DETERMINATION OF GENETIC DIVERSITY IN CUCUMBER (*Cucumis sativus L.*) GERMPLASMS

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Molecular Biology and Genetics

by
İrem AYDEMİR

December 2009

ACKNOWLEDGEMENTS

I am deeply indebted to my advisor, Assoc. Prof. Dr. Sami DOĞANLAR, for his guidance during my research and study at. His perpetual energy and enthusiasm in research had motivated all his advisees, including me.

It is a pleasure to express my special thanks to Prof. Dr. Anne FRARY . She was always accessible and willing to help me with my research. As a result, research life became smooth and rewarding for me.

I would like to thank all the members of the department of Molecular Biology andGenetics, Institute of Technology and my colleagues for their kindness, help and inspiration during my study.

My deepest heartfelt gratitude to my family, my father, my mother and brother for their unflagging love and support throughout my life.

ABSTRACT

DETERMINATION OF GENETIC DIVERSITY IN CUCUMBER (*Cucumis sativus* L.) GERMPLASMS

In this study, 92 Turkish cucumber (*Cucumis sativus* L. var. *sativus*) accessions were characterized by using SRAP (Sequence Related Amplified Polymorphism) which is a PCR-based moleculer marker system.

A total of 45 SRAP combinations were used and 31 of these combinations amplified well and also showed polymorphism. Thus, 153 SRAP fragments were obtained and 138 fragments were polymorphic. These data were used to determine genetic distance and draw genetic distance dendrograms of Turkish cucumber accessions. Dendrograms were drawn using both UPGMA (Unweighted Pair Group Method) arithmetical averages and Unweighted Neighbour-Joining methods. According to the UPGMA dendrogram, the cucumber accessions clustered into four groups. Group B was composed of the genetically most related accessions with a minimum similarity of 0,82. Group D was composed of genetically most distinct accessions. The genetic distances of the dendrogram varied between 0,16 and 0,99. The neighbour-joining dendrogram showed similar clustering of the cucumber accessions. The results showed that Turkish cucumber is genetically quite diverse and has the potential for broadening the genetic base of cucumber.

ÖZET

HIYAR (*Cucumis sativus* L.) GERMPLAZMLARINDA GENETİK ÇEŞİTLİLİĞİN BELİRLENMESİ

Bu çalışmada 96 hıyar (*Cucumis sativus* L. var. *sativus*) hattı, PCR tabanlı moleküler işaretleyici sistemlerden biri olan SRAP (Sequence Related Amplified Polymorphism) kullanılarak karakterize edilmiştir.

Toplamda 45 SRAP kombinasyonu kullanılmış, bunlardan 31 kombinasyon iyi amplifiye olmuş ve polimorfizm göstermiştir. 153 SRAP fragmenti elde edilmiş ve 138 fragment polimorfik bulunmuştur. Bu veriler Türk hıyar hatlarının genetik uzaklığını belirlemekte ve genetik uzaklık ağacını çizmekte kullanılmıştır. Genetik ağaçlar, UPGMA (Unweighted Pair Group Method) ve Unweighted Neighbour-Joining metodları kullanılarak çizilmiştir. UPGMA methodu ile çizilen genetik ağaca göre hıyar hatları 4 gruba ayrılmıştır. Grup B genetik olarak birbirine en benzer hatlardan oluşmuş ve en küçük benzerlik değeri 0,82 dir. Grup D ise birbirine genetik olarak birbirinden en farklı hatlardan oluşmuştur. Genetik ağacın genetik aralığı 0,16 ile 0,99 arasında değişiklik göstermiştir. Neighbour-joining metodu ile çizilen ağaçtada hıyar hatları benzer gruplanma göstermiştir. Bu sonuçlar Türk hıyarının genetik olarak oldukça çeşitli olduğunu ve hıyar genetik çeşitliliğini arttırmak için bir potansiyele sahip olduğunu göstermiştir.

TABLES OF CONTENTS

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

CHAPTER 1

INTRODUCTION

1.1. General Information About *Cucumis sativus*

Cucurbits (the *Cucurbitaceae* family) are composed of 118 genera and 825 species. Members of this family are distributed primarily in tropical and subtropical regions of the world (Wang, et al. 2007). The most economically important cucurbits according to world total production are watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) (FAO 2006). The *Cucurbitaceae* includes two subfamilies *Zanonioideae* and the *Cucurbitoideae. Cucurbitoideae* comprises eight tribes one of which is *Melothrieae* which includes the genus *Cucumis*. The genus includes 30 wild and cultivated species that are spread throughout the world and has two major species: cucumber and melon. Cucumber has a small chromosome complement with n = x = 7 and a small haploid genome of 367 Mbp/C. The plant possesses unique properties with its genome. The three genomes of *C. sativus* show different transmission, maternal for chloroplast, paternal for mitochondrial and biparental for the nuclear DNA (Havey, et al. 1998). The mitochondrial genome is the largest of all eukaryotes. Nevertheless cucumber has a narrow genetic base, with a genetic variability of only 3-8%.

