses are given in Appendix D.

+ The number coding each accession corresponds to the numbers in the figures.

A, Accessions studied in allozyme analysis.

R, Accessions included in RAPD analysis.

2.2. Enzyme electrophoresis

Seeds of accessions were used to prepare extracts. They are good sources of enzymes. Besides, life stages of donor genotype in chickpea appear to influence the levels of allozyme variation, hence, the topologies of the dendrograms seem to vary with the life stages. The most suitable stage for allozyme polymorphism survey was observed to be the seed stage (Triest & Kabir, 2000). Thus, extracts were prepared from 40-50 mg cotyledon sections of seeds which had been presoaked in water for 24 h. The tissue pieces were homogenized in 1.5 mL Eppendorf tubes with 200 - 250 μL. extraction buffer (50 mM Tris-HCl, 10 mM DTT, % 5 sucrose, pH 7.5) on ice with a glass rod (Wendel & Weeden, 1989). Following a quick spin, filter paper wicks with the dimensions of 2x9 mm were dipped into the supernatant and excess liquid was blotted on a paper for few seconds, then, applied to the gel. Gels were prepared by boiling 10 % starch with appropriate gel buffer. Electrophoresis was carried out on a water jacket cooled to 4 °C.

Seven enzyme systems were studied using Histidine-Citrate (HC) and Morpholine-Citrate (MC) buffers. Alcohol dehydrogenase (ADH, E.C.1.1.1.1), 6-Phosphogluconate dehydrogenase (PGD, E.C.1.1.1.44) Phosphoglucomutase (PGM, E.C.2.7.5.1), Isocitrate dehydrogenase (IDH,

E.C.1.1.1.42), Phosphoglucoisomerase (PGI, E.C.5.3.1.9) and Shikimate dehydrogenase (SKDH, EC 1.1.1.25) isozymes were resolved with HC system (pH 6). Malate dehydrogenase (MDH, E.C.1.1.1.37) isozymes were resolved using MC. buffer (pH 7). After electrophoresis, gels were cut horizontally into 2 mm slices, and each slice was incubated in a staining solution containing substrate for activity staining for the respective enzyme and other ingredients. Recipes for the electrophoresis and staining buffers which were adapted from Wendel and Weeden (1989) are given in Tables 2 and 3.

Table 2. Electrophoresis and gel buffers.

Buffer	Electrode buffer	Gel Buffer
Histidine Citrate	65 mM L Histidine, free base. 19 mM Citric acid, monohydrate. pH adjusted to 6 by adding ~2.5 g/L solid Citric acid monohydrate.	9 mM L Histidine, free base. 6 mM Citric acid, monohydrate. The gel buffer was 1:6 dilution of electrode buffer with ddH_20.
HC gels were run at 25 mA for 5:30 h.		
Morpholine Citrate	40 mM Citric acid, monohydrate. pH adjusted to 7 with N-(3-Aminoproply)-morpholine, ~10 ml/L.	The gel buffer was 1:19 dilution of electrode buffer with ddH_20.
MC gels were run at 30 mA for 5:30 h.		

Table 3. Gel staining recipes

Enzyme system	Recipe		Staining
Alcohol dehydrogenase ADH (E.C.1.1.1.1) NAD^+ oxidoreductase	50 mM Tris HCl, pH 8	25 ml	These ingredients were combined in the fume hood, and poured onto the gel in a staining tray which was incubated at 37 °C for 30'.
	NAD	5 mg	
	Ethanol 96 %	200 ul	
	MTT	5 mg	
	PMS	1 mg	
6-Phosphogluconate dehydrogenase 6PDG (E.C. 1.1.1.44)	50 mM Tris HCl, pH 8	25 ml	These ingredients were combined and poured onto the gel in a staining tray which was later incubated at 37 °C for 30'.
	6-Phosphogluconic acid	10 mg	
	$MgCl_2$	25 mg	
	NADP	2 mg	
	MTT	5 mg	
	PMS	1 mg	
Phosphogucoisomerase PGI (E.C.5.3.1.9)	50 mM Tris HCl, pH 8	25 ml	These ingredients were combined, poured onto the gel in a staining tray and the gel incubated at 37 °C for 30'.
	Fructose-6-phosphate Na_2 salt	10 mg	
	G6PDH	10 U	
	NADP	2 mg	
	MTT	5 mg	
	PMS	1 mg	
Phosphoglucomutase PGM (E.C.2.5.7.1)	50 mM Tris HCl, pH 8.5	25 ml	These ingredients were dissolved in the buffer and poured onto the gel in a staining tray. The tray was incubated at 37 °C for 30'.
	Glucose-1-phosphate Na_2 salt	50 mg	
	$MgCl_2$	25 mg	
	G6PDH	10 U	
	NADP	2 mg	
	MTT	5 mg	
	PMS	1 mg	
Isocitrate dehydrogenase IDH (E.C. 1.1.1.41)	50 mM Tris HCl, pH 8	25 ml	These ingredients were dissolved in the buffer, poured onto the gel in a staining tray and incubated at 37 °C for 30'.
	Isocitric acid Na_3 salt	50 mg	
	$MgCl_2$	25 mg	
	NADP	2 mg	
	MTT	5 mg	
	PMS	1 mg	

Table 3. Gel staining recipes (continued)

Enzyme system	Recipe		Staining
Shikimate	50 mM Tris HCl, pH 8.5	25 ml	These ingredients were
dehydrogenase	Shikimic acid	25 mg	combined, poured onto the gel
SKDH (E.C.1.1.1.25)	NADP	2 mg	in a staining tray and the gel
	MTT	5 mg	was incubated at 37 °C for 30'.
	PMS	1 mg	
Malate dehydrogenase	50 mM Tris HCl, pH 8.5	25 ml	These ingredients were
MDH (E.C.1.1.1.37)	L-Malic acid	75 mg	combined in the buffer, poured
	NAD	5 mg	onto the gel in a staining tray
	MTT	5 mg	and incubated at 37 °C for 30'.
	PMS	1 mg	

2.3. DNA extraction for RAPD analysis

Leaf material was used to extract DNA from *Cicer* accessions. Seeds of accessions for annual species and two perennial species (*C. anatolicum, C. incisum*) were germinated and grown in a growth chamber. After 4-5 weeks of growth period, the youngest leaves of the seedlings were harvested to extract DNA. Attempts to germinate seeds of the accessions for the remaining perennial species were unsuccessful. The plant material for them was the youngest dried leaflets which were ground in liquid nitrogen and used to extract DNA (Weising *et. al.* 1995). No significant degradation of DNA and differences in RAPD patterns were detected between fresh frozen

and dried material when compared in *C. anatolicum* and *C. incisum* for which both types of material were available.

CTAB DNA extraction procedure, as described in Hulbert and Bennetzen (1991), was used to isolate DNA from *Cicer* samples. The procedure was scaled down to carry out in 1.5 mL Eppendorf tubes. Briefly, extraction procedure was as follows; leaf material was ground in liquid nitrogen until it becomes a fine powder using a pre-cooled pointed screwdriver in a 1.5 mL Eppendorf tube. The powder was suspended in 600 - 700 µL of 2 x CTAB extraction buffer (1.4 M NaCl, 2 % hexadecyltrimethylamonium bromide (Sigma) 1 % β-mercapto ethanol, 100 mM Tris-HCl, pH 8) and incubated in a water bath at 65 °C for 30-45 min with occasional mixing. Equal volume of chloroform-isoamyl alcohol (24:1) was added to the mixture, inverted for few times and left at room temperature for 15 min. The emulsion was centrifuged at 10 000 x g for 10 min and the supernatant was transferred into a new centrifuge tube. Chloroform extraction was repeated twice and before the last extraction RNA was digested by adding 0.5 µL RNAse A (10 mg/ml) and incubating at 37 °C for an hour. Supernatant was transferred into a fresh tube, and DNA was precipitated by adding 0.8 volume of isopropyl alcohol and inverting it several times. DNA pellet was precipitated with a brief spin followed by a 70 %

ethanol wash. The DNA pellet was air dried and dissolved in appropriate volume of sterile ddH_2O. Concentrations of DNA samples were determined by running on 1 % agarose gel with 1 μg of *Hind* III digested λ DNA.

2.3. RAPD assay

Fifty 10-mer random oligonucleotide primers (10-mer Set 50/1(1)), obtained from Dr. John Hobbs (Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., Canada), were used to amplify *Cicer* genomic DNA. Following an initial screening, seven primers that amplified clear and reproducible bands in all *Cicer* species were used to detect RAPD variation in accessions (Table 2). Amplification reaction volume was 25 μL, each containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1 % Triton X-100, 1.5 mM $MgCl_2$, 100 μM of each dATP, dCTP, dGTP, and dTTP, 2 μM primer, 0.8 units of *Taq* DNA polymerase (Promega) and 25 ng genomic DNA. PCR reactions were performed in a thermocycler with a heated lid (Techne Inc.) programmed for one cycle consisting of an initial denaturation at 94 °C for 3 min, annealing at 36 °C for 1 min and an extension step at 72 °C for 2 min followed by 39 cycles each consisting of a denaturation step at 94 °C for 30 sec. an annealing step at 36 °C for 1 min and an extension step at 72 °C for 2 min. Amplification was terminated by a final extension step of 5 min at 72

°C. PCR products were electrophoresed in 1.5 % agarose gels and visualized by staining with ethidium bromide (0.4 ug/ml) and UV illumination. Molecular weight markers (Sigma PCR Marker; 50 bp - 2 kb) were used to estimate the sizes of amplification products and compare duplicate reactions.

2.4. Analysis of allozyme data

Inheritance and the functional subunit numbers for the enzyme systems, ADH, PGM, PGI, MDH and PGD used in this study were previously characterized in *Cicer* by Gaur and Slinkard (1992a, b). Interpretations of banding patters of SKD and IDH were as in Ahmad (1992), and Weeden and Wendel (1989). Different activity zones appearing on gels were considered as putative loci, and the bands within these zones with different mobilities were considered as putative alleles (allozymes). Symbolic designations were given to loci as numbers starting from the most anodal band as '*1*', '*2*' in order of decreasing electrophoretic mobility. The alleles were designated in letters according to their relative mobilities, the most anodal one is being '*a*' and subsequent bands *b*, *c*, and so on.

The electrophoretic data derived from enzyme systems were analyzed using computer software packages; BIOSYS-1 version 1.7 (Swofford and

Selander, 1981) and NTSYS-pc version 1.7 (Rohlf, 1992). Data were entered as genotypes and a hierarchical arrangement of the accessions was established at the species level so that the data could be analyzed within and among species level and summarized by species.

Genetic variation measures in accessions and species were calculated as percentage of polymorphic loci, the average number of alleles per locus and observed and expected heterozygosity using following expressions.

In our calculations, a locus is considered polymorphic if the frequency of most common allele does not exceed 0.95. It is simply estimated from the allele frequency data by determining the number of polymorphic loci and dividing it by the total number loci examined which is then converted into percentage for each unit (accession or species). Percentage of polymorphic loci is not a good measure of genetic variation. A better estimate of genetic variation is the average heterozygosity, or gene diversity as Nei calls it. Unbiased estimate of heterozygosity for a single (*k*th) locus was calculated according to Nei (1978).

$$h_k = 2n(1-\Sigma x^2_i) / 2n-1$$

where n is the sample size and Σx^2_i is the sum of squared allele frequencies of the ith allele. Average of unbiased heterozygosity over the r loci studied is given by;

$$H = \Sigma h_k / r$$

where and h_k is the value of h for the kth locus.

Genetic distance is another measure of genetic diversity and differentiation among populations and species. It is commonly estimated according to Nei (1972). To illustrate this calculation, assume two populations, X and Y, and for a given locus, let x_i and y_i be the frequencies of the ith allele in populations X and Y respectively. Nei's (1972) standard genetic distance is expressed as;

$$D = -ln [J_{xy} / \sqrt{(J_x J_y)}$$

where J_x and J_y are the means of Σx^2_i, Σy^2_i and $\Sigma x_i y_i$ over all loci in the genome, respectively.

However, a bias is introduced when the sample sizes are small with the same order of magnitude as it is the case in heterozygosity. Nei (1978)

developed an unbiased estimate formula for genetic distance by replacing gene identities Σx_i^2 and Σy_i^2 with unbiased estimates, $(2n_x\Sigma x_i^2-1/(2n_x-1)$ and $(2n_y \Sigma y_i^2-1/(2n_y-1)$ over the r loci respectively. Variances for each of these estimates are given in Nei (1978).

Pairwise genetic distances among accessions and species were calculated according to the unbiased estimate of Nei (1978). Cluster analysis using UPGMA was carried out to construct dendrograms for *Cicer* accessions and species. A dendrogram showing phylogenetic relationships among accessions and species was also constructed according to distance Wagner procedure in BIOSYS-I using Prevosti distance.

Ordination analyses are commonly used to determine data structures by replacing original variables with a new set of variables which explain much of the variation. PCO procedure finds the eigenvalues (measure of the variance accounted for by the corresponding eigenvectors) and eigenvectors (coordinates) of a matrix containing the distances between objects. Principle co-ordinate analysis (PCO) of pairwise genetic distances among *Cicer* accessions was carried out using MVSP 3.1 computer program (Kovach, 2000) to determine the ability of allozyme based genetic distances in displaying relationships among accessions and species.

2.5. Analysis of RAPD data

The amplification products at each band (gel positions) were treated as a separate character and scored as 1 (present) / 0 (absent), and a rectangular binary data matrix of 43 x 95 was prepared. Statistical analyses of the data matrix were carried out using the NTSYS-pc computer program (Ver. 1.7, Rohlf 1992). A similarity matrix was obtained using Dice's coefficient as in Nei and Li (1979), and it was converted into distances (*GD*) using the formula;

$$GD_{ab} = - ln(S_{ab})$$

where S_{ab} is the measure of genetic similarity between accessions *a* and *b* and it is defined as;

$$S_{ab} = 2N_{ab}/N_a+N_b$$

where N_{ab} is the number of common bands present in accessions *a* and *b*, and N_a and N_b are the sum of the scored bands in accessions a and *b* respectively. The distance matrix was then used for cluster analysis of *Cicer* accessions using the unweighted pair-group method with arithmetic mean (UPGMA) procedure.

Using same data matrix, percent of polymorphic loci, mean gene diversity (Nei, 1973), and mean number of alleles per loci were estimated. Genetic distances among accessions were also computed according to Nei (1978) using POPGENE computer program (Yeh *et. al.* 1999). Cluster analysis was carried out using UPGMA and dendrograms were visualized using TREEVIEW computer program (Page, 1996).

Principle coordinate analysis (PCO) of pairwise genetic distances among *Cicer* accessions was carried out using MVSP computer program (Ver. 3.1, Kovach, 2000) to determine the ability of 95 RAPD gel positions to display relationships among accessions and species. A comparison between the similarity matrices obtained by using the coefficient of Nei and Li (1979) and Jaccard's (1908) was made using the matrix comparison procedure of SYN-TAX – pc program (Ver. 5, Podani, 1993).

CHAPTER 3

RESULTS

3.1. Allozyme results

Surveying allozyme variation with seven enzyme systems revealed 13 isozyme loci. Only 12 of them had scorable staining intensity and reproducibility. Although *Adh-2* locus was detected in most of the species, it was excluded from the analysis due to the difficulty in reliably distinguishing null allele from faintly stained bands in scoring. Most of the accessions were monomorphic for the loci studied in general. Polymorphism among species was common and many of the species could be distinguished from each other based on allozyme polymorphisms. All the isozymes had anodal migration. Functional subunit number and the monogenic inheritance of these enzyme systems in *Cicer* studied before (Gaur and Slinkard 1990a, b). Isozyme loci that were scored informative are *Adh-1, Pgd-1, Pgd-2, Pgm-1,*

Skdh-2, *Skdh-3*, *Idh*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Mdh-4*, and *Pgi*. Alleles and their relative mobilities detected in *Cicer* accessions are given in Table 6.

3.1.1 Isozyme phenotypes

3.1.1.1. Alcohol dehydrogenase (ADH, E.C. 1.1.1.1)

This enzyme is an oxidoreductase which catalyzes following reaction, and it proceeds in the direction of oxidation of ethanol to acetaldehyde at pH 8. Histochemically, it is detected using tetrazolium based dyes (NBT, MTT) which become insoluble and form a colored band in the gel where the enzyme activity occur when reduced by pyridine nucleotide, Nicotinamide adenine dinucleotide, NAD.

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3COH + NADH + H$$

$$NADH + NBT+PMS \rightarrow Formazan\ (Blue\ precipitate).$$

In *Cicer* three activity zones were observed in almost all species examined. It was previously characterized that functional ADH enzyme is a dimer and encoded by two loci. The band in the middle is the hybrid band (heterodimer) formed between the products of these two loci. These activity

zones are *Adh-1,* hybrid band (between *Adh-1* and *Adh-2*) and *Adh-2* respectively (Gaur and Slinkard 1990a). The most anodal band (*Adh-1*) had reliable reproducibility and scorable sharpness. Annual and perennial species had different alleles for *Adh-1,* and polymorphism was detected in two *C. reticulatum* accessions. The allelic variation observed at this locus in *Cicer* accessions and species and their frequencies are given in Tables 4 and 5.

3.1.1.2. 6-phosphogluconate dehydrogenase (PGD, E.C.1.1.1.44)

This enzyme catalyzes the conversion of 6-phosphoglucanate to Ribulose 5-phosphate as shown in the following reaction.

$$\text{6-phosphoglucanate} + NADP^+ \rightleftharpoons \text{D-Ribulose-5-phosphate} + NADPH + CO_2$$

$$NADPH + NBT + PMS \rightarrow \text{Formazan (Blue precipitate).}$$

In *Cicer,* this enzyme was shown to be a functionally dimer and from seed extracts two isozymes were detected in all *Cicer* species (Figure 3). These isozymes were designated as *Pgd-1* and *Pgd-2* (Gaur and Slinkard, 1990a). Except *C. montbretii, C. bijugum* and *C. arietinum,* the rest of the species had same allele at *Pgd-1* locus. Polymorphism was detected in

accessions of *C. montbretii,* and two *C. reticulatum* accessions (ILWC 36 and ILWC182) were monomorphic for an alternative allele at the *Pgd-1* locus (Table 4). One of the most informative loci among *Cicer* species was *Pgd-2* locus, and many of the *Cicer* species had different alleles at *Pgd-2.* Accession Bergama was fixed for alternative alleles (Table 4). *C. pinnatifidum, C. bijugum, C. echinospermum, C, reticulatum* and *C. arietinum* had unique alleles, but monomorphic within species. Observed allelic variation and their frequencies in each accession and species are given in Tables 4 and 5. A sample gel picture is given in Figure 3.

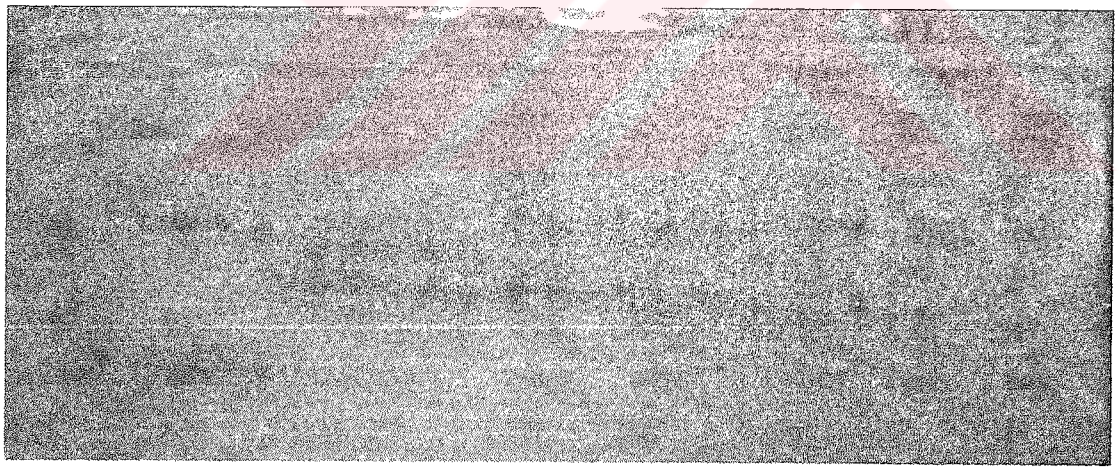

Figure 3. A sample gel picture shoving the alleles detected at two putative *Pgd* loci (loci from the anode *Pgd-1*, *Pgd-2* respectively) using PGD enzyme system in annual species.