As a vegetable crop, *Cucumis sativus* has great economic importance. Most cucumbers are grown all around the world for fresh market and China is the greatest producer. It is very popular as a fresh market vegetable in Europe and in the United States. Thus, cucumber is mainly used in salads, but young and ripe fruits are also used as cooked vegetables. In Asia young shoots are consumed as a leafy vegetable and seeds are used as a source of edible oil. Cucumber is also used by native people to cure many illnesses in some countries. In Africa ripe raw cucumber fruits are used as a cure for sprue, a disease that causes flattening of the villi and inflammation of the lining of the small intestine; and in Indo-China, cooked immature fruits are used to treat dysentery in children (Grubben and Denton 2004).

Cucumber is thought to have been first domesticated in India, then distributed to Greece and Italy and introduced into China around 100 BC (Plader, et al. 2007). Cucumber has been cultivated for food for at least 3000 years. It appeared in France in the ninth century, England in the fourteenth century and North America in the middle of the sixteenth century (Plader, et al. 2007). In addition cucumber is cultivated because its extract has soothing, cleansing and softening properties which are important for the cosmetics industry. Cucumber also serves as a pesticide because of its steroid content including cucurbitacins (Wang, et al. 2007).

Cucumber is also cultivated for genetic studies. It is a good model organism for experiments because of its small haploid genome of 367 Mbp/C and its diverse array of unisexual or bisexual flowering sex phenotypes (Nam, et al. 2005). Sex expression is animportant factor which has an positive effect on yield and constitutes a major component of cucumber improvement programs (Serquan, et al. 1997).

1.2. Origin and Distribution of *Cucumis sativus*

The subgenus *Cucumis* includes Sino–Himalayan species like *C. sativus* (2n = 2x = 14) and *C. hystrix Chakr*. (2n = 2x = 24). The wild *C. hystrix* is only found in Yunnan province of Southern China and has unique genetic traits (Prohens and Nuez 2008). *C. sativus* has several botanical groups like var. *sativus,* the cultivated cucumber and *var. hardwickii,* the wild form. Commercial cucumber, referred to as *Cucumis sativus* is thought to have originated in the southern Himalayan foothills region of Asia. *C. sativus var. hardwickii* (Royle) Alef. is a wild free-living variety of *C.s. var. sativus* that can be seen in Himalayan foothills.

Cucumber is believed to have been domesticated in India for 3000 years and in Eastern Iran and China probably for 2000 years. China is a secondary center of genetic diversification of *C. sativus*. Cucumber was introduced from India to China, North Africa and South Europe, and from Europe to New World by early travellers and explorers. It was introduced to Tropical Africa by the Portuguese. Now it is grown all around the world (Grubben and Denton 2004, Wang, et al. 2007, Staub, et al 1999). Turkey is not the diversification center of cucumber and genetic diversity is generally low (Sarı, et al.2008). However, cucumber has great importance in Turkey. With an

annual production of approximately 1.67 million tonnes, Turkey is the third largest producer after China with 2.80 million and Iran with 1.72 million tonnes (FAOSTAT 2009).

Table 1.1. World cucumber production in 2007 based on FAO (2009). (Countries are ranked based on their total production).

Country	**Production (tonnes)**
China	28,049,900
Iran, Islamic Republic	1,720,000
Turkey	1,674,580
Russian Federation	1,386,810
United States of America	920,000
Japan	639,800
Egypt	615,000
Ukraine	599,200
Indonesia	581,206
Spain	510,000

1.3. Ecology , Nutrient and Mineral Compounds of *Cucumis sativus*

Cucumber requires a warm climate, in cold countries it can grow only in greenhouses or in open field if there are hot summer days. The optimum day temperature is 30ºC , and optimum night temperature is 18-21ºC. Minimum temperature is 15ºC for efficient development. Sensitivity to day light affects the yield of cucumber by defining the sex characters and also type of growth. For example, short day length promotes vegetative growth and female flower production. Cucumber needs a plentiful amount of water but waterlogging is not good for its growth. Low humidity causes loss of water because of its large leaf area. Also high humidity promotes the formation of downy mildew which is a fungus that first appears as tiny tan or bright pink speckles on the leaves. The soil should be fertile, well-drained with a pH of 6.0-7.0. Cucumber can germinate in 3 days if temperatures are at optimum levels. Flowering starts 40-45 days

after sowing. Male flowers develop earlier than female flowers. Fruits can be harvested 1-2 weeks after flowering (Grubben and Denton 2004, Wang, et al. 2007).

The nutritional composition of a 100 g portion of cucumber includes most of its weight in water with proteins, fat, carbohydrate as primary metabolites and also dietary fibre that is important for the digestive system (Table 1.2). Cucumber contains some essential vitamins and antioxidants which are effective on human health. In addition cucurbitacin C occurs in *Cucumis sativus*. Cucurbitacin is a terpene that is formed in foliage and fruits. It protects the fruit from insect attack (Grubben and Denton 2004, Wang, et al.2007).

Table 1.2. Nutritional composition of 100 g edible portion of cucumber fruit.

Compound	**Amount**
Water	96,4 g
Energy	42 kj (10 kcal)
Protein	0,7 g
Fat	0,1 g
Carbohydrate	1,5 g
Dietary Fibre	0,6 g
Ca	18 mg
Mg	8 mg
P	49 mg
Fe	0,3 mg
Zn	0,1 mg
Carotene	60 mg
Thiamin	0,03 mg
Riboflavin	0,01 mg
Niacin	0,2 mg
Folate	9 mg
Ascorbic acid	2 mg

1.4. Importance of Plant Diversity

Genetic diversity is the amount of heritable variability between varieties or populations of organisms. Variability occurs from differences in DNA sequences, biochemical characteristics like protein structure or isoenzyme properties, physiological properties like resistance to illnesses and growth rate, and morphological characters such as leaf type and flower colour. Selection, mutation, genetic drift and gene flow also affect genetic diversity in different populations by acting on the alleles in these populations. Selection is an important effector and can be divided into two types: natural and artificial. Artificial selection plays an important role in the variation of crop species (Rao and Hodgkin 2002).

Today modern agriculture and human existence depend on a few highly productive crop species. Domestication of these crops first occurred 10,000 years ago during the transition from nomadic hunter-gatherers to life in agrarian societies. During early and modern domestication, selection of some traits had effects on genetic diversity which caused rapid and radical changes in plant species. Early agriculturists choose traits like nonshattering of seeds, compact growth habit and loss of germination inhibition (Tanksley and McCouch 1997). Selection of lines with these traits caused decreases in the genetic base of following populations. Today there is another important threat for genetic diversity. Modern breeding methodologies produce high-yielding crops which are important for the agriculturist. However the genetic variation of crop plants becomes narrower day by day because new varieties are formed from crosses between genetically related modern species (Tanksley and McCouch 1997). Unfortunately crop species have been driven into a genetic bottleneck. The allelic variation of genes in a population starts to decrease in a bottleneck event so this event brings a dramatic loss of heterogeneity.

The narrow genetic base of some plant species poses several threats to these species. Crop species with low genetic variation are more susceptible to diseases and insect attacks and also environmental changes. There are some remarkable examples of these events. In Ireland there was a potato famine in the 1840s because potatoes were not resistant to leaf blight disease. Similarly in India there was a rice famine because of rice brown spot disease in 1943. In the southern states of the USA, southern corn leaf blight disease decreased corn production about 25% in 1970 (Rao and Hodgkin 2002).

In order to overcome these problems associated with the limited genetic diversity of crop plants, researchers started to construct gene banks and preserve core collections within these banks. However the collections in these banks need to be characterized. Molecular marker systems are an important tool for the characterization and utilization of germplasm banks (Tanksley and McCouch 1997). Marker systems also help in the efficient management of such collections by eliminating redundant accessions in gene banks.

1.5. Molecular Marker Systems

Molecular markers are particular segments of DNA which represent differences that are dispersed throughout the genome. Molecular markers may or may not be related with the expression of phenotypic traits. Molecular markers have some advantages compared to phenotypic markers: they are stable, easily detectable in all tissues and are not affected by growth, differentiation, and development. Also they are independent from environmental conditions. For these reasons, researchers and breeders prefer to use molecular marker systems in their studies (Agarwal, et al 2008, Gostimsky, et al 2005).

1.5.1. Molecular Markers in *Cucumis sativus*

Ideal molecular markers have to have some important criteria. Molecular markers must be polymorphic and distributed throughout the genome. They must provide sufficient detection of genetic differences and can generate multiple and reliable markers. Also they have to be simple, quick and cheap. Ideal molecular markers only require small amounts of DNA and preferably do not require previous information about the organism's genome (Agarwal, et al 2008).

Molecular markers are used in many research areas. One of these areas is the establishment of molecular maps of organisms (Agarwal, et al 2008, Jones, et al 1997). There are several mapping studies on cucumber. Serquan et al. (1997) established a map by using RAPD markers and found 9 linkage groups with an average distance between these markers of 8.4cM (Staub, et al. 2006). Another study was carried out by Sun et al. (2006). They constructed a map for identifying the location of genes controlling parthenocarpy (Staub, et al 2006). Molecular markers also find application areas in

ecological, evolutionary, taxonomical and phylogenic studies of plant species (Agarwal, et al. 2008). In addition molecular markers are used in breeding programs to establish new plant cultivars, gene cloning studies and also characterization of germplasm collections (Rao and Hodgkin 2002).