3.1.1.3. Phosphoglucomutase (PGM, E.C. 5.4.2.2)

PGM catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate. Histochemically, it is detected using the helper enzyme, Glucose-6-Phosphate Dehydrogenase, which converts glucose-6-phosphate to 6-phosphogluconate as it is shown in the following scheme;

PGM

a-D-Glucose-1-Phosphate → a-D-Glucose-6-Phosphate

G6PD

Glucose-6-Phosphate + NAD^+ → 6-Phosphogluconate + NADH

NADH + NBT + PMS → Formazan (Blue precipitate).

In *Cicer,* this enzyme has been shown to be a functionally monomer. Although two activity zones were observed in seed extracts, the less anodal faint band was not scored. In annual *Cicer* species, using leaf extracts, two isozymes (one is plastid specific, *Pgm-2*, the other one is cytosolic, *Pgm-1*) for *Pgm* were characterized (Gaur and Slinkard 1990). The most anodal isozyme probably corresponding to *Pgm-1* is presumably expressed in seed. *Pgm* was the one of the most variant enzyme system within and among species when compared to others. Alternative alleles and heterozygotes were detected in the accessions of *C. isauricum*, *C. judaicum*, *C. pinnatifidum*, *C. bijugum*, *C. echinospermum,* and *C. reticulatum*. *C.*

pinnatifidum and *C. echinospermum* had four alleles at this locus. Accessions of *C. arietinum* was monomorphic and interestingly had the same allele with *C. montbretii*. *Pgm* was the only locus we observed polymorphism in *C. judaicum*. Observed allelic variation and their frequencies are given in Table 4 and 5.

3.1.1.4 Isocitrate dehydrogenase (IDH, E.C. 1.1.1.42)

Isocitrate dehydrogenase is one of the Krebs cycle enzymes which converts isocitric acid to 2-oxoglutarate.

$$\text{DL-Isocitric acid} + NADP^+ \rightarrow \text{2-oxoglutarate} + NADPH + CO_2$$

$$NADPH + NBT + PMS \rightarrow \text{Formazan (Blue precipitate).}$$

At *Idh* locus, polymorphism was detected in accessions of *C. anatolicum, C. pinnatifidum*, and *C. echinospermum*. Accessions of *C. reticulatum* and *C. arietinum* had the same allozymes at *Idh*, and no polymorphism were detected in both species. *C. incisum*, and accessions of *C. judaicum* and *C. pinnatifidum* had same allozymes for *Idh*. Only the accession TR23039, which was identified as *C. pinnatifidum*, but shared same allele with *C. echinospermum*, *C. reticulatum* and *C. arietinum*. Observed allelic variation and their frequencies are given in Tables 4 and 5, and a sample of gel picture is given in Figure 4.

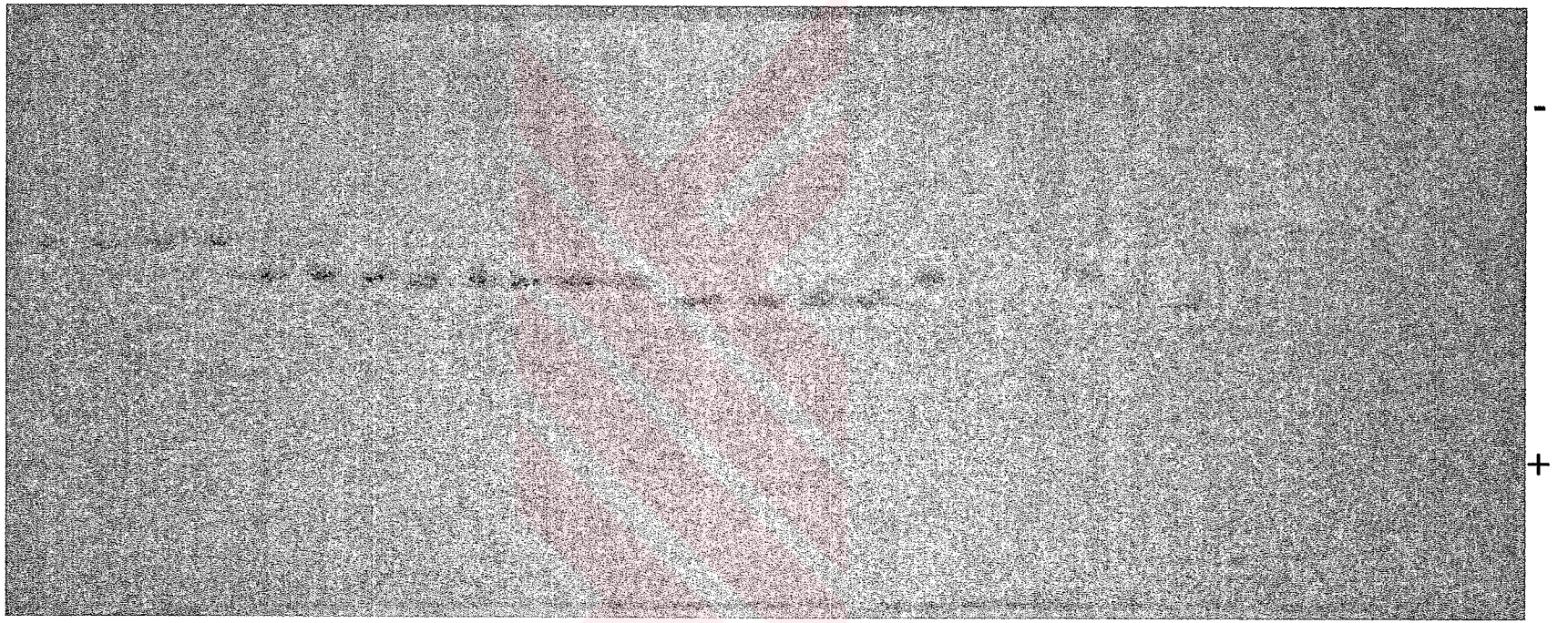

Figure 4. A sample gel picture shoving the alleles detected in annual species using IDH enzyme system. Activity zone is the putative *Idh* locus.

3.1.1.5. Shikimate Dehydrogenase (SKDH, E.C. 1.1.1.25)

$$\text{Shikimate} + NADP^{+} \rightarrow \text{5-dehyroskimate} + NADPH$$

$$NADPH + NBT + PMS \rightarrow \text{Formazan (Blue precipitate)}$$

For this enzyme, three activity zones were detected, however, the most anodal one did not have scorable intensity and reproducibility in all species, and only two less anodal isozymes were scored. Annual and perennial species had different alleles at these two isozyme loci, and we have observed polymorphism among species and within few species. *C. incisum* had unique alleles at these two loci. Observed allelic variation and their frequencies are given in Tables, 4 and 5.

3.1.1.6. Malate Dehydrogenase (MDH, E.C. 1.1.1.37)

This enzyme catalyzes following reaction, and it is detected in gel using a tetrazolium based staining.

$$\text{L-Malic acid} + NAD^+ \rightarrow \text{Oxaloacetate} + NADH$$

$$NADH + NBT + PMS \rightarrow \text{Formazan (Blue precipitate).}$$

For MDH enzyme system, all of the *Cicer* species had four isozymes except six *C. pinnatifidum* accessions (Ereğli, Avcılar, Mut, ILWC78, ILWC250, ILWC251). These accessions had one extra band between isozymes of *Mdh-3* and *Mdh-4* invariably in all individuals. Only the *C. reticulatum* was found to be polymorphic at *Mdh-2* and *Mdh-3* loci. In addition, we have detected polymorphism in accessions of *C. anatolicum*, *C. reticulatum, C. echinospermum, C. pinnatifidum* and *C. bijugum* at *Mdh-4* locus. As in other enzyme systems, except *C. incisum,* annual and perennial species had different alleles for these isozymes as a group. *C. incisum* shared all four allozymes with annual species and differentiated from perennials. Observed allelic variation at these loci and their frequencies are given in Table 4 and 5.

3.1.1.7. Phosphoglucoisomerase (PGI, E.C. 5.3.1.9)

PGI is an isomerase interconverting aldoses and ketoses. It is detected using an helper enzyme, Glucose-6-Phosphate Dehydrogenase and tetrazolium salt based dyes as it is illustrated in the following reaction.

a-D-Fructose-6-phosphate → Glucose-6-phosphate

G6PD

Glucose-6-phosphate + NAD^+ → 6-Phosphogluconate + NADH

NADH + NBT + PMS → Formazan (Blue precipitate).

For PGI enzyme system, only one activity zone was detected using seeds as an enzyme source. As in the other enzyme systems, at *Pgi* locus annuals and perennials could be differentiated as different groups. Alternative alleles were detected in accessions of *C. bijugum*, *C. echinospermum* and *C. reticulatum*. Rest of the species were monomorphic. *C. incisum* shared the same allele with the annuals (Tables 4 and 5).

Table 4. Allozymes and their frequencies observed in *Cicer* accessions.

Species / Accession	*Idh*	*Pgd-1*	*Pgd-2*	*Pgm*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Mdh-4*	*Sdh-2*	*Sdh-3*	*Adh*	*Pgi*
C. montbretii												
Kaz dagı	*d*	*a/ d*(.9 / .1)	*e*	*e*	*B*	*d*	*f*	*d*	*h*	*g*	*d*	*d*
Bergama	*d*	*a/ d*(.9 / .1)	*a*	*e*	*b*	*d*	*f*	*d*	*h*	*g*	*d*	*d*
C. isauricum												
Kurucaova	*d*	*a*	*a*	*b*	*b*	*d*	*f*	*d*	*g*	*f*	*e*	*d*
Ermenek	*d*	*a*	*a*	*b*	*b*	*d*	*f*	*d*	*b / g* (.86 / .14)	*b / e* (.14 / .86)	*e*	*d*
Akseki	*d*	*a*	*a*	*b/c/d* (.55 / .35 / .1)	*b*	*d*	*f*	*d*	*e / g* (.38 / .62)	*e/ f* (.88 / .12)	*e*	*d*
C. anatolicum												
Kızılcahamam	*d*	*a*	*c*	*c*	*b*	*c*	*e*	*d/e* (.1 / .9)	*b/ e* (.86 / .14)	*b/ e* (.04 / .96)	*e*	*d*
Kazancı	*d/ f* (.86 / .14)	*a*	*c*	*c*	*b*	*c*	*e*	*e*	*b/e* (.13 / .27)	*b/f* (.17 / .83)	*e*	*d*
Erciyes	*d*	*a*	*c*	*c*	*b*	*c*	*e*	*e*	*f*	*f*	*e*	*d*
C. incisum												
Yahyalı	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*b*	*d*	*d*	*d*	*d*
C. judaicum												
ILWC168	*c*	*a*	*e*	*b/ c*(.9 / .1)	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC148	*c*	*a*	*e*	*b/ c*(.9 / .1)	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*

Table 4. (continued)

Species / Accession	*Idh*	*Pgd-1*	*Pgd-2*	*Pgm*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Mdh-4*	*Sdh-2*	*Sdh-3*	*Adh*	*Pgi*
C. pinnatifidum												
Besni	*c*	*a*	*e*	*b/c/d* (.87 / .06 / .06)	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
Eregli	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*a/ b* (.5 / .5)	*b*	*b*	*f*	*b*
Avcılar	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*a/b* (.5 / .5)	*b*	*b*	*f*	*b*
Erkenek	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC33	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*b*	*b/c* (.9 / .1)	*b/c* (.9 / .1)	*f*	*b*
ILWC236	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC 9	*c*	*a*	*e*	*b/c* (.5 / .5)	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC250	*c*	*a*	*e*	*b/c* (.9 / .09)	*a*	*a*	*a*	*a/b* (.5 / .5)	*b*	*b*	*f*	*b*
ILWC29	*d*	*a*	*e*	*b/c* (.9 / .1)	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC78	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*a/b* (.5 / .5)	*b*	*b*	*f*	*b*
ILWC248	*c*	*a*	*e*	*a*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC251	*c/d* (.9 / .1)	*a*	*e*	*b*	*a*	*a*	*a*	*a/b* (.5/.5)	*b*	*b*	*f*	*b*
Kır obası	*c*	*a*	*e*	*b*	*a*	*a*	*a*	*a/ b* (.5 / .5)	*b*	*b*	*f*	*b*
TR 23039	*d*	*a*	*a*	*c/d* (.45 / .55)	*a*	*a*	*a*	*b*	*b*	*b*	*b/f* (.83 / .17)	*b*

Table 4. (continued)

Species /Accession	*Idh*	*Pgd-1*	*Pgd-2*	*Pgm*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Mdh-4*	*Sdh-2*	*Sdh-3*	*Adh*	*Pgi*
C. echinospermum												
ILWC239	*d*	*a*	*b*	*b/d* (.7 / .3)	*a*	*a*	*a/f*(.9 / .05)	*b*	*b*	*b*	*b*	*b*
ILWC245	*b/e/f* (.5 / .1 / .4)	*a*	*b*	*b*	*a*	*a*	*a*	*a/b* (.05 / .95)	*b*	*b*	*b*	*b*
ILWC246	*b/e/f* (.64 / .18 / .18)	*a*	*b*	*d*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
ILWC235	*d*	*a*	*b*	*c/e* (.7 / .3)	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*a*
ILWC238	*d*	*a*	*b*	*d*	*a*	*a*	*a*	*a/b* (.05 / .95)	*b*	*b*	*b*	*b*
TR 39241	*d*	*a*	*b*	*c*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
TR 54962	*d*	*a*	*b*	*c*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
C. bijugum												
ILWC240	*a*	*c*	*e*	*b*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b/d* (.91 / .09)
ILWC241	*a*	*c*	*e*	*b*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC243	*a*	*c*	*e*	*b*		*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC8	*a*	*c*	*e*	*b/c/e* (.8 / .1 / .1)	*a*	*a*	*a*	*b*	*b*	*a*	*f*	*b*
ILWC177	*a*	*c*	*e*	*b*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b*
ILWC62	*a*	*c*	*e*	*b*	*a*	*a*	*c*	*b*	*b*	*b*	*f*	*b*
ILWC32	*a*	*c*	*e*	*b*	*a*	*a*	*a*	*b*	*b*	*b*	*f*	*b/d*(.71 / .29)

Table 4. (continued)

Species / Accession	*Idh*	*Pgd-1*	*Pgd-2*	*Pgm*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Mdh-4*	*Sdh-2*	*Sdh-3*	*Adh*	*Pgi*
C. reticulatum												
ILWC36	*d*	*b*	*c*	*c*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*c*
ILWC182	*d*	*b*	*c*	*e*	*a*	*a*	*b/c* (.9 / .1)	*b*	*b*	*b*	*b/c* (.5/.5)	*c*
ILWC216	*d*	*a*	*c*	*c/d/e* (.14/.64/.22)	*a*	*a*	*a*	*b*	*b*	*b*	*a/b* (.83 / .17)	*b*
ILWC218	*d*	*a*	*c*	*c/e/f* (.78/.1/.1)	*a*	*a/ b* (.95/.05)	*a/c/d* (.9 /.05 /.05)	*b*	*b*	*b*	*b*	*b*
ILWC81	*d*	*a*	*c*	*c/e*(.2 / .8)	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
ILWC237	*d*	*a*	*c*	*c/e*(.9 / .1)	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
ILWC247	*d*	*a*	*c*	*c/e*(.9 / .1)	*a*	*a*	*a*	*b*	*a/b*(.1 / .9)	*b*	*b*	*b*
TR 54961	*d*	*a*	*c*	*c*	*a*	*a*	*a*	*b/c* (.95 / .05)	*b*	*b*	*b*	*b*
TR 54963	*d*	*a*	*c*	*c/e*(.8 / .2)	*a*	*a*	*a*	*b*	*b*	*a/b*(.1/.9)	*b*	*b*
C. arietinum												
ILC 195	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
Kırmızı nohut	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
Akçin 91	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
Menemen 91	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
Diyar 95	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
Akn 51; Eser 87	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*
ILC 482; Canıtez 89	*d*	*b*	*d*	*e*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*

Table 5. Observed alleles and their averaged frequencies in *Cicer* species.

Alleles		*C. mon.*	*C. isa.*	*C. ana.*	*C. inc.*	*C. jud.*	*C. pin.*	*C. ech.*	*C. bij.*	*C. ret.*	*C. ari.*
Idh	*a*	-	-	-	-	-	-	-	1.00	-	-
	b	-	-	-	-	-	-	0.163	-	-	-
	c	-	-	-	1.00	1.00	0.99	-	-	-	-
	d	1.00	1.00	0.953	-	-	0.007	0.71	-	1.00	1.00
	e	-	-	-	-	-	-	0.04	-	-	-
	f	-	-	0.047	-	-	-	0.083	-	-	-
Pgd-1	*a*	0.9	1.00	1.00	1.00	1.00	1.00	1.00	-	0.78	-
	b	-	-	-	-	-	-	-	-	0.22	1.00
	c	-	-	-	-	-	-	-	1.00	-	-
	d	0.1	-	-	-	-	-	-	-	-	-
Pgd-2	*a*	0.5	1.00	-	-	-	0.07	-	-	-	
	b	-	-	-	-	-	-	1.00	-	-	-
	c	-	-	1	-	-	-	-	-	1.00	-
	d	-	-	-	-	-	-	-	-	-	1.00
	e	0.5	-	-	1.00	1.00	0.93	-	1.00	-	-
Pgm	*a*	-	-	-	-	-	0.07	-	-	-	-
	b	-	0.85	-	1.00	0.9	0.80	0.24	0.97	-	-
	c	-	0.12	1.00	-	0.1	0.086	0.39	0.014	0.746	-
	d	-	0.03	-	-	-	0.04	0.33	-	0.071	-
	e	1.00	-	-	-	-	-	0.021	0.014	0.10	1.00
	f	-	-	-	-	-	-	-	-	0.01	-
Mdh-1	*a*	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b	1.00	1.00	1.00	-	-	-	-	-	-	-
Mdh-2	*a*	-	-	-	1.00	1.00	1.00	1.00	1.00	0.99	1.00
	b	-	-	-	-	-	-	-	-	0.01	-
	c	-	-	1.00	-	-	-	-	-	-	-
	d	1.00	1.00	-	-	-	-	-	-	-	-
Mdh-3	*a*	-	-	-	1.00	1.00	1.00	0.99	0.86	0.88	1.00
	b	-	-	-	-	-	-	-	-	0.10	-
	c	-	-	-	-	-	-	-	0.14	0.015	-
	d	-	-	-	-	-	-	-	-	0.005	-
	e	-	-	1.00	-	-	-	-	-	-	-
	f	1.00	1.00	-	-	-		0.01	-	-	-
Mdh-4	*a*	-			-	-	0.21	0.014	-	0.005	-
	b	-	-	-	-	-	0.79	0.96	1.00	0.995	1.00
	c	-	-	-	1.00	1.00	-	-	-	-	-
	d	1.00	1.00	0.03	-	-	-	-	-	-	-
	e	-	-	0.97	-	-	-	-	-	-	-
Skdh-2	*a*	-	-	-	-	-	-	-	-	0.001	-
	b	-	0.29	0.07	-	1.00	0.993	1.00	1.00	0.999	1.00
	c	-	-	-	-		0.007	-	-	-	-
	d	-	-	-	1.00	-	-	-	-	-	-

Table 5 (continued)

Alleles		*C. mon.*	*C. isa.*	*C. ana.*	*C. inc.*	*C. jud.*	*C. pin.*	*C. ech.*	*C. bij.*	*C. ret.*	*C. ari.*
Skdh-2	*e*	-	0.13	0.32	-				-	-	-
	f	-	-	0.61	-	-	-	-	-	-	-
	g	-	0.59	-	-	-	-	-	-	-	-
	h	1.00	-	-	-	-	-	-	-	-	-
Skdh-3	*a*	-	-	-	-	-	-	-	0.14	0.001	-
	b	-	0.05	0.07	-	1.00	0.993	1.00	0.86	0.999	1.00
	c	-	-	-	-		0.007	-	-	-	-
	d	-	-	-	1.00	-	-	-	-	-	-
	e	-	0.58	0.32	-				-	-	-
	f	-	0.37	0.61	-	-	-	-	-	-	-
	g	1.00	-	-	-	-	-	-	-	-	-
	h	-	-	-	-	-	-	-	-	-	-
Adh-1	*a*	-	-	-	-	-	-	-	-	0.091	-
	b	-	-	-	-	-	0.012	1.00	-	0.85	0.9
	c	-	-	-	-	-	-	-	-	0.05	-
	d	1.00	-	-	1.00	-	-	-	-	-	-
	e	-	1.00	1.00	-	-	-	-	-	-	-
	f	-	-	-	-	1.00	0.987	-	1.00	-	0.1
Pgi	*a*	-	-	-	-	-	-	0.17	-	-	-
	b	-	-	-	-	1.00	1.00	0.83	0.95	0.78	1.00
	c	-	-	-	-	-	-	-	-	0.22	-
	d	1.00	1.00	1.00	1.00	-	-	-	0.054	-	-

*Abbreviations for species: *C. mon, C. montbretii, C.isa, C. isauricum, C.ana, C. anatolicum, C. inc, C. incisum, C. jud, C. judaicum, C. pin, C. pinnatifidum, C. bij, C. bijugum, C. ech, C. echinospermum, C. ret, C. reticulatum, C. ari, C. arietinum.*

Table 6. Relative mobilities of alleles observed in *Cicer* accessions.

Alleles	*Idh*	*Pgd-1*	*Pgd-2*	*Pgm*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Mdh-4*	*Sdh-2*	*Sdh-3*	*Adh*	*Pgi*
a	130	100	100	106	100	100	100	116*	106	114	108	107
b	121	91	90	100	88	95	98	100	100	100	100	100
c	118	86	84	92		88	96	79	85	90	96	93
d	100	83	65	86		96	92	75	80	80	93	90
e	98		53	82			87	52	74	70	90	62
f	94						96		70	60	87	
g							85		68	20		
h									40			

* Allele *Mdh-4*^{116} designated as allele *a*, but its mobility was between *Mdh-4*^{100} and *Mdh-4*^{79}.

3.1.2. Allozyme variation

In general, *Cicer* accessions were monomorphic at the majority of isozyme loci indicating that they were fixed for one allele while some of the accessions were polymorphic and had a considerable allelic variability. Even though it was rare, some accessions were fixed for an alternative allele and differentiated from other accessions of the same species as it would be expected in predominantly self-pollinating species. For example, at the same locus, *Pgd-1,* two *C. reticulatum* accessions (ILWC36 and ILWC182) were fixed for the allele '*b*' while the rest of the accessions were monomorphic for allele '*a*' (Table 4). Likewise, one of the *C. pinnatifidum* accession, ILWC 248, had an alternative allele for *Pgm*, and *C. echinospermum* accessions ILWC 246 and ILWC 238 were fixed for allele *d* while other accessions had alternative alleles and some of them were polymorphic (Table 4).

Genetic variation in *Cicer* accessions and species was assessed in terms of percent of polymorphic loci, average number of alleles per locus, observed and expected heterozygosity (Table 7 and 8). The percent of polymorphic loci in *Cicer* species excluding *C. incisum* and *C. arietinum* varied from 3.6 % to 22.2 %. The highest average percent of polymorphic loci was observed in two perennial species *C. isauricum* and *C. anatolicum*

(22.2 and 16.6 % respectively). Consistent with the previous observations, among annual species *C. reticulatum* had the highest percentage of polymorphic loci (12.9 %) and this was followed by *C. echinospermum* and *C. pinnatifidum* (9.5 % and 8.9 %). However, the observed level of polymorphism was not as high as the previously reported values (Table 8; Ahmad *et. al.* 1992; Tayyar and Waines 1996).

Heterozygotes were detected at certain isozyme loci in accessions of both annual and perennial species excluding *C. montbretii, C. incisum, C. bijugum, C. judaicum* and *C. arietinum*. However, the observed mean heterozygosity was low, and nearly all of the accessions showed significant deviations from HW expectations nearly at all loci. Contrary to previous reports of allozyme variation, heterozygosity was detected in four accessions of *C. echinospermum*, but highest heterozygosity was detected in one accession of *C. isauricum* and in accessions of *C. reticulatum* (Table 7). Finally, nine cultivars representing *C. arietinum* revealed no polymorphism indicating that it is poor in terms of allozyme variation. This is also consistent with the previous reports of allozyme variation.

Table 7. Genetic variability at 12 loci in 57 accessions of *Cicer* species.

Population		Mean sample size for each	Mean number of alleles for	Percentage of polymorphic	Mean heterozygosity	
No	Name	Locus	each locus	Loci*	Direct count	** HW Exp.
1.	Bergama	9.9 ± 0.4	1.1 ± 0.1	8.3	0.000 ± 0.000	0.016 ± 0.16
2.	Kaz dağı	7.3 ± 0.5	1.1 ± 0.1	8.3	0.000 ± 0.000	0.016 ± 0.016
3.	Kuruca ova	9.6 ± 0.5	1.0 ± 0.0	.0	0.000 ± 0.000	0.000 ± 0.000
4.	Ermenek	10.1 ± 0.7	1.2 ± 0.1	16.7	0.000 ± 0.000	0.042 ± 0.029
5.	Akseki	8.8 ± 0.3	1.3 ± 0.2	25.0	0.042 ± 0.029	0.111 ± 0.062
6.	Kızılcahamam	9.2 ± 0.4	1.3 ± 0.1	25.0	0.029 ± 0.020	0.050 ± 0.027
7.	Kazancı	10.9 ± 0.8	1.3 ± 0.2	25.0	0.006 ± 0.006	0.093 ± 0.053
8.	Erciyes	7.6 ± 0.5	1.0 ± 0.0	.0	0.000 ± 0.000	0.000 ± 0.000
9.	Yahyalı	7.7 ± 0.5	1.0 ± 0.0	.0	0.000 ± 0.000	0.000 ± 0.000
10.	ILWC168	8.5 ± 0.4	1.1 ± 0.1	8.3	0.000 ± 0.000	0.019 ± 0.019
11.	ILWC148	8.8 ± 0.5	1.1 ± 0.1	8.3	0.000 ± 0.000	0.017 ± 0.017
12.	Besni	8.8 ± 0.5	1.2 ± 0.2	8.3	0.010 ± 0.010	0.020 ± 0.020
13.	Ereğli	10.4 ± 0.9	1.1 ± 0.1	8.3	0.000 ± 0.000	0.043 ± 0.043
14.	Avcılar	11.0 ± 1.0	1.1 ± 0.1	8.3	0.000 ± 0.000	0.043 ± 0.043
15.	Erkenek	9.5 ± 0.9	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
16.	ILWC 33	9.9 ± 0.2	1.2 ± 0.1	16.7	0.000 ± 0.000	0.032 ± 0.021
17.	ILWC 236	9.5 ± 0.5	1.0 ± 0.1	0.0	0.000 ± 0.000	0.000 ± 0.000
18.	ILWC 9	9.2 ± 0.3	1.1 ± 0.1	8.3	0.000 ± 0.000	0.033 ± 0.033
19.	ILWC 250	9.5 ± 0.9	1.2 ± 0.1	16.7	0.000 ± 0.000	0.057 ± 0.044
20.	ILWC 29	9.1 ± 0.5	1.1 ± 0.1	8.3	0.017 ± 0.017	0.016 ± 0.016
21.	ILWC 78	8.7 ± 0.9	1.1 ± 0.1	8.3	0.000 ± 0.000	0.043 ± 0.043
22.	ILWC 248	9.2 ± 0.2	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
23.	ILWC 251	10.1 ± 0.9	1.2 ± 0.1	16.7	0.000 ± 0.000	0.059 ± 0.044
24.	Mut	11.0 ± 0.7	1.1 ± 0.1	8.3	0.000 ± 0.000	0.043 ± 0.043
25.	TR23039	10.3 ± 0.4	1.2 ± 0.1	16.7	0.028 ± 0.028	0.067 ± 0.048
26.	ILWC 239	9.3 ± 0.3	1.2 ± 0.1	16.7	0.009 ± 0.009	0.044 ± 0.035
27.	ILWC 245	9.4 ± 0.3	1.3 ± 0.2	16.7	0.009 ± 0.009	0.060 ± 0.051
28.	ILWC 246	9.3 ± 0.2	1.2 ± 0.2	8.3	0.000 ± 0.000	0.046 ± 0.046
29.	ILWC 235	9.4 ± 0.2	1.1 ± 0.1	8.3	0.000 ± 0.000	0.044 ± 0.044
30.	ILWC 238	9.1 ± 0.6	1.2 ± 0.1	16.7	0.014 ± 0.010	0.024 ± 0.017
31.	TR39241	7.1 ± 1.1	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
32.	TR54962	7.9 ± 0.8	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
33.	ILWC 240	8.4 ± 0.4	1.1 ± 0.1	8.3	0.000 ± 0.000	0.014 ± 0.014
34.	ILWC 241	9.5 ± 0.3	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
35.	ILWC 243	9.6 ± 0.4	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
36.	ILWC 8	9.7 ± 0.3	1.2 ± 0.2	8.3	0.000 ± 0.000	0.030 ± 0.030
37.	ILWC 177	9.3 ± 0.5	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
38.	ILWC 62	9.2 ± 0.3	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
39.	ILWC 32	9.4 ± 0.4	1.1 ± 0.1	8.3	0.000 ± 0.000	0.037 ± 0.037
40.	ILWC 36	7.2 ± 0.4	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000

Table 7. (continued)

Population		Mean sample size for each	Mean number of alleles for each	Percentage of polymorphic	Mean heterozygosity	
No	Name	Locus	Locus	Loci*	Direct count	** HW Exp.
41.	ILWC 182	7.4 ± 0.5	1.2 ± 0.1	16.7	0.019 ± 0.019	0.061 ± 0.046
42.	ILWC 216	7.9 ± 0.4	1.3 ± 0.2	16.7	0.012 ± 0.012	0.072 ± 0.051
43.	ILWC 218	9.3 ± 0.4	1.5 ± 0.2	33.3	0.017 ± 0.011	0.094 ± 0.047
44.	ILWC 81	9.5 ± 0.2	1.1 ± 0.1	8.3	0.017 ± 0.017	0.028 ± 0.028
45.	ILWC 237	7.8 ± 0.4	1.1 ± 0.1	8.3	0.000 ± 0.000	0.017 ± 0.017
46.	ILWC 247	9.3 ± 0.4	1.1 ± 0.1	8.3	0.000 ± 0.000	0.016 ± 0.016
47.	TR54961	10.6 ± 0.3	1.1 ± 0.1	0.0	0.008 ± 0.008	0.008 ± 0.008
48.	TR54963	8.1 ± 0.9	1.3 ± 0.1	25.0	0.000 ± 0.000	0.058 ± 0.031
49.	ILC 195	7.0 ± 1.8	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
50.	Diyar 95	8.4 ± 0.9	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
51.	ILC 482	8.2 ± 1.4	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
52.	Kırmızı nohut	8.7 ± 0.9	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
53.	Akçin 91	9.5 ± 0.8	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
54.	Canıtez 89	8.2 ± 1	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
55.	Eser 87	9.3 ± 0.5	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
56.	Menemen 91	9.2 ± 0.8	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000
57.	Akn 51	8.0 ± 0.9	1.0 ± 0.0	0.0	0.000 ± 0.000	0.000 ± 0.000

*A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

** Unbiased estimate (Nei, 1978).

Table 8. Genetic variability at 12 loci averaged for species.

Cicer species	The number of accessions	Mean sample size for each locus	Percentage of polymorphic loci	Mean number of alleles per locus
C. montbretii	2	8.6 ± 0.45+	8.3	1.10 ± 0.10+
C. isauricum	3	9.5 ± 0.50	22.2	1.16 ± 0.10
C. anatolicum	3	9.2 ± 0.53	16.6	1.30 ± 0.10
C. incisum	1	7.7 ± 0.50	0	1.00 ± 0.00
C. judaicum	2	8.6 ± 0.45	8.3	1.10 ± 0.10
C. pinnatifidum	14	9.7 ± 0.62	8.9	1.10 ± 0.10
C. bijugum	7	9.3 ± 0.37	3.6	1.17 ± 0.03
C. echinospermum	7	7.5 ± 0.50	9.5	1.04 ± 0.10
C. reticulatum	9	8.5 ± 0.33	12.9	1.18 ± 0.12
C. arietinum	9	8.2 ± 1.03	0	1.00 ± 0.00

+Standard error.

3.1.3 Allozyme based intra and inter-species genetic distances and cluster analysis

Electrophoretic data obtained from each enzyme system were used to calculate the unbiased genetic distance coefficients (GD) according to Nei (1978) for all pairwise combinations of accessions representing ten *Cicer* species (Appendix A). Using UPGMA analysis, accessions were clustered to generate a dendrogram depicting the relationships among *Cicer* accessions (Figure 5). *Cicer* accessions, except that of *C. reticulatum* and *C. echinospermum,* were generally grouped together and formed species clusters in the dendrogram. Accessions representing *C. reticulatum* separated into two groups by *C. echinospermum* accessions (ILWC235, ILWC245, and ILWC246). The other unusual grouping observed among accessions of *C. pinnatifidum* was the grouping of accession TR23039 which clustered with accessions of *C. echinospermum* and *C. reticulatum*. The experts in the gene bank identified this sample as *C. pinnatifidum*, but it had the seed morphology of *C. echinospermum*, and this grouping confirmed it. Two of the *C. reticulatum* accessions (ILWC36 and ILWC182) clustered with the chickpea lines, and ILWC182 had the lowest distance to the chickpea (0.214). Both accessions also shared the same allele with cultivated species at the *Pgd-1* locus.

Overall, the dendrogram of *Cicer* accessions had two branches, one of which contained the species clusters formed by accessions of three perennial species (*C. montbretii, C. isauricum* and *C. anatolicum*), and the other one had two clusters of annual species (*C. pinnatifidum, C. judaicum, C. bijugum, C. echinospermum, C. reticulatum* and *C. arietinum*) and the accession representing *C. incisum* (Figure 5).

Same procedure was followed to develop a dendrogram for species. Using hierarchical analysis routine of BIOSYS-1, genetic distance coefficients for species were computed from the coefficients of accessions, and a dendrogram for ten *Cicer* species was constructed using UPGMA procedure of the NTSYS-pc (Table 9; Figure 13). As in the grouping of *Cicer* accessions, the dendrogram of species comprised two main branches each bearing the species corresponding to the clusters observed in the accession dendrogram. Overall, in one branch of the dendrogram three perennial species *C. montbretii, C. anatolicum*, and *C. isauricum* formed a perennial species cluster. In the other branch of the dendrogram one perennial species, *C. incisum*, and six annual species formed a cluster. This cluster had two branches one comprising the two clusters for annual species, and the other bearing *C. incisum*. In the cluster of annual species, three closely related species, *C. pinnatifidum, C. judaicum* and *C. bijugum,* formed one

group, and *C. reticulatum C. arietinum* and *C. echinospermum* formed the other group. The observed distance values among annual *Cicer* species were generally small and close to the previous reports, however, relatively large distance values were detected between perennials and annuals (Table 9). The closest wild species to the cultivated species was *C. reticulatum* (GD=0.27). *C. reticulatum* and *C. echinospermum* were closer to each other (GD=0.25) than the cultivated species. *C. pinnatifidum, C. judaicum* and *C. bijugum* formed the second group within annual species cluster indicating that they are genetically close to one another.

Table 9. Unbiased genetic distances among 10 *Cicer* species computed according to Nei (1978).

	C. mont.	*C. isa.*	*C. anat.*	*C. incis.*	*C. jud.*	*C. pin.*	*C. ech.*	*C. bij.*	*C. ret.*	*C. ariet.*
C. mont.	0.088 (0.088- 0.088)									
C. isa.	0.548 (0410 - 0.680)	0.089 (0.078- 0.106)								
C. anat.	1.095 (1.065-1.116)	0.950 (0.761-1.099)	0.095 (0.012- 0.167)							
C. incis.	2.582 (2.582-2.582)	1.850 (1.770-1.988)	2.460 (2.436 - 0.485)	0.0000 (0.000–0.000)						
C. jud.	2.573 (2.572-2.574)	1.839 (1.678-1.994)	2.202 (2.072- 2.371)	0.411 (0.411- 0.411)	0.000 (0.000-0.000)					
C. pin.	2.145 (1.377-2.582)	1.822 (1.224 – 2.485)	2.179 (1.441 - 2.485)	0.457 (0.389 - 0.658)	0.126 (0.088- 0.335)	0.078 (0.000- 0.391)				
C. ech.	1.999 (1.603 – 2.559)	1.743 (1.320 – 2.461)	1.717 (1.286 - 2.461)	0.668 (0.516 - 0.853)	0.252 (0.159 - 0.367)	0.379 (0.110 – 0.569)	0.119 (0.000 – 0.242)			
C. bij.	2.406 (2.207 – 2.604)	2.350 (2.193 – 2.504)	2.206 (2.099 – 2.403)	0.754 (0.693 – 0.887)	0.342 (0.273 – 0.417)	0.273 (0.182 – 0.631)	0.555 (0.381 –0.853)	0.077 (0.000 –0.187)		
C. ret.	1.801 (1.469 – 2.477)	1.807 (1.556 – 2.485)	1.197 (1.007 – 1.760)	0.770 (0.656 – 1.099)	0.448 (0.356 – 0.671)	0.458 (0.115 – 0.758)	0.247 (0.087 – 0.576)	0.604 (0.412 – 0.875)	0.137 (0.000 – 0.368)	
C. ariet.	1.784 (1.784 – 1.784)	2.374 (2.212- 2.485)	2.347 (2.265 - 2.485)	0.693 (0.693 - .693)	0.516 (0.396- 0.530)	0.526 (0.272 - 0.591)	0.331 (0.271- 0.516)	0.569 (0.405 - 0.693)	0.279 (0.188- 0.453)	0.000 (0.000 - 0.000)

*Abbreviations for species: *C. mont, C montbretii, C. isau, C. isauricum, C.anat, C. anatolicum, C. incis, C. incisum, C. jud, C. judaicum, C. pin, C. pinnatifidum, C. bij, C. bijugum C. ech, C. echinospermum, C. ret, C. reticulatum, C. ari, C. arietinum.* The ranges are given below the each coefficient.

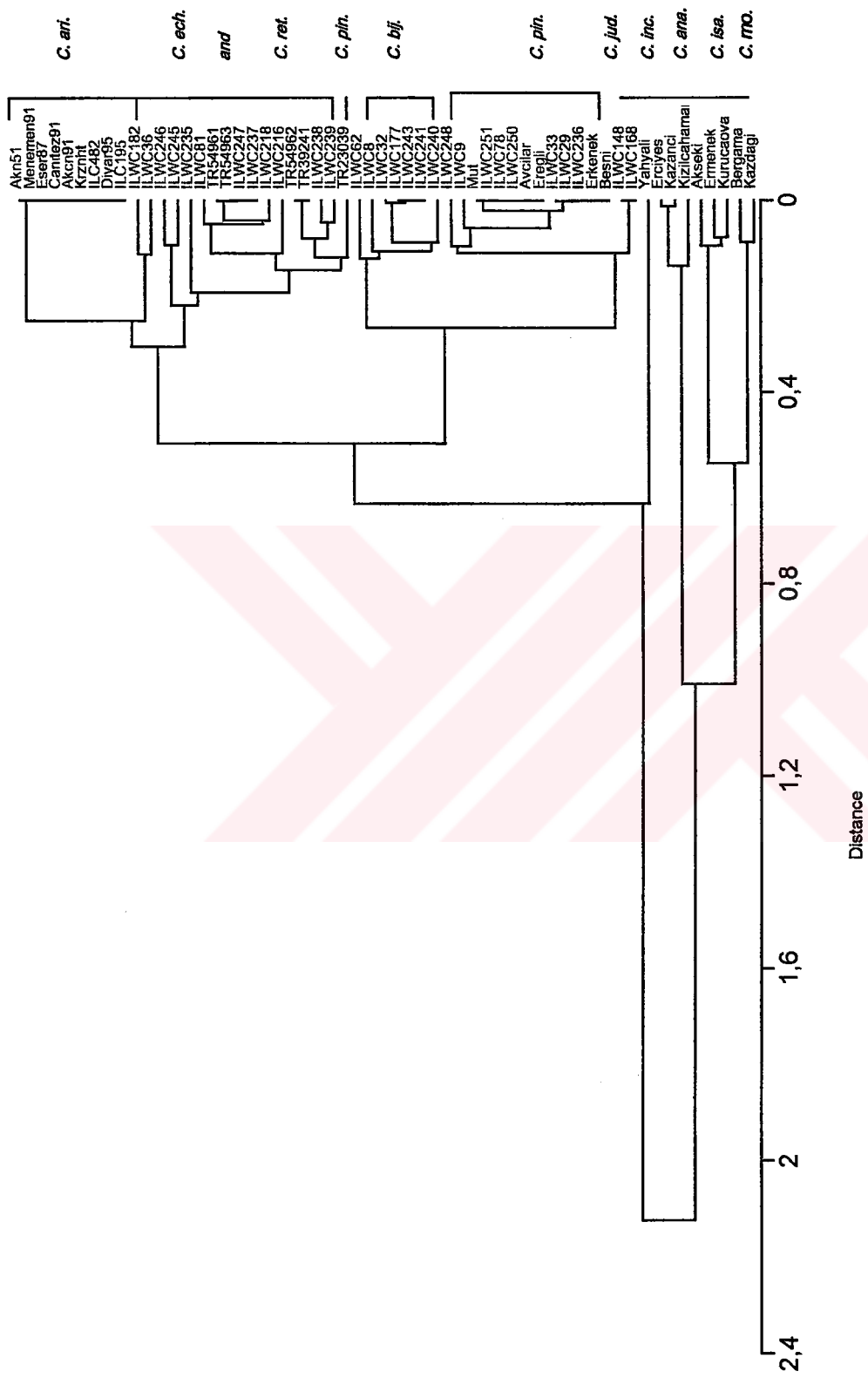

Figure 5. UPGMA phenogram of 57 *Cicer* accessions based on genetic distances of Nei (1978).