In the earliest genetic diversity studies of cucumber, protein-based isozyme markers and RFLP (Restriction Fragment Length Polymorphism) markers were used. In two different studies, Knerr et al. (1989) used 18 isozyme loci and Meglic et al. (1996) used 21 isozyme loci for identifying genetic diversity of cucumber lines. Also the genetic diversity of Chinese cucumber germplasm was characterized by the application of isozymes (Staub, et al. 1999). However this marker showed a low level of polymorphism. Hybridization-based RFLP markers were also used in identification of genetic diversity in cucumber germplasm (Dijkhuizen, et al. 1996). RAPD (Random Polymorphic DNA) markers are PCR-based molecular markers and also were used in cucumber. Xixiang et al. (2002) determined the genetic diversity of 50 cucumber lines which were from China, Japan, Africa, Holland by using RAPD markers. In another study, the genetic diversity of cucumber lines from African countries were detected by application of RAPD markers (Mliki, et al. 2003). SSR (Simple Sequence Repeats) or microsatellites are also PCR-based molecular markers and have been used in genetic diversity studies in cucumber and also some other cucurbit species like watermelon and melon (Watcharawongpaiboon and Chunwongse 2007). In 2008, the genetic relatedness of 59 cucumber line were determined with application of SSR markers by Sheng-ki et al. As a result, the germplasm fell into seven groups. Danin-Poleg et al. (2001) found that 80% of cucumber SSRs were polymorphic in their studies. Another PCR based marker system is AFLP (Amplification Fragment Length Polymorphism) which is also used in genetic diversity studies. In 2004 the genetic diversity of 70 accessions of *C.sativus* from various countries were determined by using AFLP markers (Xixiang, et al. 2004). More recently, sequence-related amplified polymorphism (SRAP) markers have become popular molecular markers. Because SRAP markers were applied to cucumber in this research project, these markers are discussed in detail in the next section.

1.5.2. Sequence-Related Amplified Polymorphism (SRAP)

SRAP is a PCR-based technique which depends on the amplification of open reading frames (ORFs). It is based on two-primer amplification. The sequences of primers are random and they are 17-21 nucleotides in length (Agarwal, et al. 2008 and Li and Quiros 2001). The primers are composed of:

1. AT or GC-rich core sequences, that are 13-14 base pairs long. The 10 or 11 bases starting at the 5'-end are called "filler " sequences. These sequences are random sequences but must be different from each other. Filler sequences are followed by the sequence CCGG in the forward primer and AATT in the reverse primer.
2. The core region of primers is followed by three selective nucleotides at the 3'-end.

SRAP markers are dominant markers. SRAP markers have the advantage of the easiness of being a PCR technique. The application of this technique is simple and is also cheap. This technique gives reliable results and also multiple polymorphic bands (Agarwal, et al. 2008).

In this study, 34 PCR-based SRAP molecular markers were used for the determination of genetic diversity among 96 cucumber accessions. These accessions were obtained from USDA. Cucumber accesions were collected from different places of Turkey and represent varieties in Turkish cucumber accessions.

1.6. Goals

The aim of this study was the determination of the amount of genetic diversity in the cucumber accessions in Turkey and the amount of genetic relatedness in the material. Results of the study have importance for breeders by serving as a source for obtaining new cultivars. Germplasm collections can be also developed and managed with the help of this study. Redundant accessions can be eliminated by determining which accessions to conserve and where to conserve. Also results will lead to reorganization of core collection of Turkish cucumber accessions. In addition this study helps us to understand the origin and genetic relatedness of cucumber accessions in Turkey.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

In this study 96 cucumber accessions were used. These accessions were obtained from USDA-ARS Plant Germplasm Inspection Station, Beltsville, Maryland, USA. Their origin is Turkey and they were collected and preserved in US gene banks. These genotypes were selfed two generations to avoid risks because of possible seed mixture. These processes were done by Yüksel Tohumculuk. In Table 2.1 the cucumber accessions are shown with their pedigree number, accession name and their location of collection. For this study ten seeds of each accessions were planted in peat and perlite in seedling plates. They were germinated in a growth chamber.

Table 2.1. Table of cucumber accessions from USDA with their pedigree number, accession name and location.

Pedigree Number	**Accession Name**	**Source**	**Location**
PI 105263	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 109063	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 109481	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 109482	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 109483	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 109950	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 109951	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 109952	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 165029	*Cucumis sativus var.sativus*	USDA	Yerli hıyar
PI 165046	*Cucumis sativus var.sativus*	USDA	Ankara
PI 177361	*Cucumis sativus var.sativus*	USDA	Çorum
PI 165509	*Cucumis sativus var.sativus*	USDA	India
PI 167043	*Cucumis sativus var.sativus*	USDA	Hatay
PI 167050	*Cucumis sativus var.sativus*	USDA	Hatay
PI 167052	*Cucumis sativus var.sativus*	USDA	Hatay
PI 167079	*Cucumis sativus var.sativus*	USDA	Adana
PI 167358	*Cucumis sativus var.sativus*	USDA	İçel
PI 167389	*Cucumis sativus var.sativus*	USDA	Adana
PI 167198	*Cucumis sativus var.sativus*	USDA	İçel
PI 167197	*Cucumis sativus var.sativus*	USDA	İçel
PI 169304	*Cucumis sativus var.sativus*	USDA	Manisa
PI 169334	*Cucumis sativus var.sativus*	USDA	Manisa
PI 169350	*Cucumis sativus var.sativus*	USDA	Çanakkale
PI 169351	*Cucumis sativus var.sativus*	USDA	Çanakkale
PI 169352	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 169353	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 169377	*Cucumis sativus var.sativus*	USDA	Manisa
PI 169380	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 169381	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 169382	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 169384	*Cucumis sativus var.sativus*	USDA	İstanbul
PI 169385	*Cucumis sativus var.sativus*	USDA	Kocaeli
PI 169386	*Cucumis sativus var.sativus*	USDA	Kırklareli
PI 169389	*Cucumis sativus var.sativus*	USDA	Edirne
PI 169388	*Cucumis sativus var.sativus*	USDA	Çanakkale
PI 169390	*Cucumis sativus var.sativus*	USDA	Çanakkale
PI 169393	*Cucumis sativus var.sativus*	USDA	Çanakkale

(cont. on next page)

Table 2.1. (cont.)

Pedigree Number	Accession Name	Source	Location
PI 169392	*Cucumis sativus var.sativus*	USDA	Çanakkale
PI 169394	*Cucumis sativus var.sativus*	USDA	Bursa
PI 169397	*Cucumis sativus var.sativus*	USDA	Bursa
PI 169398	*Cucumis sativus var.sativus*	USDA	Kocaeli
PI 169399	*Cucumis sativus var.sativus*	USDA	Kocaeli
PI 172840	*Cucumis sativus var.sativus*	USDA	Kars
PI 172841	*Cucumis sativus var.sativus*	USDA	Van
PI 172842	*Cucumis sativus var.sativus*	USDA	Van
PI 172844	*Cucumis sativus var.sativus*	USDA	Urfa
PI 172843	*Cucumis sativus var.sativus*	USDA	Mardin
PI 172847	*Cucumis sativus var.sativus*	USDA	Gaziantep
PI 172845	*Cucumis sativus var.sativus*	USDA	Urfa
PI 172846	*Cucumis sativus var.sativus*	USDA	Urfa
PI 172848	*Cucumis sativus var.sativus*	USDA	Maraş
PI 172849	*Cucumis sativus var.sativus*	USDA	Maraş
PI 172851	*Cucumis sativus var.sativus*	USDA	Malatya
PI 172852	*Cucumis sativus var.sativus*	USDA	Elazığ
PI 173674	*Cucumis sativus var.sativus*	USDA	Muş
PI 174160	*Cucumis sativus var.sativus*	USDA	Kars
PI 174166	*Cucumis sativus var.sativus*	USDA	Urfa
PI 174167	*Cucumis sativus var.sativus*	USDA	Urfa
PI 169401	*Cucumis sativus var.sativus*	USDA	Kocaeli
PI 169402	*Cucumis sativus var.sativus*	USDA	Hatay
PI 171600	*Cucumis sativus var.sativus*	USDA	Zonguldak
PI 171601	*Cucumis sativus var.sativus*	USDA	Zonguldak
PI 171607	*Cucumis sativus var.sativus*	USDA	Tokat
PI 171611	*Cucumis sativus var.sativus*	USDA	Trabzon
PI 171612	*Cucumis sativus var.sativus*	USDA	Trabzon
PI 172838	*Cucumis sativus var.sativus*	USDA	Urfa
PI 197086	*Cucumis sativus var.sativus*	USDA	India
PI 204567	*Cucumis sativus var.sativus*	USDA	Yozgat
PI 204568	*Cucumis sativus var.sativus*	USDA	Kayseri
PI 204569	*Cucumis sativus var.sativus*	USDA	Kayseri
PI 204690	*Cucumis sativus var.sativus*	USDA	Malatya
PI 204692	*Cucumis sativus var.sativus*	USDA	Malatya
PI 206425	*Cucumis sativus var.sativus*	USDA	Trabzon
PI 206952	*Cucumis sativus var.sativus*	USDA	Trabzon
PI 172847	*Cucumis sativus var.sativus*	USDA	Gaziantep

(cont. on next page)

Table 2.1. (cont.)

Pedigree Number	Accession Name	Source	Location
PI 206954	*Cucumis sativus var.sativus*	USDA	Giresun
PI 206955	*Cucumis sativus var.sativus*	USDA	Eskişehir
PI 227209	*Cucumis sativus var.sativus*	USDA	Japan
PI 263079	*Cucumis sativus var.sativus*	USDA	Russia
PI 174174	*Cucumis sativus var.sativus*	USDA	Gaziantep
PI 174177	*Cucumis sativus var.sativus*	USDA	Malatya
PI 175679	*Cucumis sativus var.sativus*	USDA	Sinop
PI 175680	*Cucumis sativus var.sativus*	USDA	Balıkesir
PI 175683	*Cucumis sativus var.sativus*	USDA	Erzincan
PI 175686	*Cucumis sativus var.sativus*	USDA	Türkiye
PI 175692	*Cucumis sativus var.sativus*	USDA	Kırşehir
PI 175693	*Cucumis sativus var.sativus*	USDA	Kayseri
PI 175694	*Cucumis sativus var.sativus*	USDA	Kayseri
PI 169297	*Cucumis sativus var.sativus*	USDA	Bursa
PI 175695	*Cucumis sativus var.sativus*	USDA	Kayseri
PI 176956	*Cucumis sativus var.sativus*	USDA	Konya
PI 177359	*Cucumis sativus var.sativus*	USDA	Ankara
PI 178886	*Cucumis sativus var.sativus*	USDA	Çankırı

2.2. Methods

2.2.1. DNA Extraction

The genomic DNA was extracted from young leaf tissues when the seedlings were at the 4-leaf stage. Extraction was performed by using a CTAB-DNA extraction protocol modified according to Fulton et al. (1995) and also by using the Promega Wizard Genomic purification kit. After extraction, genomic DNAs were stored at -20°C in TE buffer for molecular characterization.