The noteworthy observation in clustering of *Cicer* species is the grouping of *C. incisum* with the annual species in both dendrograms of accessions and species (Figures 5 and 12). Small genetic distances detected between this species and annual species indicate that *C. incisum* is genetically closer to the examined annual species than the other perennial species based on allozyme variation. Classification of this species with one annual species in the *Chamocicer* section also supports the observed relationship here.

Allozyme data were subjected to distance Wagner Analysis using Prevosti distance. A dendrogram presumed to depict phylogenetic relationships among accessions thereby species was developed. Similar to UPGMA generated tree, distance wagner procedure grouped accessions together with few exceptions and displayed relationships among accessions (Figure 13). In addition, using allozyme based genetic distances (Nei 1978), Principle co-ordinate analysis (PCO) was carried out to visualize grouping of *Cicer* accessions in an ordination analysis. Unlike the RAPD based PCO analysis, allozyme based ordination analysis did not reveal a good graphical illustration of relationships among accessions and species (Figure 14).

3.2. RAPD results

Genomic DNA isolated from accessions of different *Cicer* species was successfully amplified following the optimization of template DNA, *Taq* DNA polymerase and $MgCl_2$ concentrations and thermal profile of the amplification reaction. Fifty 10-mer primers were screened on individuals representing each species. Seven primers showing consistently reproducible and simple amplification products were selected to screen *Cicer* accessions (Table 10). Assaying RAPD variation in accessions of *Cicer* species with these primers yielded 95 bands (ranging 0.15 – 2.5 kb in size), 92 of which were polymorphic. Four of these RAPD patterns obtained using primers UBC #1-30, UBC #1-44, UBC# 1- 23 and UBC #1-38 are given in Figures 6, 7, 8 and 9. Sequences of primers, their percentage of GC and the number of RAPD fragments they amplified and approximate sizes of products are given in Table 10. The number of bands varied from 8 - 18 with the primer used. The primer UBC # 1-44 has amplified the lowest number of RAPD bands, and primer UBC # 1-30 amplified the highest number of RAPD markers (Figure 7; Table 10).

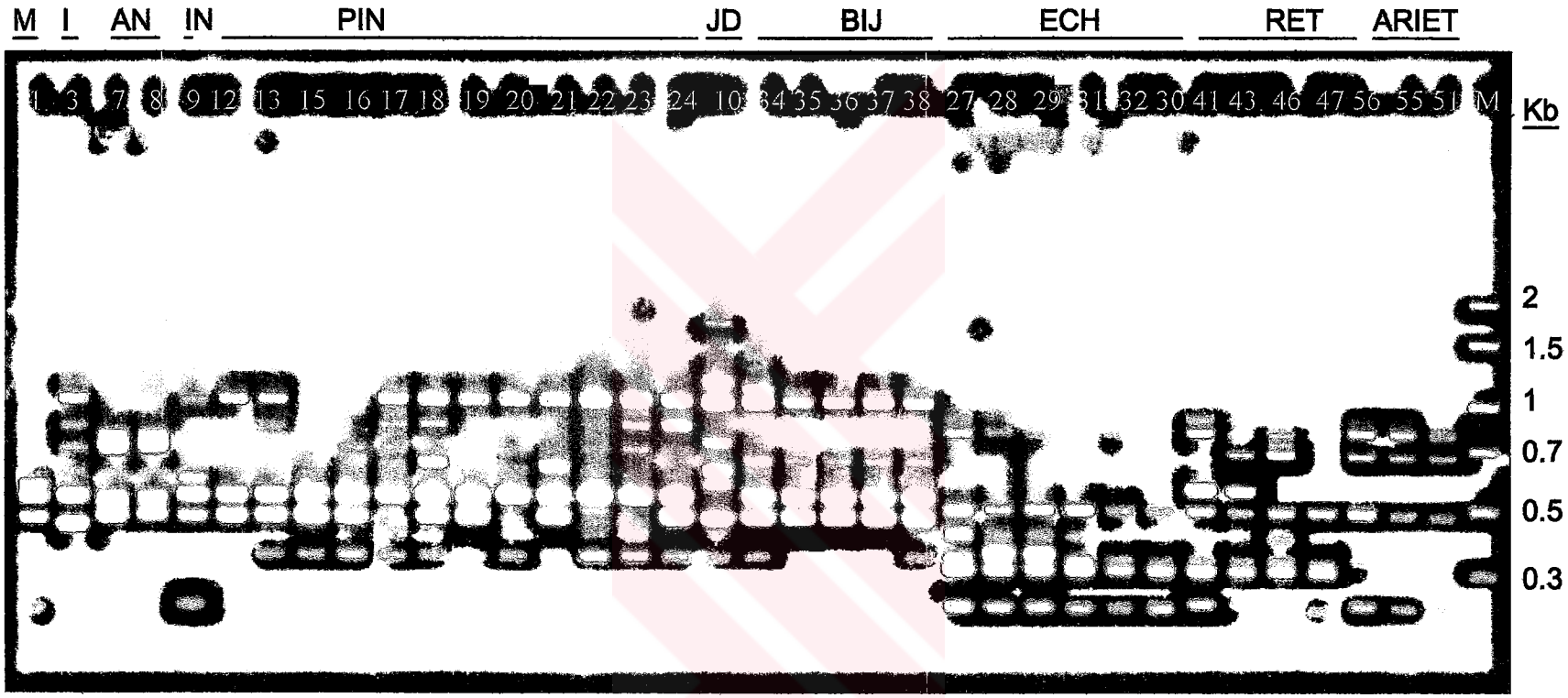

Figure 6. Amplification patterns of cultivated and wild *Cicer* accessions generated by using arbitrary primer UBC # 1-30. M is the size marker, and the number given above each lane corresponds to the accession code listed in Table 1. Abbreviations for species are as follows: M; *C. montbretii*, I; *C. isauricum*, AN; *C. anatolicum*, IN; *C. incisum*, PIN; *C. pinnatifidum*, JD; *C. judaicum*, BIJ; *C. bijugum*, ECH; *C. echinospermum*, RET; *C. reticulatum*, ARIET; *C. arietinum*.

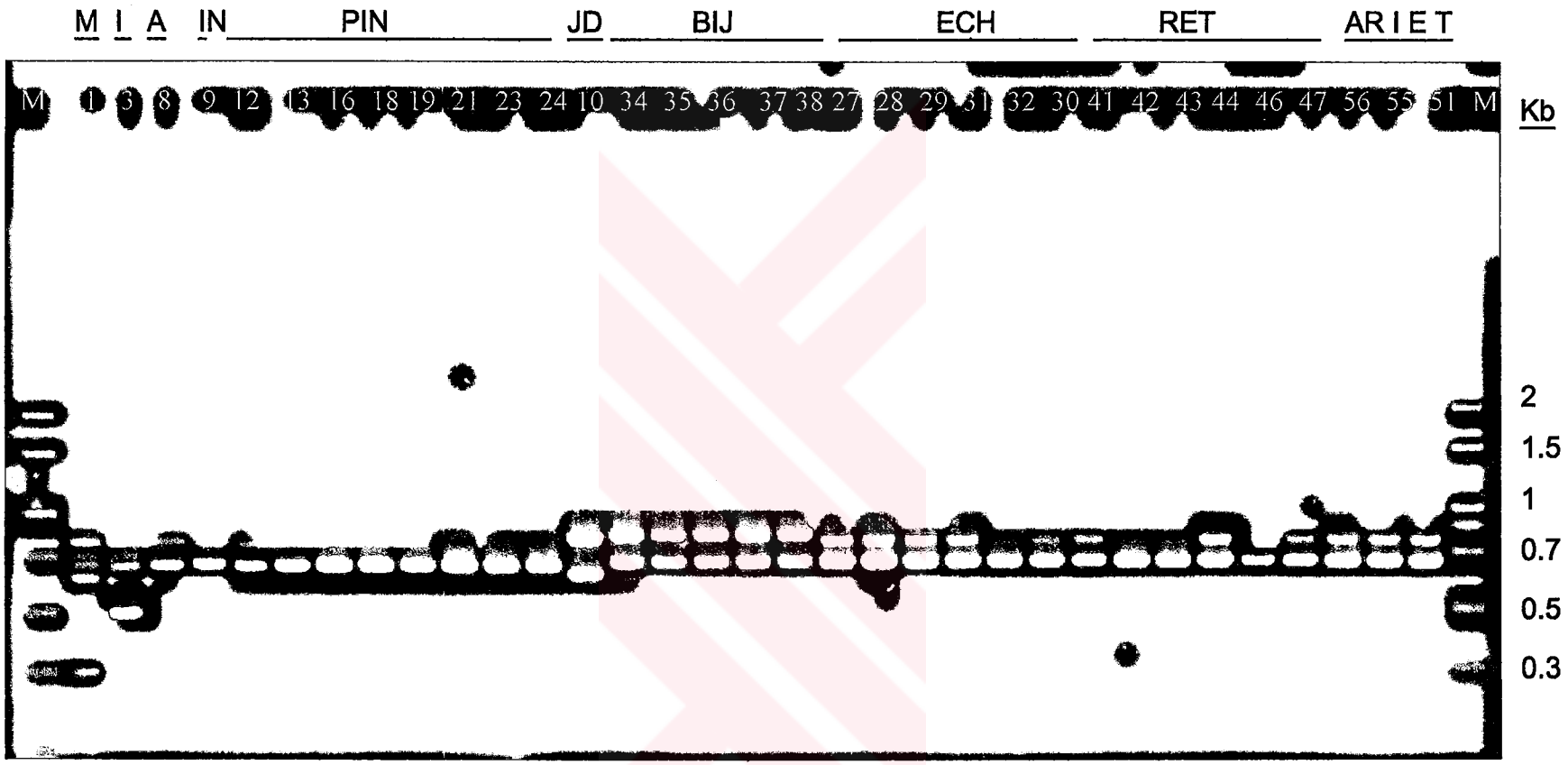

Figure 7. Amplification patterns of cultivated and wild *Cicer* accessions generated by using arbitrary primer UBC # 1-44. M indicates the size marker, and the number given above each lane corresponds to the accession code listed in Table 1. Abbreviations for species are as follows: M; *C. montbretii*, I; *C. isauricum*, A; *C. anatolicum*, IN; *C. incisum*, PIN; *C. pinnatifidum*, JD; *C. judaicum*, BIJ; *C. bijugum*, ECH; *C. echinospermum*, RET; *C. reticulatum*, ARIET; *C. arietinum*.

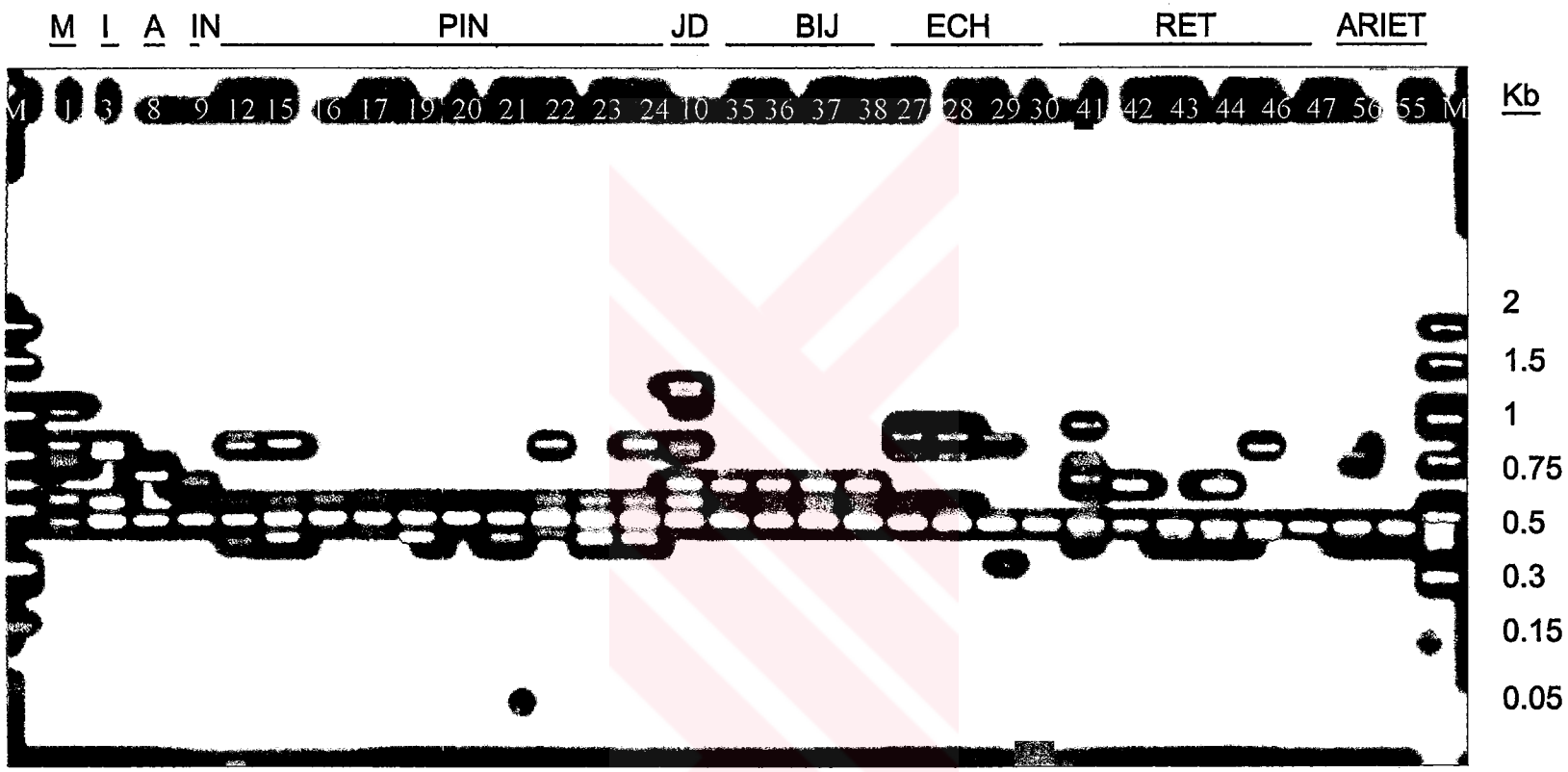

Figure 8. Amplification patterns of cultivated and wild *Cicer* accessions generated by using arbitrary primer UBC # 1-23. The number given above each lane corresponds to the accession code listed in Table 1. M is the size marker. Abbreviations for species are as follows: M; *C. montbretii*, I; *C. isauricum*, A/AN; *C. anatolicum*, IN; *C. incisum*, PIN; *C. pinnatifidum*, JD; *C. judaicum*, BIJ; *C. bijugum*, ECH; *C. echinospermum*, RET; *C. reticulatum*, ARIET; *C. arietinum*.

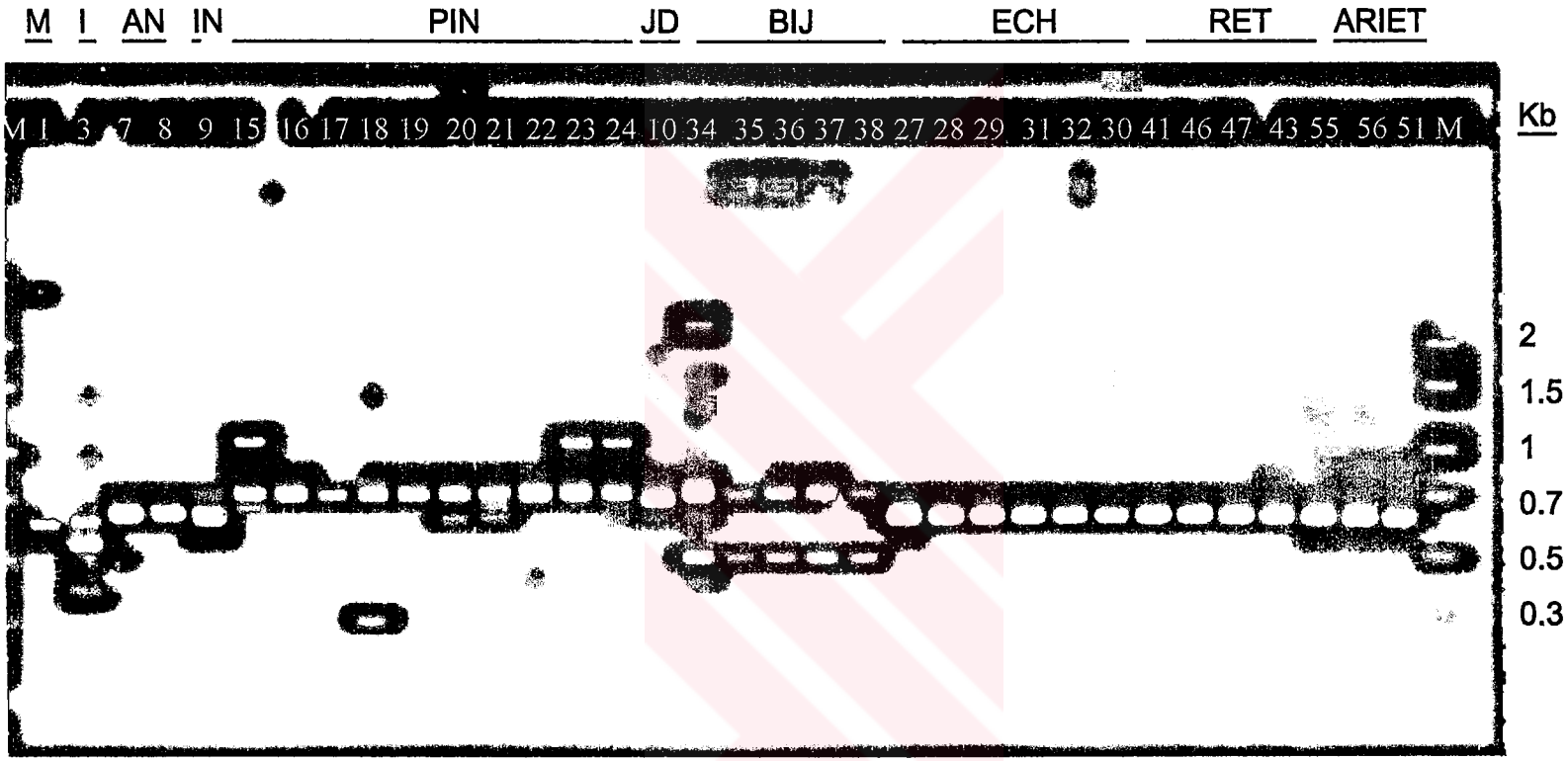

Figure 9. Amplification patterns of cultivated and wild *Cicer* accessions generated by using arbitrary primer UBC # 1-38. The number given above each lane corresponds to the accession code listed in Table 1, and M is the size marker. Abbreviations for species are as follows: M; *C. montbretii*, I; *C. isauricum*, AN; *C. anatolicum*, IN; *C. incisum*, PIN; *C. pinnatifidum*, JD; *C. judaicum*, BIJ; *C. bijugum*, ECH; *C. echinospermum*, RET; *C. reticulatum*, ARIET; *C. arietinum*.

Genetic variation in species represented by more than one accession was measured in terms of percent of polymorphic loci, mean number alleles per loci and mean gene diversity. The interspecific RAPD variation was very high among accessions of different *Cicer* species, but variation among accessions within species was relatively low. *C. pinnatifidum* represented by 13 accessions was the most polymorphic species followed *C. reticulatum* and *C. echinospermum* (Table 11). The observed levels of variation were comparable to the previous isozyme reports.

Table 10. Sequences of selected random primers used to amplify *Cicer* genomic DNA and deduce relationships.

Primer	Sequence 5'→3'	Number of RAPD markers	G C %	Approx. size (bp) Min. ´ Max.
UBC # 5	CCT GGG TTC C	14	70	250 – 1500
UBC # 6	CCT GGG TTC C	13	70	300 – 2000
UBC # 23	CCC GCC TTC A	12	70	450 – 1300
UBC # 30	CCG GCC TTA G	18	70	200 – 1500
UBC # 34	CCG GCC CCA A	16	80	150 – 1500
UBC # 38	CCG GGG AAA A	14	60	200 – 2500
UBC # 44	TTA CCC CGG C	8	70	300 - 850
Total/Aver.		95	70	264 - 1590

Table 11. Genetic variability at 95 RAPD loci in five *Cicer* species which were represented by more than one accession.

Cicer species	Mean number of observed alleles	Percent of polymorphic loci	Mean gene diversity^
C. pinnatifidum	1.284	28.42	0.0847 (0.150)
C. bijugum	1.073	7.37	0.0220 (0.006)
C. echinospermum	1.136	13.68	0.0455 (0.118)
C. reticulatum	1.168	16.84	0.0610 (0.143)
C. arietinum	1.01	1.05	0.0039 (0.038)
For 43 accessions	1.94	96.84	0.2412 (0.030)

^Nei, 1973, standard errors are given in parenthesis.

3.2.1. RAPD based intra and inter-species genetic distances and cluster analysis

To make inferences about relationships among accessions and species statistically, RAPD data matrix was used to compute pairwise genetic distances for accessions according to both Nei and Li's coefficient (1979) (Appendix B). Genetic distances among *Cicer* accessions were also computed according to Nei (1978) from the product frequencies. These distance matrices were used to develop dendrograms using unweighted pair-group method using arithmetic averages (UPGMA) (Figures 10, 11). Mean inter and intra-species genetic distance estimates of Nei and Li (1979) are given in Table 12. In both dendrograms, *Cicer* accessions formed two clusters, one of which contained accessions of both perennial and annual

species, and the other group had the accessions of three genetically close species. Within these groups, except *C. reticulatum* and *C. arietinum*, accessions of *C. bijugum*, *C. pinnatifidum*, *C. echinospermum* and *C anatolicum* clustered as separate groups. Accessions of *C. arietinum* and *C. reticulatum* did not form separate clusters indicating the close relatedness between them. Overall, genetic relatedness among *Cicer* species displayed in the dendrogram generally corroborated our grouping based on allozyme variation. However, some differences were obvious. For instance, *C. judaicum*, which had distinct RAPD profiles with almost every primer used, grouped outside the cluster containing the *C. pinnatifidum*, *C. bijugum* and the perennial species *C. incisum*. In addition, these species were found to be more closely related to perennial species than the group containing *C. echinospermum, C. reticulatum*, and *C. arietinum*, which had very similar RAPD profiles and formed a separate cluster in dendrograms (Figures 10, 11). Moreover, an unrooted tree were constructed to display relationships among accessions and species (Figure 12).

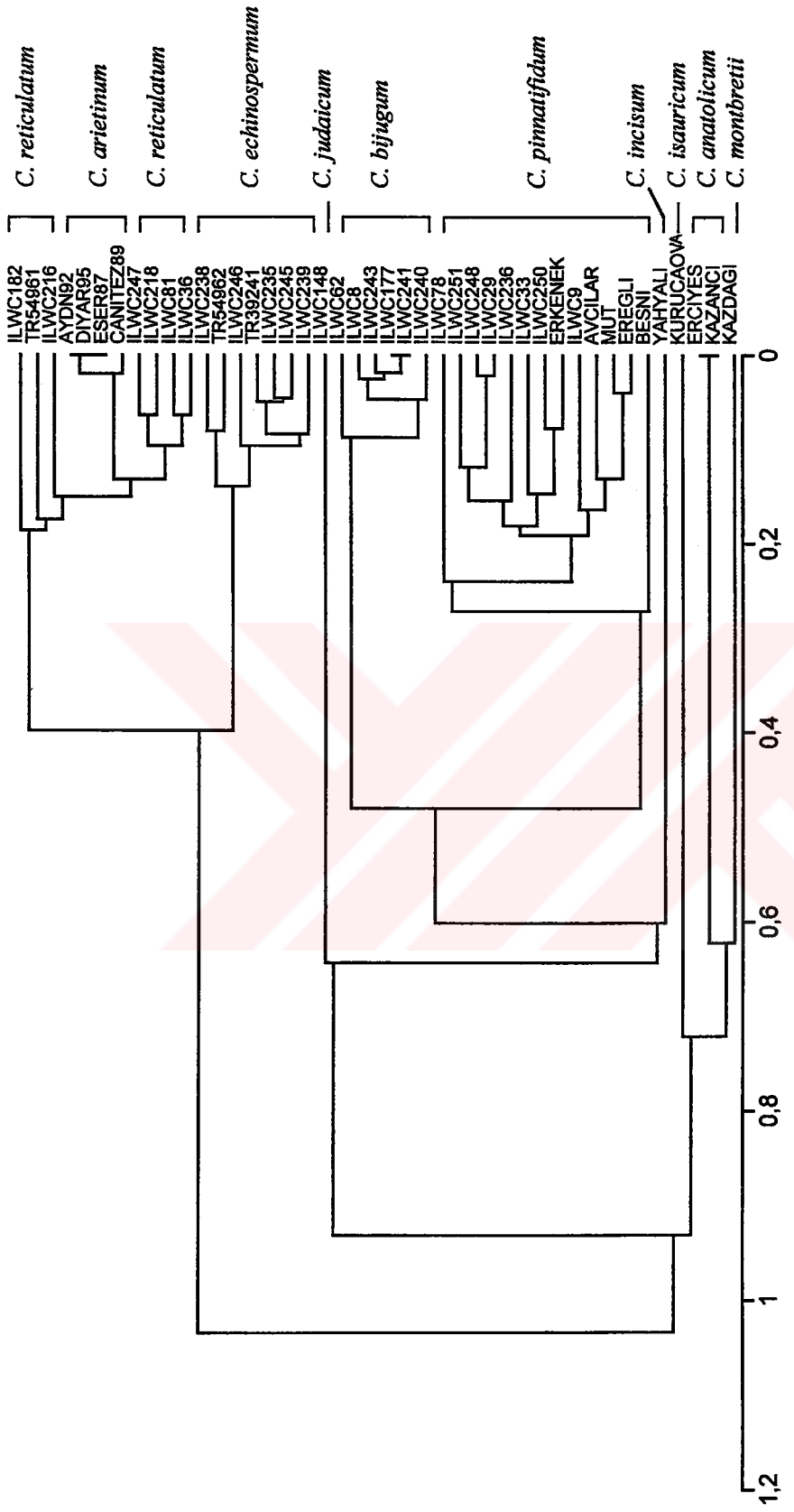

Figure 10. UPGMA dendrogram of *Cicer* accessions based on genetic distance estimates of Nei and Li (1979) computed from RAPD data.

Table 12. Estimates of mean genetic distances between *Cicer* species computed by averaging the genetic distances among respective accessions.

Cicer species	*C. mon.*	*C. isa*	*C. ant*	*C. inc.*	*C. pin.*	*C. jud.*	*C. bij.*	*C. ec.*	*C. re*	*C. ari.*
C. montbretii	0.000									
C. isauricum	0.734	0.000								
C. anatolicum	0.622	0.715	0.000							
C. incisum	0.738	0.821	0.942	0.000						
C. pinnatifidum	0.895	0.715	0.986	0.598	0.142*					
C. judaicum	0.936	1.011	1.191	0.753	0.641	0.000				
C. bijugum	0.806	0.883	1.145	0.606	0.612	0.629	0.030			
C. echinospermum	0.979	1.155	0.964	0.945	0.928	1.124	0.990	0.06		
C. reticulatum	1.161	1.237	1.148	1.139	1.102	1.243	1.012	0.39	0.11	
C. arietinum	1.095	1.173	1.116	1.006	1.049	1.104	0.960	0.41	0.14	0.006

* Mean genetic distance within species.

Mean genetic distances between *Cicer* species were computed by averaging the genetic distances among respective accessions (Table 12). These genetic distances ranged from 0.144 between *C. arietinum* and *C. reticulatum* to 1.243 between *C. reticulatum* and *C. judaicum*. Ranges of mean genetic distances among perennial and annual species were between 0.622 to 0.942 and 0.144 to 1.243 respectively. Among perennials, *C. incisum* had the lowest mean genetic distance values to annual species and closely clustered with annuals. *C. reticulatum* was the closest species to the chickpea (0.144). Three species (*C. echinospermum, C. reticulatum*, and *C. arietinum*) placed in the first branch of the dendrogram had considerably lower mean genetic distances ranging from 0.144 to 0.405 when compared to

distances between species in the remaining clusters (Table 12). In addition, mean genetic distances within species with more than one accessions were calculated by taking the averages of distances for accessions. They ranged from 0.006 to 0.142 indicating that mean genetic distances could be in some cases as high as the distance observed between different species as in the case of *C. arietinum* and *C. reticulatum* .

In order to determine the ability of the 95 RAPD bands to distinguish and asses the grouping of the accessions and species, Principle co-ordinate analysis (PCO) was employed. Accessions were plotted for the first tree coordinates which accounted for the 66,78, 18,91 and 9,86 % of the variation respectively (Figure 16). In addition, similarity matrices obtained using Nei and Li's formula (1979) and Jaccard's coefficient was compared to assess the equivalence of two similarity calculation methods using matrix comparison procedure of SYN-TAX-pc, and nearly a total correlation was found (r = 0.993). Furthermore, cophenetic correlation analysis also showed a significant correlation between dendrogram and distance matrix (r= 0.98).

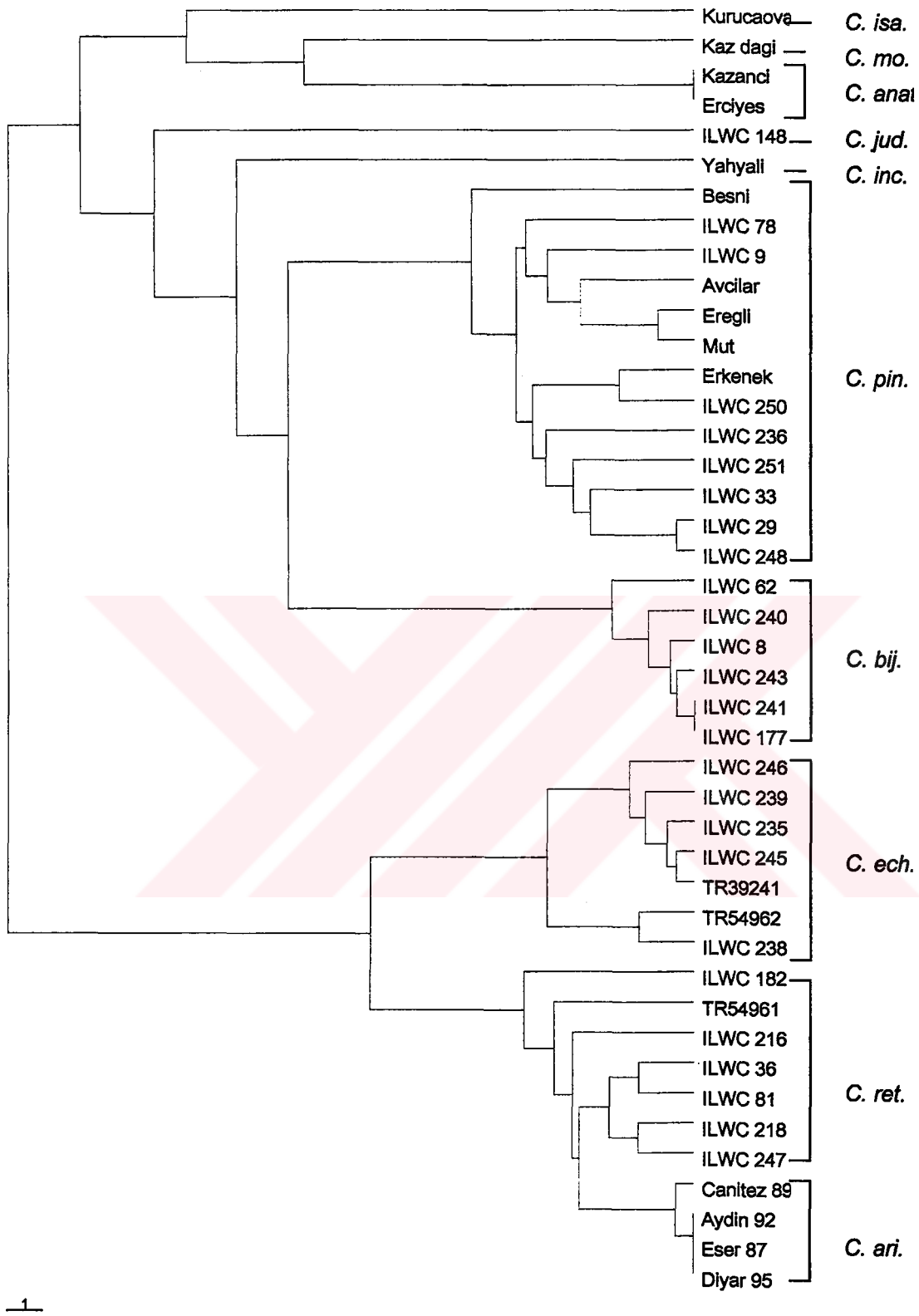

Figure 11. UPGMA dendrogram of *Cicer* accessions based on genetic distance estimates of Nei (1978) computed from RAPD data.

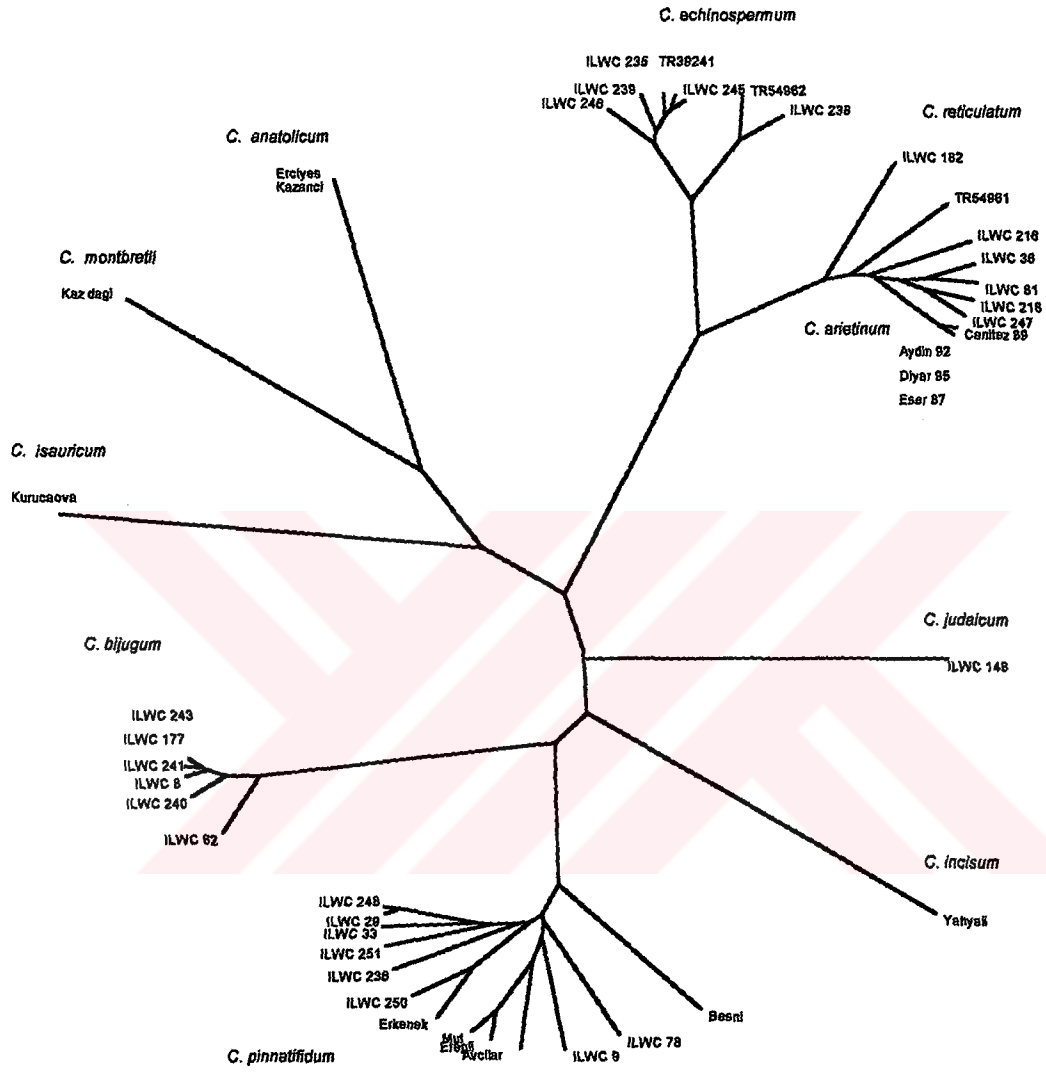

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Figure 12. Unrooted tree of *Cicer* accessions based on genetic distances of Nei (1978) computed from RAPD data.

CHAPTER 4

DISCUSSION

Genetic markers are commonly used to determine phylogenetic relationships, patterns of evolution and to characterize the plant genomes. They represent a sample of the genome, but they are used to compare entire genomes. Allozymes and Random Amplified Polymorphic DNA (RAPD) fragments are two marker systems frequently used in determination of genetic variation in natural populations.

In the present study, genetic variation and relationships among perennial and annual *Cicer* species with distribution in Turkey were assed using allozyme and RAPD markers. The level of variation detected with both marker systems was low in general, and the level of RAPD variation appear to be similar to the allozyme variation.

Cluster analysis based on allozyme and RAPD data revealed similar grouping of *Cicer* species. In addition, groupings of annual and perennial species were in agreement with their classical classification into three sections. Consistent with previous studies of relationships, *C. reticulatum* was found to be the closest annual species to the chickpea. On the other hand, *C. incisum* appears to be the closest perennial to the annuals among the perennials included in both studies.

4.1. Pattern of allozyme variation in *Cicer* appears to be correlated with the patterns observed in other annual and perennial selfer species

The observed level of allozyme variation was relatively lower than the previous reports in annual species, but higher levels of polymorphisms were detected in some perennial species. Presence of limited variation in annual *Cicer* species were previously known and our results are consistent with the previous observations. This aspect of the annual species was generally attributed to highly self-pollinated nature of the annual species (Kazan and Muelbauer, 1991; Ahmad *et. al* 1992; Tayyar and Waines, 1996). In addition, in annual self-pollinating plant species, genetic variation is generally partitioned into among rather than within populations of species,

and alternative alleles may be found either in high frequencies or fixed in different populations. Another pattern is the virtual lack of variation either within or among populations in self-pollinating species (Crawford 1989). Moreover, using isozyme data compiled from a broad spectrum of plant species Hamrick and Godt (1990) have shown that the amounts of variation are generally correlated with a series of life traits such as duration of life (perennial/annual), geographic distribution and breeding system. High levels of variation are usually found in perennials than in annuals (Hamrick 1989; Hamrick and Godt 1990). *Cicer* species usually have restricted geographic distribution and the population sizes are relatively small. The level and distribution of isozyme variation observed in our study are in agreement with these aspects of the allozyme diversity in plants and the previous reports of allozyme variation in *Cicer* (Kazan and Muelbauer, 1991; Ahmad *et. al.*, 1992; Tayyar and Waines, 1996).

The percent of polymorphic loci and mean number of alleles for *C. reticulatum* and *C. pinnatifidum* were close to the values reported for self-pollinated species and the previously calculated values for these species except Ahmad *et. al.* (1992) which had considerably higher percent of polymorphic loci for *C. reticulatum* and *C. pinnatifidum* (59 % and 32 % respectively). For the first time, polymorphism were detected in *C.*

echinospermum at four loci and the percent of polymorphic loci and mean number of alleles were 9.5 % and 1.07 respectively. Relatively low percent of polymorphic loci was observed in other annuals (*C. judaicum* and *C. bijugum*; Table 8). Ahmad *et al.* (1992) found no polymorphism within these species except *C. bijugum* (5 %). Tayyar and Waines (1996) observed no polymorphism in *C. echinospermum* and *C. bijugum* although they detected polymorphism in *C. judaicum* (13.04 %). In previous studies of allozyme variation no polymorphism was detected in perennial species contrary to our observation of polymorphism in three perennial species (Table 8). Percent of polymorphic loci and the mean number of alleles per locus were relatively higher in two perennial species (*C. isauricum* and *C. anatolicum*) than in annual species. The levels of polymorphism in perennial and in annual *Cicer* species are similar to the patterns of allozyme distribution reported by Hamrick and Godt (1990).

Previous surveys of allozyme variation in *C. arietinum* utilizing the large numbers of accessions indicated that it has limited allozyme variation (Tuwafe *et. al.*, 1987; Kazan and Muelbauer, 1991; Ahmad *et al.*,1992; Tayyar and Waines, 1996). Tuwafe *et. al.*, (1987) studied isozyme variation in 1,392 accessions collected from 25 countries and found polymorphism at only four isozyme loci. Tayyar and Waines, 1996 surveyed isozyme

variation in 25 accessions and detected polymorphism at 3 loci. Ahmad *et. al.* (1992) assayed isozyme polymorphism in 25 accessions of cultivated species and found no polymorphism. Because of this poor genetic variability, several investigators have developed interspecific hybrids between *C. arietinum* and *C. reticulatum* and *C. echinospermum* to construct mapping populations (Gaur and Slinkard, 1990a, b; Kazan *et al.*, 1993). The objective of this study was mainly to assess variation and relationships among the species with distribution in Turkey, and included nine *C. arietinum* accessions revealed no polymorphism.