2.2.2. SRAP Analysis

In this study 45 SRAP primer combinations were used for the PCR amplification. In table 2.2 the primer list with their sequences was shown. The PCR reaction solution was composed of 2µl 10X buffer, 2 µl $MgCl_2$, 0.7 µl dNTP, 0.3 µl Taq

polymerase, 9.5 µl dH_2O, 2 µl ME (forward primers), 2 µl EM (reverse primer) and 1.5 µl 50-100ng DNA.

The amplification conditions were: 5 min. initial denaturation at 94°C, then 5 cycles composed of denaturating at 94°C for 1 min, annealing at 35°C for 1 min, extension at 72°C for 1 min and followed by 35 cycles; heating at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for1 min and 10 min final extension at 72°C.

Table 2.2. SRAP primers and their sequences used in this study.

Forward primers 5'-3'	**Reverse primers 3'-5'**
me1: TGAGTCCAAACCGGATA	em2: GACTGCGTACGAATTTGC
me3: TGAGTCCAAACCGGAAT	em3: GACTGCGTACGAATTGAC
me4: TGAGTCCAAACCGGACC	em4: GACTGCGTACGAATTTGA
me6: TGAGTCCAAACCGGTAG	em5: GACTGCGTACGAATTAAC
me8: TGAGTCCAAACCGGTGT	em6: GACTGCGTACGAATTGCA
me9: TGAGTCCAAACCGGTCA	em7: GACTGCGTACGAATTATG
me10: TGAGTCCAAACCGGGAC	em8: GACTGCGTACGAATTAGC
me11: TGAGTCCAAACCGGGTA	em9: GACTGCGTACGAATTACG
me12: TGAGTCCAAACCGGGGT	em10: GACTGCGTACGAATTTAG
me13: TGAGTCCAAACCGGCAG	em11:GACTGCGTACGAATTTCG
me14: TGAGTCCAAACCGGCTA	em12: GACTGCGTACGAATTGTC
	em13: GACTGCGTACGAATTGGT
	em14:GACTGCGTACGAATTCAG

2.2.3. Data Analysis

The genomic DNA fragments which were obtained from SRAP primer combinations were scored as present (1) and absent (0) of each fragment. These data were used to calculate genetic distance and also to draw genetic distance dendrograms of Turkish cucumber accessions. Dendrograms were drawn using NTSYS-pc version 2.2 based on DICE matrix and UPGMA (Unweighted Pair Group Method) arithmetical averages in SAHN module. The concordance between genotypic data and the dendrogram was determined by using Mantel test (Mantel 1967). In addition, genetic distance dendrograms were drawn using DARwin5 software program which was based on DICE matrix and UPGMA in Hierarchical Clustering module, and also based on Unweighted Neighour-Joining in Neighbour-Joining module.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. SRAP Results

In this study we used 45 SRAP combinations for determination of genetic diversity among 92 cucumber accessions. SRAP combinations are shown in table 3.1.

According to the results, 31 SRAP combinations amplified well. However, 14 combinations did not amplify well and so were excluded from further analysis. All 31 combinations were polymorphic. We obtained 153 bands and 138 of them (90.2%) showed polymorphism. The combinations ME4-EM3 and ME8-EM7 gave the most polymorphic bands with 7 bands each while combinations ME1-EM5 and ME11-EM8 gave only 1 polymorphic band each. Overall, each SRAP primer combination gave an average of 4.4 polymorphic bands. The number of polymorphic bands for each combination are shown in table 3.2. The polymorphism characteristics of the SRAP combinations are shown in table 3.3.

Table 3.1. SRAP combinations that were used in this study.

Well Amplified Combinations	**PoorlyAmplified Combinations**
ME1-EM3	ME1-EM1
ME1-ME5	ME9-EM4
ME1-EM7	ME6-EM5
ME3-EM5	ME3-EM7
ME4-EM3	ME9-EM8
ME4-EM5	ME11-EM9
ME6-EM3	ME12-EM9
ME9-EM9	ME12-EM10
ME10-EM4	ME13-EM10
ME10-EM5	ME13-EM11
ME11-EM8	ME5-EM12
ME11-EM10	ME11-EM12

(Cont. on next page)

Table 3.1. (Cont.)

Well Amplified Combinations	**Poorly Amplified Combinations**
ME11-EM11	ME13-EM13
ME12-EM4	ME13-EM14
ME12-EM5	
ME12-EM8	
ME14-EM14	
ME6-EM8	
ME4-EM4	
ME7-EM5	
ME8-EM7	
ME9-EM2	
ME10-EM6	
ME10-EM13	
ME12-EM7	
ME10-EM7	
ME12-EM12	
ME13-EM8	
ME12-EM6	
ME1-EM3	
ME2-EM7	
ME10-EM10	

Table 3.2. Number of polymorphic bands for each SRAP combination.