4.2. Allozyme based clustering of annual and perennial species

Dendrogram of *Cicer* species obtained using unbiased genetic distances of Nei (1978) revealed two clusters; one for three perennial species and one for one perennial and six annual species (Figure 13). Perennial species cluster included *C. montbretii, C. isauricum* and *C. anatolicum*. These species belong to *Polycicer* section. Genetic similarity based on allozyme loci corroborates the classification of these three species into the section *Polycicer* based on morphology, distribution and life cycle.

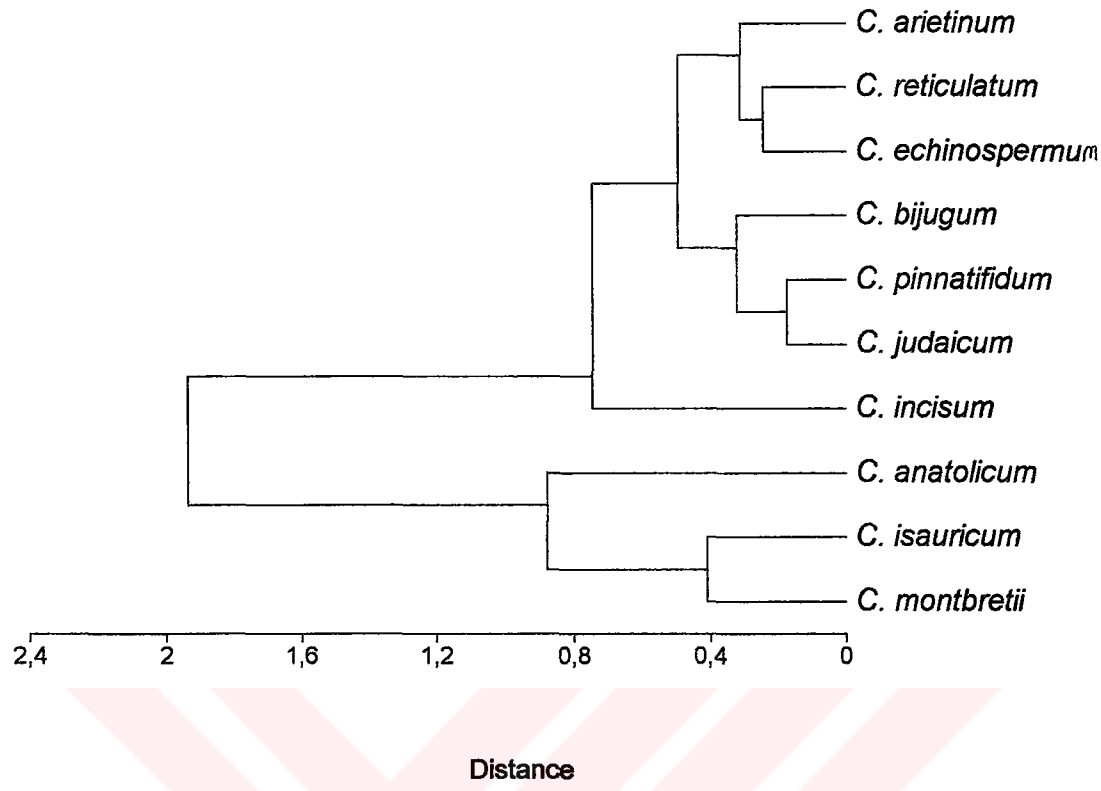

Figure 13. UPGMA phenogram of *Cicer* species based on averaged genetic distances of Nei (1978) calculated from allozyme frequencies.

Within the perennial species cluster, considerably large genetic distance (GD = 1.1) was detected between *C. anatolicum* and the group containing *C. montbretii* and *C. isauricum,* both of which were closer to each other (GD = 0.54). In the second cluster, an unusual grouping of one perennial species (*C. incisum*) with all the annual species was observed. *C. incisum* belongs to the section *Chamocicer.* Both allozyme analysis and the classification of this species in the *Chamocicer* section with the annual species *C. chorassanicum* reveal same information that it could be the closest perennial to annuals. However, the species spectrum of the present

study is not sufficient to generalize that *C. incisum* is the closest perennial to the annual species without studying remaining annual and perennials in the genus. Previous groupings of *C. chorassanicum, C. yamashita* and *C. cuneatum* have revealed that they are relatively distant to the annual species surveyed in this study (Kazan and Muelbauer, 1991; Ahmad *et al.*, 1992; Tayyar and Waines, 1996). Only one study clustered *C. chorassanicum* and *C. yamashita* with perennial species *C. anatolicum* and *C. songoricum* (Tayyar & Waines 1996). Species relationships based on our data indicate that three perennial species including *C. anatolicum* are relatively distant, and *C. incisum* is the closest perennial to the cluster of six annual species. Kazan and Muelbauer (1991) grouped *C. anatolicum* with *C. arietinum*, *C. reticulatum* and *C. echinospermum* whereas both our analysis and the relationships deduced by Tayyar and Waines (1996) contradict to that grouping. In chickpea, surveying allozyme variation in germplasm collections at three life stages using six enzyme systems, Triest and Kabir (2000) have found that the amount of allozyme variation decreases from seed to mature plant, and the topology of the dendrogram changes. In both Tayyar and Waines (1996) and our analysis, seeds were used as enzyme source whereas leaflets of seedlings were used in Kazan and Muelbauer (1991). This might be the one the reason for the observed difference in grouping *C. anatolicum*.

Six annual species belonging to *Monocicer* section were grouped together within the second cluster in the dendrogram (Figure 13). This grouping was fully consistent with the relationships given in Tayyar and Waines (1996) and in agreement with Kazan and Muelbauer (1991) and Ahmad *et. al.* (1992) in general. The first cluster included *C. pinnatifidum, C. judaicum* and *C. bijugum* and the second cluster included *C. arietinum, C. reticulatum*, and *C. echinospermum*. The observed genetic distances within these clusters were relatively small (Table 9). *C. judaicum* and *C. pinnatifidum* were closer to each other (GD = 0.12) than the *C. bijugum*. Although grouping of these three species agrees with the grouping observed by Ahmad *et. al.* (1992), *C. judaicum* is the closest species to *C. pinnatifidum* in our study. This difference may also be due to the enzyme source utilized in both studies similar to the case observed with *C. anatolicum*.

4.3. Distance wagner tree and principle coordinate analysis (PCO)

Based on allozyme data, a phylogenetic three was constructed using distance Wagner procedure and Prevosti distance (Figure 15). This procedure also grouped accessions into species clusters as in UPGMA. Overall, the wagner tree had two main branches one bearing accessions of

perennial species and the other one containing accessions for annual species and *C. incisum*. In addition to the similar grouping, the distance wagner procedure also clearly indicated that *C incisum* is the closest perennial to annuals and *C. reticulatum* is the closest annual to chickpea. Wagner tree appears to show a step wise differentiation of annual and perennial species.

Subjecting allozyme based genetic distances to ordination analysis did not provide a good resolution of relationships among accessions and species (Figure 14). Only the accessions belonging to three perennial species (*C. montbretii, C. isauricum* and *C. anatolicum*) and *C. bijugum* could be separated as distinct groups. Accession for the remaining species grouped very closely in the center of the plot.

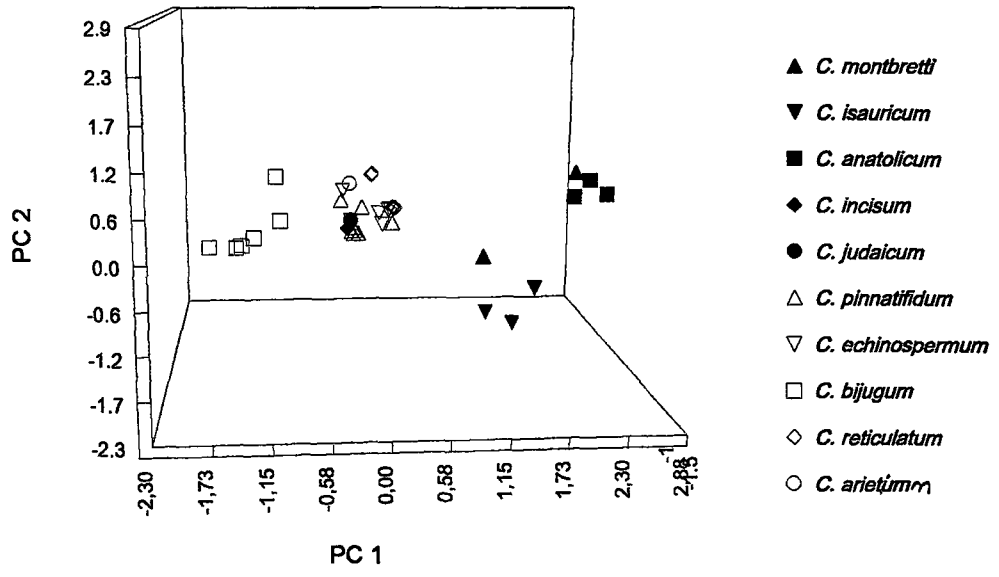

Figure 14. Associations among *Cicer* accessions as revealed by Principle coordinate analysis. First three coordinates (PC) account for the 66.8, 18.9 and 9.9% of the variation respectively.

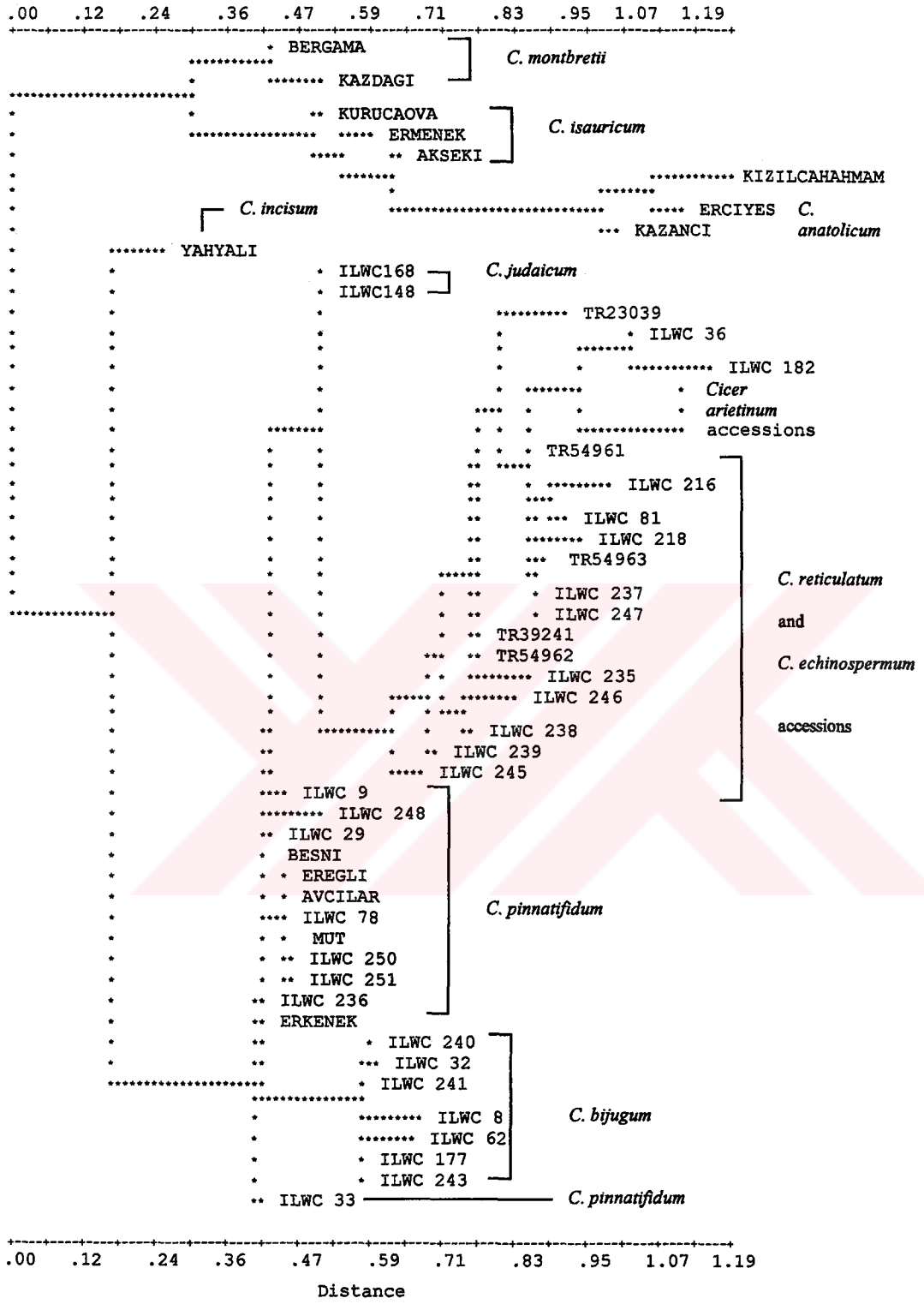

Figure 15. Allozyme based dendrogram of *Cicer* accessions developed according to distance wagner procedure using Prevosti distance.

4.4. RAPD variation in *Cicer*

RAPD analysis is another approach used to study inter- and intra-species DNA variation and genetic relationships in a number of plant taxa to detect (Skroch *et al.* 1993; Sharma *et. al.*1995a; Abo-elwafa *et. al.* 1995; Link *et. al.* 1995; Butos *et. al.* 1998). In *Cicer,* Ahmad (1999) studied relationships among annual species with RAPD markers using bulked DNA samples from a single accessions for each species recently.

In the present study, RAPD markers were used to deduce intra and interspecies relationships and examine RAPD variation more comprehensively in 43 wild and cultivated accessions representing ten *Cicer* species (annuals and perennials). This is the first study covering annual and perennial species to asses inter-species DNA variation (in some intra-species variation) and genetic relationships in the genus.

A total of fifty 10-mer primers were screened for their ability to amplify *Cicer* genomic DNA, and amplification products were obtained in about 15 of them. Among these primers, a few failed to amplify DNA in some species, and others did not have clear RAPD profiles, and they were excluded from the analysis. Apparently, some of them required different amplification conditions than were used in this study. Seven primers with clear and

scorable patterns were used to screen *Cicer* accessions (Table 10). However, if optimized, may be individually, the other primers could also be used.

4.5. Some aspects of using RAPDs in diversity assessments

There are some concerns regarding the use of RAPDs in analysis of genetic relationships. First, their reproducibility have been questioned because of the sensitivity of DNA amplification with arbitrary primers to reaction conditions. Maintenance of consistent reaction conditions is critical for reproducible RAPD profiles. Second, the dominant inheritance of RAPD markers leads to the loss of information when compared to co-dominant markers. Last, the uncertainty of homology among the co-migrating amplification products observed among different individuals or species. Homology of co-migrating amplified fragments can be determined by either Southern analysis using one of the band as a probe (Thormann et. al. 1994; Weising *et. al.* 1995) or cleaving shared RAPD band with 4 bp or 6 bp cutter restriction enzymes to see that same cleavage patterns for the same band could be obtained among different genotypes (Bustos *et al.* 1998). These studies indicate that nearly 20 % of the co-migrating bands appears to be not homologous. In our PCR experiments, following the optimization of

several parameters, *Cicer* genomic DNA was amplified reproducibly and clear RAPD profiles were obtained (Figures 6, 7, 8, 9). All shared bands were assumed to be homologous to each other. These aspects of RAPD analysis were taken into account during data collection and analysis.

4.6. RAPD based clustering of *Cicer* accessions and species

The RAPD based dendrogram of *Cicer* accessions displayed the genetic relationships between annual and perennial species among members of each of these groups as well as the accessions for each species (Figures 10, 11 and 12). Relationships among six annual species obtained by RAPD analysis were in agreement with the previous studies of relationships which were carried out using various approaches ranging from crossability to isozyme analysis (Ladizinsky and Adler 1976; Kazan and Muelbauer, 1991; Ahmad *et. al.* 1992; Ahmad and Slinkard, 1992; Tayyar and Waines, 1996). However, different groupings were observed in two perennials when compared to the previous studies (Kazan and Muelbauer 1991; Tayyar and Waines 1996).

Despite some differences, overall, grouping of the species is similar to the grouping observed in allozyme analysis. One of these differences was

the grouping of three annual species (*C. bijugum, C. pinnatifidum* and *C. judaicum*) with perennials, but they formed a separate cluster on the branch bearing perennials. When considering species/accessions on same cluster are closer to each other than the species/accessions on different clusters, these annuals are closer to perennials than the remaining annual species based on RAPD analysis. This similarity was also apparent in the patterns generated by four of the seven primers. Within this cluster, grouping of *C. judaicum* and *C. incisum* was another significant difference in the dendrogram. Previous allozyme and seed storage protein variation studies including our allozyme analysis have indicated that *C. judaicum* is closer to the *C. pinnatifidum*, but both Ahmad (1999) and grouping observed here surprisingly placed this species further away from the accessions of *C. pinnatifidum* (Figures 10 and 11). Other differences were in grouping of two perennial species *C. incisum* and *C. isauricum*. Consistent with the allozyme phenogram, *C. incisum* was the closest perennial to the annual species. It closely clustered with three annual species in the first branch of the dendrogram, but they were as a group closer to the cluster containing perennial species than the branch containing remaining annuals (Figures 10, 11). *C. isauricum* and the two other perennial species (*C. anatolicum* and *C. montbretii*) formed a separate cluster consistent with allozyme analysis. In contrast to allozyme study, RAPD analysis revealed that *C. anatolicum* is

closer to the *C. montbretii* than *C. isauricum*. Ahmad (1999) observed striking differences between grouping based on RAPD analysis and previous analysis of relationships among annual species. Although some differences were apparent as discussed above, generally, our grouping is compatible with our previous analysis of relationships among both annual and perennial *Cicer* species. Only the six annual species are common to both studies, and we do not know the placements of those annual species that were not studied here with respect to the annuals and perennials included in this study.

4.7. Principle coordinate analysis of RAPD data

In addition to the cluster analysis, principle co-ordinate analysis (PCO) was carried out to determine whether associations among accessions of *Cicer* species could be resolved further. PCO analysis grouped accessions according to their respective species with the exception of grouping observed among accessions of *C arietinum* and *C. reticulatum* similar to the cluster analysis (Figure 16). Unlike the allozyme based PCO, RAPD based PCO analysis using the first three principle coordinates provided a good resolution of relationships among accessions thereby species in the co-ordinate system confirming the relationships in the

dendrogram. One reason for this difference could be the number of markers employed to deduce relationships in RAPD analysis.

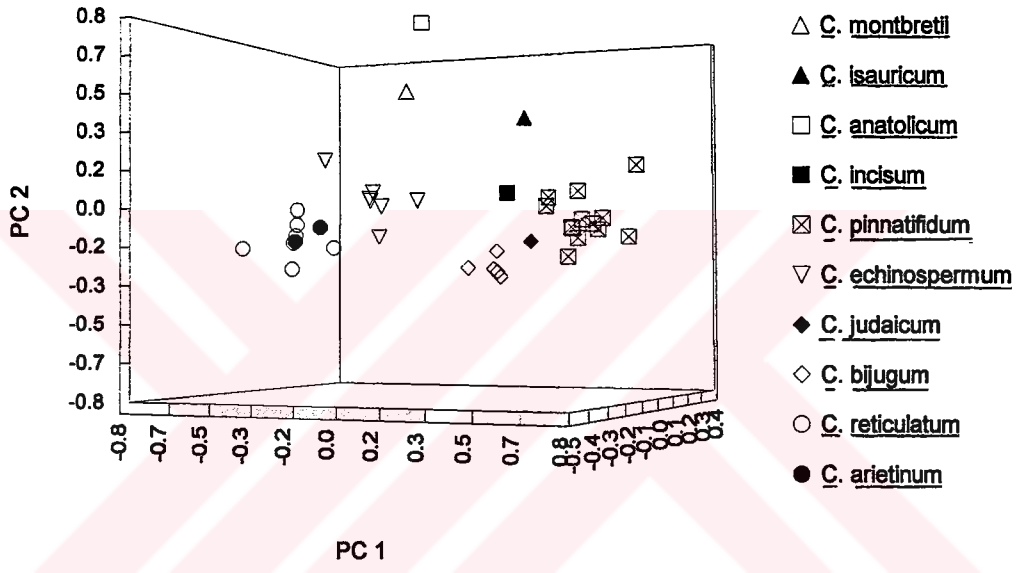

Figure 16. Associations among 43 wild and cultivated *Cicer* accessions as revealed by Principle Co-ordinate Analysis (PCO) based on genetic distance estimates calculated for 95 RAPD gel positions.

4.8. Assessment of RAPD and allozyme based tree differences

Differences observed between RAPD-based trees and allozyme based trees could be due to several reasons. The number of loci sampled in

two studies, the principle differences in two techniques in sampling variation at different levels and loci from different portions of the genome, and the error associated with each technique and the method of estimating genetic distances could cause these differences. Allozymes are products of structural genes and represent a proportion of the variation in coding regions. They may be under some selective processes. However, RAPD amplification techniques scan anonymous DNA sequences and presumed cover entire genome and represent the variation at DNA level (Williams *et al.* 1990). In this respect, RAPD analysis appears to be less restrictive in surveying polymorphism in the entire genome. Hence, it appears to be an efficient and quick method of screening DNA sequence polymorphisms at very large number of loci. Despite the fact that the error associated with RAPD analysis could be large due to the characteristics of RAPDs, the number of markers used to deduce relationships and the features of RAPDs mentioned above suggest that RAPD-based tree could be more reliable.

4.9. RAPD variation in annual species

RAPD analysis also enabled us to measure genetic diversity among accessions of different species and among accessions within species, which were represented by more than one accession. The percent of polymorphic

loci and mean heterozygosity are the two parameters commonly used to measure genetic diversity. In general, estimated values of these parameters among accessions of different species were considerably high. However, genetic diversity within species was limited as in the case of allozyme analysis. *C. arietinum* had the lowest while *C. pinnatifidum* had the highest genetic variability when compared to other three annual species (Table 11). The level of polymorphism observed in *C. pinnatifidum* is comparable to the previous reports of allozyme variation. For the perennial species in RAPD analysis we had DNA from only one accession for each species, thus, it was impossible to measure these parameters and compare with allozyme analysis. Distribution of RAPD variation somewhat reflects the presence of limited intra species variation, and previous studies also suggest that there is a correlation between the level of intra-specific RAPD variation and reproductive system. Autogamous species are generally characterized by a large inter-population RAPD variation (Kazan *et al.* 1993). In this respect, RAPD variation parallels the level of allozyme variation, and the magnitude of it appears to be close to the values reported for lentil species (Sharma et. al.1995).

4.10. Based on allozyme and RAPD analysis derived relationships, assessment of evolution of chickpea and life cycle in the genus *Cicer*

Previous studies utilizing different approaches from crossability to allozyme analysis have suggested that two wild species showing distribution in southeastern Turkey are in same crossability group and closely related to each other. Species in this cluster are regarded as primary gene pool of *C. arietinum* L. (Ladizinsky and Adler, 1976; Kazan and Muelbauer, 1991; Ahmad *et al.*, 1992; Ahmad and Slinkard, 1992; Muelbauer *et al.*, 1994 Tayyar and Waines, 1996). Our study of genetic relationships have also confirmed this grouping and revealed that some of the *C. reticulatum* accessions closer to the *C. arietinum*. Genetic distance between *C. echinospermum* and *C. reticulatum* was very small (GD = 0.25) indicating that they are closer to each other than *C. arietinum*. The closest wild species to the cultivated species was *C. reticulatum* (GD = 0.27). Allozyme based clustering of *C. reticulatum* accessions revealed two groups; one included ILWC 36 and ILWC 182 which were grouped with *C. arietinum* accessions, and the other group (6 accessions, Figure 5) clustered with the accessions of *C. echinospermum*. Grouping of *C. reticulatum* accessions suggests that some of the accessions may be closer to cultivated species and may have played a role in the evolution of chickpea from this species.

C. reticulatum is regarded as the wild progenitor of chickpea based on morphology, crossability, and electrophoresis of seed storage proteins and isozymes (Ladizinsky and Adler, 1976; van der Maesen, 1987; Kazan and Muelbauer, 1991; Ahmad *et al.*, 1992; Ahmad and Slinkard, 1992). Based on the presence of closely related species in southern Turkey, several investigators have proposed this region to be the possible center of origin for chickpea. Chickpea is also considered as one of the founder crops domesticated in Fertile Crescent. The distribution ranges of *C. reticulatum* and *C echinospermum* accessions in southern Turkey and their close clustering with accessions of *C. arietinum* correlate with the postulation of this region as the domestication site. *C. reticulatum* has considerable allelic variability in allozyme analysis whereas *C. arietinum* had no polymorphism and many of the alleles present in *C. reticulatum* were not detected in *C. arietinum* indicating that 'founder effect' operating in domestication of chickpea have reduced the actual variability to a narrow spectrum.

Based on the results obtained in both allozyme and RAPD analysis, one could also speculate on the evolution of annual habit from perennial state in the genus *Cicer*. In both studies, among perennial species, *C. incisum* is the genetically closest species to the annuals. Classification of this species with one annual species (*C. chorassanicum*) in the *Chamocicer*

section based on morphology and life cycle also supports our grouping as in allozyme analysis. Thus, both allozyme and RAPD variation appears to reveal same information regarding the evolution of life cycle in the genus *Cicer* and closest wild species of *C. arietinum*. However, the observed relationships were estimated using accessions collected from Turkey, and it only includes approximately a quarter of known wild species in the genus. Thus the species spectrum is insufficient to generalize the observed relationships to the entire genus. In addition, only the four *C. arietinum* lines included to asses the grouping of this species with respect to annuals and perennials. Observed RAPD variation was not sufficient to say that RAPD analysis can be used to differentiate chickpea accessions. Among four varieties, only one could be differentiated from the other three, and the observed low level of variation somewhat reflects the limited genetic variation in this species.

CHAPTER 5

CONCLUSIONS

- Both allozyme and RAPD markers are useful in studying variation and relationships in *Cicer* species. The levels of variation detected with both marker systems are similar in *Cicer*. However, when the number of markers and their genomic coverage are considered, RAPDs appear to be more informative.
- In contrast to the previous allozyme studies, polymorphism was detected in annual species; *C. echinospermum*, *C. bijugum* and *C. judaicum*.
- Among studied perennial species *C. incisum* is the closest species to the studied annuals.
- As it is observed in previous studies of relationships, *C. reticulatum* is the closest wild species to the chickpea, in allozyme analysis, two *C. reticulatum* accessions were found to be closer to the chickpea than the remaining accessions of this species.

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Table Allozyme based pairwise genetic distances among accessions.

Species	Accessions	Kaz dagı	Bergama	Kurucaova	Ermenek	Akseki	Kizilcahamam	Kazancı	Erciyes	Yahyalı	ILWC168	ILWC148	Besni	Eregli
C. montbretil	Kaz dagi	0												
C. montbretil	Bergama	0,088	0											
C. isauricum	Kurucaova	0,545	0,41	0										
C. isauricum	Ermenek	0,68	0,524	0,078	0									
C. isauricum	Akseki	0,643	0,487	0,106	0,083	0								
C. anatolicum	Kizilcahamam	1,065	1,065	1,048	0,843	0,814	0							
C. anatolicum	Kazancı	1,104	1,104	1,086	1,053	0,761	0,106	0						
C. anatolicum	Erciyes	1,116	1,116	1,099	1,077	0,773	0,167	0,012	0					
C. incisum	Yahyalı	2,582	2,582	1,792	1,77	1,988	2,459	2,436	2,485	0				
C. judaicum	ILWC168	2,572	2,572	1,846	1,683	1,994	2,157	2,072	2,357	0,411	0			
C. judaicum	ILWC148	2,574	2,574	1,84	1,678	1,994	2,169	2,083	2,371	0,411	0	0		
C. pinnatifidum	Besni	1,825	2,572	1,846	1,683	2,004	2,205	2,116	2,414	0,411	0,088	0,088	0	
C. pinnatifidum	Eregli	1,813	2,56	1,77	1,615	1,966	2,243	2,152	2,463	0,448	0,114	0,114	0,021	0
C. pinnatifidum	Avcilar	1,813	2,56	1,77	1,615	1,966	2,243	2,152	2,463	0,448	0,114	0,114	0,021	0
C. pinnatifidum	Erkenek	1,835	2,582	1,792	1,637	1,988	2,265	2,173	2,485	0,405	0,089	0,088	0	0,021
C. pinnatifidum	ILWC33	1,819	2,566	1,776	1,633	1,972	2,267	2,181	2,469	0,389	0,091	0,091	0,001	0,022
C. pinnatifidum	ILWC236	1,835	2,582	1,792	1,637	1,988	2,265	2,173	2,485	0,405	0,089	0,088	0	0,021
C. pinnatifidum	ILWC9	1,818	2,565	2,245	2,017	2,073	1,767	1,701	1,908	0,487	0,125	0,126	0,036	0,071
C. pinnatifidum	ILWC250	1,806	2,553	1,809	1,648	1,97	2,164	2,076	2,368	0,453	0,113	0,113	0,02	0
C. pinnatifidum	ILWC29	1,827	2,574	1,835	1,673	1,993	2,178	2,091	2,382	0,41	0,088	0,088	0	0,021
C. pinnatifidum	ILWC78	1,813	2,56	1,77	1,615	1,966	2,243	2,152	2,463	0,448	0,114	0,113	0,021	0
C. pinnatifidum	ILWC248	1,835	2,582	2,485	2,212	2,426	2,265	2,173	2,485	0,539	0,173	0,174	0,077	0,112
C. pinnatifidum	ILWC251	1,754	2,447	1,713	1,564	1,895	2,156	2,079	2,359	0,453	0,115	0,115	0,022	0
C. pinnatifidum	Mut	1,813	2,56	1,77	1,615	1,966	2,243	2,152	2,463	0,448	0,114	0,113	0,021	0
C. pinnatifidum	TR23039	1,8	1,377	1,351	1,239	1,224	1,443	1,441	1,552	0,658	0,333	0,335	0,332	0,391
C. echinospermum	ILWC239	1,784	1,784	1,439	1,32	1,494	1,642	1,645	1,769	0,565	0,193	0,193	0,301	0,34
C. echinospermum	ILWC245	2,551	2,551	1,761	1,606	1,957	2,234	2,099	2,454	0,516	0,16	0,159	0,267	0,292
C. echinospermum	ILWC246	2,559	2,559	2,461	2,188	2,307	2,242	2,13	2,461	0,67	0,254	0,255	0,364	0,425
C. echinospermum	ILWC235	1,603	1,603	1,769	1,614	1,618	1,418	1,416	1,524	0,853	0,365	0,367	0,502	0,569
C. echinospermum	ILWC238	1,823	1,823	1,78	1,625	1,672	1,652	1,655	1,78	0,689	0,26	0,261	0,369	0,423

Table (continued)

Species	Accessions	Kaz dagi	Bergama	Kurucaova	Ermenek	Akseki	Kizilcahamam	Kazanci	Erciyes	Yahyali	ILWC168	ILWC148	Besni	Eregli
C. echinos.	TR39241	1,835	1,835	1,792	1,637	1,572	1,292	1,286	1,386	0,693	0,264	0,267	0,388	0,448
C. echinos.	TR54962	1,835	1,835	1,792	1,637	1,572	1,292	1,286	1,386	0,693	0,264	0,267	0,388	0,448
C. bijugum	ILWC240	2,383	4,868	2	3,709	3,469	2,186	2,099	2,391	0,887	0,384	0,387	0,273	0,326
C. bijugum	ILWC241	2,477	4,6	2,485	2,212	3,024	4	3,64	3,3	0,693	0,292	0,291	0,185	0,212
C. bijugum	ILWC243	2,477	4,6	2,485	2,212	3,024	4	3,64	3,7	0,693	0,292	0,291	0,185	0,212
C. bijugum	ILWC8	2,366	4,764	2,693	2,507	3,156	3,86	3,876	4,772	0,712	0,417	0,417	0,296	0,332
C. bijugum	ILWC177	2,477	4,6	2,485	2,212	3,024	4	3,64	3,7	0,693	0,292	0,291	0,185	0,212
C. bijugum	ILWC62	2,477	4	2,485	2,212	3,024	4	3,64	3,6	0,875	0,411	0,411	0,291	0,323
C. bijugum	ILWC32	2,207	3,711	2,466	2,193	3,005	3,134	2,952	3,719	0,723	0,306	0,305	0,195	0,224
C. reticulatum	ILWC36	2,477	2,477	2,485	2,212	2,126	1,292	1,286	1,386	1,099	0,663	0,666	0,673	0,758
C. reticulatum	ILWC182	1,752	1,752	2,453	2,18	2,395	1,633	1,635	1,76	1,095	0,67	0,671	0,67	0,747
C. reticulatum	ILWC216	1,691	1,691	1,754	1,599	1,64	1,211	1,205	1,302	0,656	0,356	0,357	0,352	0,411
C. reticulatum	ILWC218	1,729	1,729	1,742	1,632	1,556	1,051	1,041	1,106	0,669	0,445	0,448	0,451	0,519
C. reticulatum	ILWC81	1,469	1,469	1,778	1,622	1,684	1,217	1,211	1,308	0,679	0,378	0,38	0,379	0,434
C. reticulatum	ILWC237	1,769	1,769	1,783	1,628	1,58	1,039	1,029	1,118	0,684	0,373	0,376	0,38	0,439
C. reticulatum	ILWC247	1,776	1,776	1,784	1,629	1,579	1,037	1,027	1,116	0,685	0,374	0,376	0,38	0,44
C. reticulatum	TR54961	1,831	1,831	1,788	1,633	1,568	1,017	1,007	1,095	0,697	0,382	0,385	0,389	0,447
C. reticulatum	TR54963	1,714	1,714	1,762	1,62	1,57	1,041	1,034	1,115	0,664	0,378	0,381	0,384	0,446
C. arietinum	ILC195	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Diyar 95	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	ILC 482	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Kırmzı nohut	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Akcin 91	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Canıtez 91	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Eser 87	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Menemen 91	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591
C. arietinum	Akn 51	1,784	1,784	2,485	2,212	2,426	2,265	2,29	2,485	0,693	0,529	0,53	0,529	0,591

Table (continued)

Accessions	Avcilar	Erkenek	ILWC33	ILWC236	ILWC9	ILWC250	ILWC29	ILWC78	ILWC248	ILWC251	Mut	TR23039	ILWC239	ILWC245	ILWC246
ILWC33	0,022	0,001	0												
ILWC236	0,021	0	0,001	0											
ILWC9	0,071	0,048	0,049	0,048	0										
ILWC250	0	0,021	0,023	0,021	0,059	0									
ILWC29	0,021	0	0,001	0	0,035	0,02	0								
ILWC78	0	0,021	0,022	0,021	0,071	0	0,021	0							
ILWC248	0,112	0,087	0,089	0,087	0,07	0,104	0,079	0,112	0						
ILWC251	0	0,021	0,023	0,021	0,072	0	0,022	0	0,113	0					
Mut	0	0,021	0,022	0,021	0,071	0	0,021	0	0,112	0	0				
TR23039	0,391	0,35	0,359	0,35	0,292	0,378	0,337	0,391	0,35	0,37	0,391	0			
ILWC239	0,34	0,302	0,31	0,302	0,35	0,34	0,303	0,34	0,39	0,319	0,34	0,132	0		
ILWC245	0,292	0,263	0,269	0,263	0,333	0,295	0,266	0,292	0,381	0,284	0,292	0,247	0,069	0	
ILWC246	0,425	0,382	0,391	0,382	0,365	0,417	0,374	0,424	0,382	0,416	0,424	0,188	0,115	0,093	0
ILWC235	0,569	0,517	0,53	0,517	0,442	0,554	0,501	0,569	0,517	0,545	0,569	0,221	0,143	0,241	0,242
ILWC238	0,423	0,387	0,396	0,387	0,37	0,415	0,379	0,423	0,387	0,401	0,423	0,11	0,046	0,159	0,065
TR54962	0,448	0,405	0,415	0,405	0,299	0,428	0,385	0,448	0,405	0,427	0,448	0,119	0,07	0,157	0,159
ILWC241	0,212	0,182	0,186	0,182	0,243	0,214	0,184	0,212	0,288	0,203	0,212	0,481	0,425	0,381	0,515
ILWC243	0,212	0,182	0,186	0,182	0,243	0,214	0,184	0,212	0,288	0,203	0,212	0,481	0,425	0,381	0,515
ILWC8	0,332	0,295	0,29	0,295	0,34	0,332	0,295	0,332	0,39	0,323	0,332	0,608	0,571	0,53	0,654
ILWC32	0,224	0,193	0,197	0,193	0,256	0,226	0,195	0,224	0,301	0,215	0,224	0,503	0,444	0,399	0,538
ILWC36	0,758	0,693	0,711	0,693	0,558	0,734	0,669	0,758	0,693	0,732	0,758	0,335	0,39	0,516	0,515
ILWC216	0,411	0,368	0,377	0,368	0,338	0,401	0,358	0,411	0,368	0,389	0,411	0,156	0,197	0,323	0,248
ILWC218	0,519	0,468	0,471	0,468	0,373	0,501	0,45	0,519	0,468	0,496	0,519	0,167	0,205	0,313	0,314
ILWC81	0,434	0,391	0,401	0,391	0,356	0,424	0,381	0,434	0,391	0,412	0,434	0,141	0,151	0,249	0,25
ILWC247	0,44	0,398	0,407	0,398	0,3	0,422	0,378	0,44	0,398	0,419	0,44	0,116	0,157	0,255	0,256
TR54961	0,447	0,407	0,417	0,407	0,3	0,428	0,387	0,447	0,407	0,426	0,447	0,12	0,166	0,264	0,265
TR54963	0,446	0,401	0,408	0,401	0,308	0,428	0,383	0,445	0,401	0,424	0,445	0,117	0,156	0,256	0,257
C. ariet.	0,591	0,539	0,552	0,539	0,522	0,584	0,531	0,591	0,539	0,568	0,591	0,272	0,271	0,381	0,382

Table (continued)

Accessions	ILWC235	ILWC238	TR39241	TR54962	ILWC240	ILWC241	ILWC243	ILWC8	ILWC177	ILWC62	ILWC32	ILWC36
ILWC238	0,163	0										
TR39241	0,106	0,089	0									
TR54962	0,106	0,089	0	0								
ILWC240	0,575	0,526	0,41	0,41	0							
ILWC241	0,671	0,52	0,539	0,539	0,088	0						
ILWC243	0,671	0,52	0,539	0,539	0,088	0	0					
ILWC8	0,818	0,658	0,661	0,661	0,159	0,09	0,09	0				
ILWC177	0,671	0,52	0,539	0,539	0,088	0	0	0,09	0			
ILWC62	0,853	0,673	0,693	0,693	0,184	0,087	0,087	0,187	0,087	0		
ILWC32	0,652	0,543	0,562	0,562	0,091	0,005	0,005	0,098	0,005	0,095	0	
ILWC36	0,316	0,412	0,288	0,288	0,532	0,693	0,693	0,841	0,693	0,875	0,674	0
ILWC182	0,373	0,455	0,453	0,453	0,673	0,68	0,68	0,831	0,68	0,844	0,662	0,113
ILWC216	0,304	0,159	0,217	0,217	0,487	0,502	0,502	0,635	0,502	0,656	0,525	0,33
ILWC218	0,258	0,226	0,14	0,14	0,506	0,619	0,619	0,764	0,619	0,768	0,648	0,246
ILWC81	0,201	0,171	0,148	0,148	0,502	0,525	0,525	0,647	0,525	0,679	0,548	0,251
ILWC237	0,198	0,177	0,088	0,088	0,415	0,53	0,53	0,653	0,53	0,684	0,553	0,185
ILWC247	0,199	0,177	0,088	0,088	0,414	0,531	0,531	0,654	0,531	0,685	0,554	0,184
TR54961	0,206	0,186	0,087	0,087	0,412	0,542	0,542	0,665	0,542	0,697	0,565	0,183
TR54963	0,199	0,176	0,093	0,093	0,429	0,538	0,538	0,632	0,538	0,697	0,563	0,192
C. ariet. acc.	0,329	0,292	0,288	0,288	0,545	0,539	0,539	0,661	0,539	0,693	0,562	0,288

Accessions	ILWC182	ILWC216	ILWC218	ILWC81	ILWC237	ILWC247	TR54961	TR54963	ILC 195	Diyar 95	ILC482	Kirmizi nohut	Akcin 91
ILWC216	0,313	0											
ILWC218	0,368	0,152	0										
ILWC81	0,221	0,095	0,08	0									
ILWC237	0,304	0,105	0,042	0,04	0								
ILWC247	0,307	0,106	0,042	0,041	0	0							
TR 54961	0,328	0,115	0,045	0,055	0,001	0	0						
TR 54963	0,299	0,103	0,038	0,033	0	0	0,003	0					
C. ariet. accesions	0,214	0,322	0,324	0,188	0,267	0,269	0,289	0,26	0	0	0	0	0

Table RAPD based pairwise genetic distances among accessions.