SRAP Combinations	**Number of Polymorphic Bands**
ME1-EM3	2
ME1-ME5	1
ME1-EM7	6
ME3-EM5	4
ME4-EM3	7
ME4-EM5	5
ME6-EM3	2
ME9-EM9	5
ME10-EM4	6
ME10-EM5	4
ME11-EM8	1
ME11-EM10	4
ME11-EM11	2
ME12-EM4	6
ME12-EM5	4
ME12-EM8	4
ME14-EM14	6
ME6-EM8	4
ME4-EM4	6
ME7-EM5	4
ME8-EM7	7
ME9-EM2	5
ME10-EM6	2
ME10-EM13	2
ME12-EM7	6
ME10-EM7	6
ME12-EM12	6
ME13-EM8	4
ME12-EM6	6
ME1-EM3	2
ME2-EM7	5
ME10-EM10	6
Average	4.4

Table 3.3. Characteristics of SRAP combinations used in determination of genetic diversity of cucumber accessions.

Total Number of Primers	**Number of Primers that Amplified Well**	**Percentage of Amplified primers**	**Total number of Bands**	**Number of Polymorphic Bands**	**Percentage Polymorphic Bands**
45	31	69%	153	138	90%

3.1.1. Analysis of SRAP Results

According to the SRAP results, genetic distance dendrograms for the 92 cucumber accessions were drawn using NTSYS-pc version 2.2 with DICE matrix and UPGMA (Unweighted Pair Group Method) arithmetical averages in SAHN module (Figure 3.1). The correlation between genotypic data and the dendrogram was determined by using Mantel test (1967). According to this test, the correlation between the sample genotypic data and the dendrogram was very high (r value=0,985). The dendrogram scale varied from 0,16 to 0,99. Thus, the minimum genetic similarity was 16% while the maximum similarity between cucumber accessions was 99%. According to this dendrogram, the cucumber accessions fell into 4 groups: A, B, C, and D. Group A was composed of seven accessions with a minimum similarity of 0,66 which was seen between accessions PI167043 and PI177359. Group B was the largest group and was composed of 80 accessions. The minimum similarity was 0,80 which was determined between accessions PI167389 and PI167198. The accessions in this group were the genetically most-related accessions. Group C was composed of three accessions and the minimum similarity was 0,45 which was seen between PI204690 and PI174166. The last group, group D was the smallest group and was composed of two accessions. The minimum similarity was 0,16 which was determined between accessions PI109951 and PI167050. This group included the accessions that were the most genetically distinct. The accessions in these groups are shown in Table 3.4.

Principal Component Analysis (PCA) is a method that summarize the data without much loss of information based on the similarities and the differences of the data. We performed PCA to produce 2D and 3D plots. 2D and 3D plots are shown in Figures 3.2. and 3.3. The first, second and third axes accounted for 30%, 7% and 6% of the total variance, respectively. In the 2D plot, cucumber accessions clustered into 4 groups: X, Y, W, and Z. Group X was composed of 13 accessions. Group Y was the largest group and was composed of 72 accessions. Group W was composed of five accessions. Group Z was the smallest group and composed of only two accessions. In the 3D plot, cucumber accessions also clustered into 4 groups: E, F, G, and H. The number of accessions in each group matched that seen in the 2D plot. Overall, the results of PCA and dendrogram analysis using UPGMA showed good correspondence with each other.