Species	Accessions	Kaz dagı	Kurucaova	Kazancı	Erciyes	Yahyalı	Besni	Eregli	Avcilar	Erkenek	ILWC33
C. montbretii	Kaz da-ı	0									
C. isauricum	Kurucaova	0,734	0								
C. anatolicum	Kazancı	0,622	0,715	0							
C. anatolicum	Erciyes	0,622	0,715	0	0						
C. incisum	Yahyalı	0,738	0,821	0,942	0,942	0					
C. pinnatifidum	Besni	0,832	0,734	0,823	0,823	0,65	0				
C. pinnatifidum	Ere-li	0,875	0,693	0,989	0,989	0,54	0,233	0			
C. pinnatifidum	Avcılar	0,738	0,734	0,823	0,823	0,571	0,191	0,134	0		
C. pinnatifidum	Erkenek	0,842	0,675	0,832	0,832	0,673	0,294	0,233	0,147	0	
C. pinnatifidum	ILWC33	0,981	0,772	0,989	0,989	0,612	0,233	0,223	0,183	0,099	0
C. pinnatifidum	ILWC236	0,916	0,732	1,168	1,168	0,58	0,386	0,213	0,223	0,223	0,213
C. pinnatifidum	ILWC9	1,002	0,711	1,146	1,146	0,56	0,254	0,147	0,203	0,251	0,243
C. pinnatifidum	ILWC250	0,896	0,711	1,011	1,011	0,633	0,308	0,194	0,154	0,077	0,194
C. pinnatifidum	ILWC29	0,96	0,753	1,1	1,1	0,592	0,267	0,203	0,161	0,213	0,154
C. pinnatifidum	ILWC78	0,738	0,821	0,823	0,823	0,571	0,362	0,183	0,191	0,243	0,345
C. pinnatifidum	ILWC248	0,939	0,734	1,076	1,076	0,571	0,245	0,183	0,139	0,194	0,134
C. pinnatifidum	ILWC251	1,002	0,639	1,011	1,011	0,713	0,254	0,147	0,154	0,16	0,194
C. pinnatifidum	Mut	0,916	0,587	1,033	1,033	0,511	0,223	0,039	0,128	0,179	0,213
C. echinospermum	ILWC239	0,989	1,211	1	1	0,989	0,87	0,812	0,87	0,875	0,916
C. echinospermum	ILWC245	0,916	1,118	0,916	0,916	0,916	0,916	0,853	0,916	0,821	0,853
C. echinospermum	TR39241	0,87	1,079	1	1	0,87	0,989	0,812	0,87	0,875	0,916
C. echinospermum	TR54962	1,121	1,079	1	1	0,989	1,277	0,916	0,989	0,981	1,033
C. echinospermum	ILWC238	1,1	1,343	0,839	0,839	1,1	1,1	1,146	1,1	1,079	1,146
C. judaicum	ILWC148	0,936	1,011	1,191	1,191	0,753	0,6	0,639	0,6	0,624	0,639
C. bijugum	ILWC240	0,86	0,846	1,211	1,211	0,62	0,55	0,462	0,486	0,459	0,523
C. bijugum	ILWC241	0,821	0,899	1,168	1,168	0,58	0,511	0,425	0,446	0,423	0,486
C. bijugum	ILWC243	0,753	0,916	1,056	1,056	0,6	0,531	0,443	0,405	0,387	0,504

Table (continued)

Species	Accessions	Kaz dagı	Kurucaova	Kazancı	Erciyes	Yahyalı	Besni	Eregli	Avcilar	Erkenek	ILWC33
C. bijugum	ILWC8	0,801	0,879	1,146	1,146	0,56	0,56	0,405	0,426	0,462	0,531
C. bijugum	ILWC177	0,821	0,899	1,168	1,168	0,58	0,511	0,425	0,446	0,423	0,486
C. bijugum	ILWC62	0,781	0,86	1,121	1,121	0,693	0,612	0,446	0,47	0,443	0,511
C. reticulatum	ILWC36	1,191	1,273	1,228	1,228	1,056	1,343	1,1	1,056	0,936	0,981
C. reticulatum	ILWC182	1,041	1,234	1,056	1,056	1,291	1,291	1,079	1,158	1,03	1,079
C. reticulatum	ILWC216	1,146	1,231	1,178	1,178	1,146	1,483	1,191	1,146	1,002	1,056
C. reticulatum	ILWC218	1,366	1,158	1,1	1,1	1,211	1,546	1,118	1,211	1,061	1,118
C. reticulatum	ILWC81	1,118	1,197	1,146	1,146	1,002	1,252	1,041	1,118	0,994	1,041
C. reticulatum	ILWC247	1,231	1,313	1,121	1,121	1,1	1,386	1,139	1,1	0,976	1,022
C. reticulatum	TR54961	1,033	1,252	1,204	1,204	1,168	1,168	0,96	1,033	1,022	1,079
C. arietinum	Canıtez 89	1,079	1,158	1,1	1,1	1,079	1,211	1,002	1,079	0,955	1,002
C. arietinum	ESER 87	1,1	1,178	1,121	1,121	0,981	1,231	1,022	1,1	0,976	1,022
C. arietinum	Diyar 95	1,1	1,178	1,121	1,121	0,981	1,231	1,022	1,1	0,976	1,022
C. arietinum	Aydin 92	1,1	1,178	1,121	1,121	0,981	1,231	1,022	1,1	0,976	1,022

Accessions	ILWC236	ILWC9	ILWC250	ILWC29	ILWC78	ILWC248	ILWC251	Mut	ILWC239	ILWC245	ILWC246	ILWC235
ILWC9	0,233	0										
ILWC250	0,186	0,213	0									
ILWC29	0,147	0,223	0,174	0								
ILWC78	0,329	0,203	0,203	0,212	0							
ILWC248	0,128	0,203	0,154	0,021	0,245	0						
ILWC251	0,186	0,167	0,167	0,128	0,254	0,108	0					
Mut	0,205	0,142	0,142	0,194	0,223	0,174	0,142	0				
ILWC239	0,853	0,939	0,939	0,787	0,766	0,87	0,832	0,853	0			
ILWC245	0,896	0,981	0,981	0,832	0,812	0,916	0,875	0,896	0,1	0		
ILWC246	0,821	0,896	0,896	0,759	0,738	0,832	0,801	0,821	0,123	0,118	0	
ILWC235	0,896	0,981	0,981	0,832	0,812	0,916	0,875	0,896	0,1	0,046	0,069	0

Table (continued)

Accessions	ILWC236	ILWC9	ILWC250	ILWC29	ILWC78	ILWC248	ILWC251	MUT	ILWC239	ILWC245	ILWC246	ILWC235	TR39241
TR39241	0,853	0,939	0,939	0,787	0,766	0,87	0,832	0,853	0,051	0,049	0,073	0,049	0
TR54962	0,96	1,05	1,056	0,894	0,87	0,989	0,939	0,96	0,163	0,1	0,178	0,1	0,105
ILWC238	1,191	1,321	1,168	0,989	0,965	1,1	1,168	1,191	0,137	0,13	0,211	0,13	0,137
ILWC148	0,607	0,587	0,656	0,693	0,673	0,673	0,732	0,607	1,231	1,022	0,936	1,139	1,1
ILWC240	0,499	0,481	0,481	0,569	0,55	0,55	0,607	0,442	1,118	0,936	0,86	1,041	1,002
ILWC241	0,462	0,443	0,443	0,531	0,511	0,511	0,569	0,405	1,079	0,896	0,821	1,002	0,96
ILWC243	0,423	0,462	0,405	0,486	0,467	0,467	0,523	0,423	1,1	0,916	0,842	1,022	0,981
ILWC8	0,443	0,425	0,425	0,511	0,491	0,491	0,55	0,387	1,056	0,981	0,801	0,981	0,939
ILWC177	0,462	0,443	0,443	0,531	0,511	0,511	0,569	0,405	1,079	0,896	0,821	1,002	0,96
ILWC62	0,486	0,531	0,405	0,491	0,54	0,47	0,531	0,425	0,916	0,853	0,693	0,853	0,812
ILWC36	1,022	1,252	1,118	0,96	0,939	1,056	1,002	1,139	0,429	0,405	0,362	0,405	0,36
ILWC182	1,011	1,217	1,217	1,061	1,041	1,158	0,994	1,118	0,405	0,329	0,405	0,329	0,345
ILWC216	1,1	1,366	1,211	1,033	1,011	1,146	1,079	1,231	0,536	0,503	0,453	0,503	0,456
ILWC218	1,041	1,273	1,273	1,1	1,079	1,211	1,022	1,158	0,453	0,362	0,449	0,362	0,383
ILWC81	0,976	1,178	1,178	1,022	1,002	1,118	0,955	1,079	0,362	0,345	0,308	0,345	0,302
ILWC247	1,061	1,291	1,158	1,002	0,981	1,1	1,041	1,178	0,475	0,384	0,47	0,384	0,405
TR54961	0,896	1,1	1,1	0,939	0,916	1,033	0,981	1,002	0,272	0,257	0,28	0,257	0,211
Cantez 89	0,936	1,139	1,139	0,981	0,96	1,079	0,916	1,041	0,383	0,362	0,384	0,362	0,319
Eser 87	0,955	1,158	1,158	1,002	0,981	1,1	0,936	1,061	0,405	0,384	0,345	0,384	0,341
Diyar 95	0,955	1,158	1,158	1,002	0,981	1,1	0,936	1,061	0,405	0,384	0,345	0,384	0,341
Aydin 92	0,955	1,158	1,158	1,002	0,981	1,1	0,936	1,061	0,405	0,384	0,345	0,384	0,341

Table (continued)

Accessions	TR54962	ILWC238	ILWC148	ILWC240	ILWC241	ILWC243	ILWC8	ILWC177	ILWC62	ILWC36	ILWC182	ILWC216	ILWC218	ILWC81	ILWC247	TR54961	Canitez 89	Eser 87
TR54962	0																	Diyar 95
ILWC238	0,08	0																Aydin 92
ILWC148	1,231	1,211	0															
ILWC240	1,118	1,231	0,642	0														
ILWC241	1,079	1,191	0,607	0,037	0													
ILWC243	1,1	1,211	0,624	0,054	0,018	0												
ILWC8	1,056	1,168	0,656	0,057	0,019	0,038	0											
ILWC177	1,079	1,191	0,607	0,037	0	0,018	0,019	0										
ILWC62	0,916	1,011	0,639	0,118	0,08	0,099	0,061	0,08	0									
ILWC36	0,429	0,48	1,158	0,955	0,916	0,936	0,896	0,916	0,781	0								
ILWC182	0,405	0,449	1,252	1,152	1,118	1,136	1,1	1,118	0,976	0,194	0							
ILWC216	0,456	0,511	1,252	1,022	0,981	1,002	0,96	0,981	0,832	0,095	0,203	0						
ILWC218	0,319	0,429	1,465	1,197	1,158	1,178	1,139	1,158	1,002	0,112	0,167	0,17	0					
ILWC81	0,428	0,475	1,1	1,011	0,976	0,994	0,955	0,976	0,842	0,063	0,16	0,161	0,083	0				
ILWC247	0,405	0,453	1,332	1,1	1,061	1,079	1,041	1,061	0,916	0,087	0,186	0,191	0,063	0,103	0			
TR54961	0,337	0,381	1,139	1,041	1,002	1,022	0,981	1,002	0,853	0,17	0,223	0,234	0,191	0,134	0,212	0		
Canitez 89	0,453	0,503	1,178	1,079	1,041	1,061	1,022	1,041	0,896	0,161	0,167	0,17	0,134	0,083	0,154	0,139	0	
Eser 87	0,475	0,526	1,079	0,994	0,955	0,976	0,936	0,955	0,821	0,134	0,186	0,139	0,154	0,061	0,174	0,161	0,02	0
Diyar 95	0,475	0,526	1,079	0,994	0,955	0,976	0,936	0,955	0,821	0,134	0,186	0,139	0,154	0,061	0,174	0,161	0,02	0
Aydin 92	0,475	0,526	1,079	0,994	0,955	0,976	0,936	0,955	0,821	0,134	0,186	0,139	0,154	0,06	0,174	0,161	0,02	0

APPENDIX C

Reagents and their catalog numbers

Name	Manufacturer code (Sigma)
Soluble Starch	S 4501
L-Histidine	H 8000
Tetrazolium (MTT)	M 2128
Phenazine methosulfate (PMS)	P 9625
Nitro Blue Tetrazolium (NBT)	N 6876
N-(3-Aminoproply)-morpholine	A 9028
Sucrose	S 8501
Polyvinilypyrolidone	Sigma PVP
6-Phosphogluconic acid	P 7627
NAD	N 7004
NADP	N 0505
Shikimic acid	S 5375
D-Fructose 6-phosphate	F 3627
DL-Isocitric acid	I 1252
L-Malic acid (Mono sodium salt)	M 1125
Citric acid (Monohydrate)	C7129
Glucose-6-phosphate dehydrogenase	G 5760
a- D Glucose-1-phosphate	G 7000
Trizma base	T 8524
DTT	D 9779
2-mercaptoethanol	M 3148
Bromphenol blue	B 5525
EDTA	E 5134
dNTPs	DNAamp
Taq DNA polymerase	Promega
Agarose	Bioshop
PCR Marker	P 9577

APPENDIX D

Collection addresses of *Cicer* accessions

C. montbretii

Kaz dağı, Balıkesir, Edremit, Kaz dağı under *P. burita* forest.
Bergama, Izmir, 10 km from Bergama to Kozak; Pine forest; near stream.

C. isauricum

Kurucaova, Konya, Beyşehir Kurucaova between Musalla and Muslu.
Ermenek, Konya, ebove Samsali Village.
Akseki, Antalya, Antalya to Akseki Horse Energy Forest.

C. anatolicum

Kazancı, Konya on the slopes ebove the town of Kazancı.
Erciyes, Kayseri, 2 km after Hacılar to Develi, on the east side of the Mount Erciyes under patches of wild populus trees.
Kızılcahamam, Ankara, Kızılcahamam ebove Berçincatak Village.

C. incisum

Yahyali, Kayseri, Yahyali, On slopes across to Aladağ Zinc minery.

Cicer pinnatifidum

ILWC 9 ICRISAT Elazığ, Harput; road to Fatih Ahmet rubble only; not in smooth soil.
ILWC 29 Gaziantep 5 km E of Gaziantep.
ILWC 33 Adana.
ILWC 78 ICRISAT Mardin 35 km to Midyat; 14 km from Savur.

ILWC 236 Şanlı Urfa Pirhalli; 2 km N of Pirhalli; 4 km S of Borzova.
ILWC 244 Gaziantep 8 km W of Gaziantep to Adana road.
ILWC 248 Diyarbakır 35 km N of Diyarbakır on road to Elazığ.
ILWC 249 Elazığ Near Harput on road from Elazığ across road from Cemetary.
ILWC 250 Elazığ 11 km after Keban dam towards Arapkir.
ILWC 251 Diyarbakır, on Malatya-Kayseri road 4 km W of Doğanlar village and 320 km E of Kayseri.
TR23039 Elazığ, 40 km W Bingol.
Besni Adıyaman, Besni to Akpınar 20 km on the right side.
Avcılar K. Maraş, Süleymanlı, Avcılar Village.
Erkenek K Maraş, 500 m after Erkenek to Doğansehir,Tozluk mevki.
Ereğli, Konya, Ereğli, 1 km after Çakır Village.
Kırobası, Içel, on the road to Mut 1 km after Karacaoğlan köyü.

Cicer judaicum

ILWC 148 Gaziantep, 5 km E of Gaziantep.
ILWC 168 ICRISAT Elazıg, Harput; road to Fatih Ahmet rubble.

Cicer bijugum

ILWC 8 ICRISAT Diyarbakır 33 km from Diyarbakır to Ergani.
ILWC 32 ICARDA Gaziantep Near Gaziantep; field edge.
ILWC 62 ICRISAT Mardin 35 km to Midyat; 14 km from Savur.
ILWC 177 Diyarbakır 28 km E of Diyarbakır.
ILWC 240 Şanlı Urfa Payamli; 62km E of Urfa on Viransehir road.
ILWC 241 Şanlı Urfa Mecrihan; 54 km E of Urfa on Viransehir road.
ILWC 243 Şanlı Urfa Ezgil; 46 km W of Urfa on road to Birecik.

Cicer echinospermum

ILWC 235 Gaziantep, Zeytinli; 7 km S of Zeytinili on Gaziantep to Kilis road.
ILWC 238 Gaziantep, Toreli; 300 m W Toreli on road to Capali.
ILWC 239 Şanlı Urfa, Siverek; 22 km ESE Siverek on Karacadag road.
ILWC 245 Sanlı Urfa, 26 km E of Siverek on south side of Diyarbakır to Urfa road.
ILWC 246 Şanlı Urfa, 42 km W of Diyarbakir on Diyarbakir to Siverek road.

TR39241 Bingöl, 2 km W of Solhan, Harabe village.

TR54962 Diyarbakır, Karacadağ to Siverek.

Cicer reticulatum

ILWC 36 Mardin 8 km E of Savur.

ILWC 81 ICRISAT Diyarbakır 33 km from Diyarbakır to Ergani.

ILWC 182 Mardin 8 km E of Savur.

ILWC 216 ICRISAT Mardin10 km to Savur from Mardin; Pinazdare stone rubble, vineyard.

ILWC 218 ICRISAT Mardin 35 km to Midyat; 14km from Savur.

ILWC 237 Şanlı Urfa Pirhalli; 2 km N of Pirhalli; 4km S of Borzova.

ILWC 247 Adıyaman.

TR 54961 Siirt, Şırnak Eruh road.

TR 54963 Siirt Pervari to Doğanca.

Cicer arietinum

Canıtez 89	Anatolian Agricultural Research Institute, Eskişehir.
Eser 87	Anatolian Agricultural Research Institute, Eskişehir.
ILC 195	Anatolian Agricultural Research Institute, Eskişehir.
Kırmızı Nohut	Anatolian Agricultural Research Institute, Eskişehir.
Akçin 91	Anatolian Agricultural Research Institute, Eskişehir.
Akn 51	Anatolian Agricultural Research Institute, Eskişehir.
Diyar 95	Southeastern Agricultural Research Institute, Diyarbakır
Menemen 91	Anatolian Agricultural Research Institute, Eskişehir.
ILC 482	Anatolian Agricultural Research Institute, Eskişehir.

VITA

Mehmet Ali Südüpak was born in Tokat on February 2, 1968. He got his B.Sc.degree in Biology at Cumhuriyet University, in Sivas, Turkey in 1988. Then, he went to the United States to do Master and PhD in genetic engineering. In 1992, he received his Masters degree from Genetics program in Kansas State University, USA. His research area was the genetics of plant disease resistance genes. Using flanking RFLP markers, he mapped a number of *rp1* area resistance genes and studied recombination in two of the *Rp1* homozygotes which were made heterozygous at flanking RFLP marker loci. Results obtained in these studies were published in two prestigious journals; Genetics, Molecular Plant Microbe Interactions.

After leaving KSU due to the some disagreements over his life style and the work he did, he was accepted to the Biology department at Illinois Institute of Technology (IIT), Chicago as a PhD student in 1993. At IIT, as thesis project in the first semester, he was assigned to study the regulation of O_2 concentration dependent expression of *Vitreoscilla* hemoglobin gene (*vgb*) and characterization of transcription factors, if any, involved in this

regulation. Following one semester of study, he decided to continue PhD in Turkey, and returned. In 1994 spring, he was accepted to the Middle East Technical University (METU), as PhD student in the department of Biology. However, in july, 1994, he had to join the army to do his obligatory army service. He served as a translator officer in the army. When the army service was over in 1996, his PhD life has started at METU. Since then, he is a PhD student, and the last three years of this period has been spent in collecting wild *Cicer* germplasm material, and studying allozyme and RAPD variation in wild and cultivated *Cicer* accessions. At the same time, he has been working as a lecturer staff in the department of Biology at Erciyes University, in Kayseri. He taught Introductory Statistics, Evolution, Cytology, Histology, Plant Physiology, Plant Anatomy, Genetics and Molecular Biology.

His research interest area includes study of molecular aspects of plant disease resistance, recombination, mapping in plants using molecular markers and study of phylogenetic relationships and variation in the genus *Cicer* and genetic resources of chickpea using molecular markers such as allozymes, RAPDs and AFLPs. Since 1996, he has written a number of research projects, three of which have been funded, and two manuscripts, one has been accepted for publication by Theoretical and Applied Genetics.