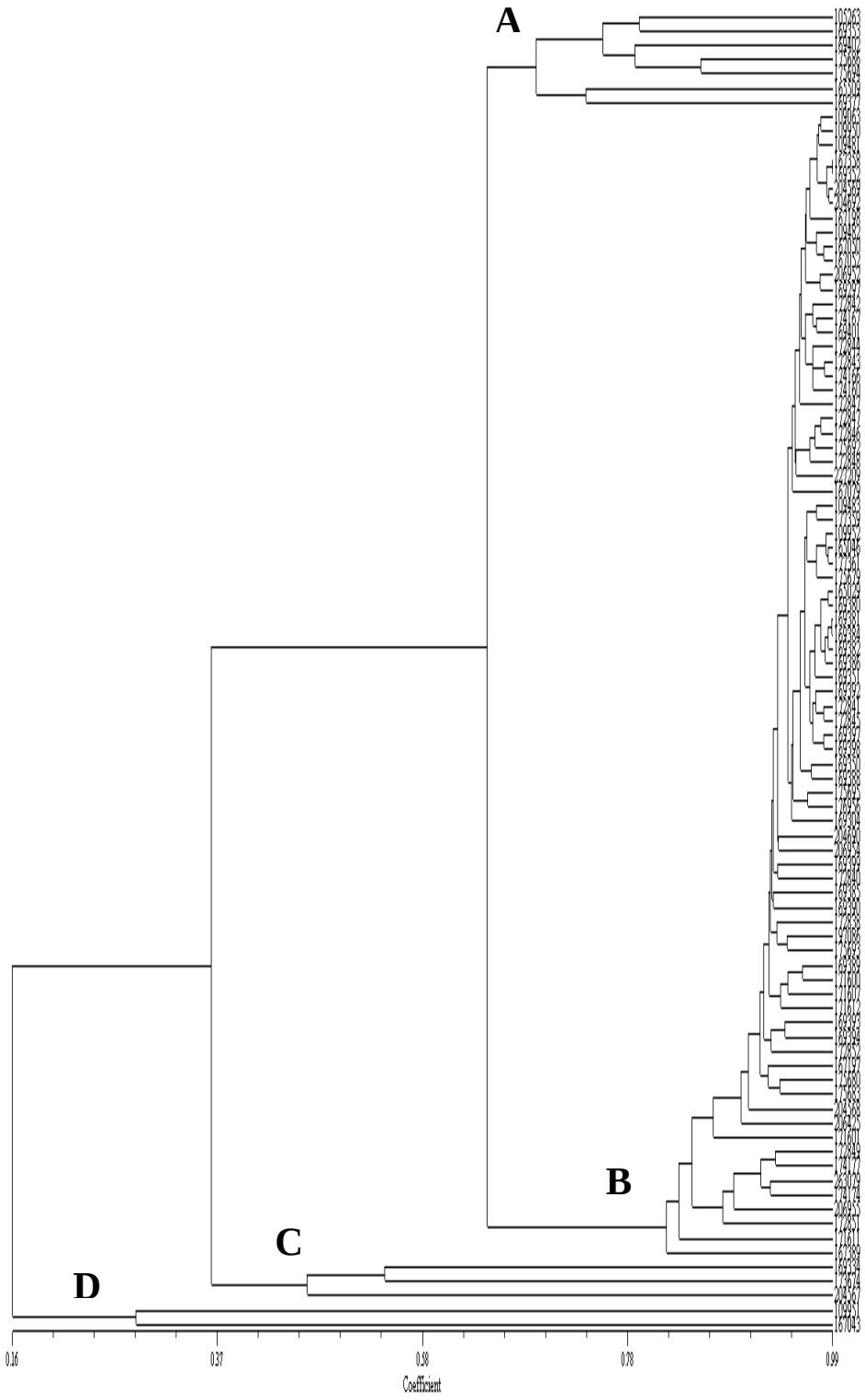

Figure 3.1. The genetic distance dendrogram of 92 cucumber accessions based on NTSYS software program.

Table 3.4. Accessions and their grouping based on the UPGMA genetic distance dendrogram.

Pedigree Number	**Location**	**Group Name**
PI105263	Türkiye	A
PI169353	Türkiye	A
PI169402	Hatay	A
PI175686	Türkiye	A
PI175694	Kayseri	A
PI165509	India	A
PI169377	Manisa	A
PI109063	Türkiye	B
PI109950	İstanbul	B
PI109481	Türkiye	B
PI167358	İçel	B
PI169352	Türkiye	B
PI204569	Kayseri	B
PI204692	Malatya	B
PI167198	İçel	B
PI109482	Türkiye	B
PI167050	Hatay	B
PI167052	Hatay	B
PI206952	Trabzon	B
PI169297	Bursa	B
PI172842	Van	B
PI174167	Urfa	B
PI169401	Koceli	B
PI172844	Urfa	B
PI172843	Mardin	B
PI174166	Urfa	B
PI174160	Kars	B
PI172847	Gaziantep	B
PI172846	Urfa	B
PI175692	Kırşehir	B
PI172848	Maraş	B
PI227209	Japan	B
PI167079	Adana	B
PI109483	Türkiye	B
PI177359	Ankara	B

(Cont. on next page)

Table 3.4. (Cont.)

Pedigree Number	Location	Group Name
PI109952	İstanbul	B
PI165046	Ankara	B
PI177361	Çorum	B
PI175679	Sinop	B
PI165029	Yerli Hıyar	B
PI169380	İstanbul	B
PI169381	İstanbul	B
PI169384	İstanbul	B
PI169382	İstanbul	B
PI169386	Kırklareli	B
PI169351	Çanakkale	B
PI169392	Çanakkale	B
PI172841	Van	B
PI172845	Urfa	B
PI169397	Bursa	B
PI169398	Kocaeli	B
PI169350	Çanakkale	B
PI169388	Çanakkale	B
PI175695	Kayseri	B
PI177956	Konya	B
PI169304	Manisa	B
PI204690	Malatya	B
PI206954	Giresun	B
PI169399	Kocaeli	B
PI172840	Kars	B
PI169385	Kocaeli	B
PI169838	Urfa	B
PI197086	India	B
PI175693	Kayseri	B
PI169389	Edirne	B
PI171600	Zonguldak	B
PI171607	Tokat	B
PI171612	Trabzon	B
PI169393	Çanakkale	B
PI169394	Bursa	B

(Cont. on next page)

Table 3.4. (Cont.)

Pedigree Number	Location	Group Name
PI172852	Elazığ	B
PI169334	Manisa	C
PI173674	Muş	C
PI204567	Yozgat	C
PI109951	İstanbul	D
PI167043	Hatay	D

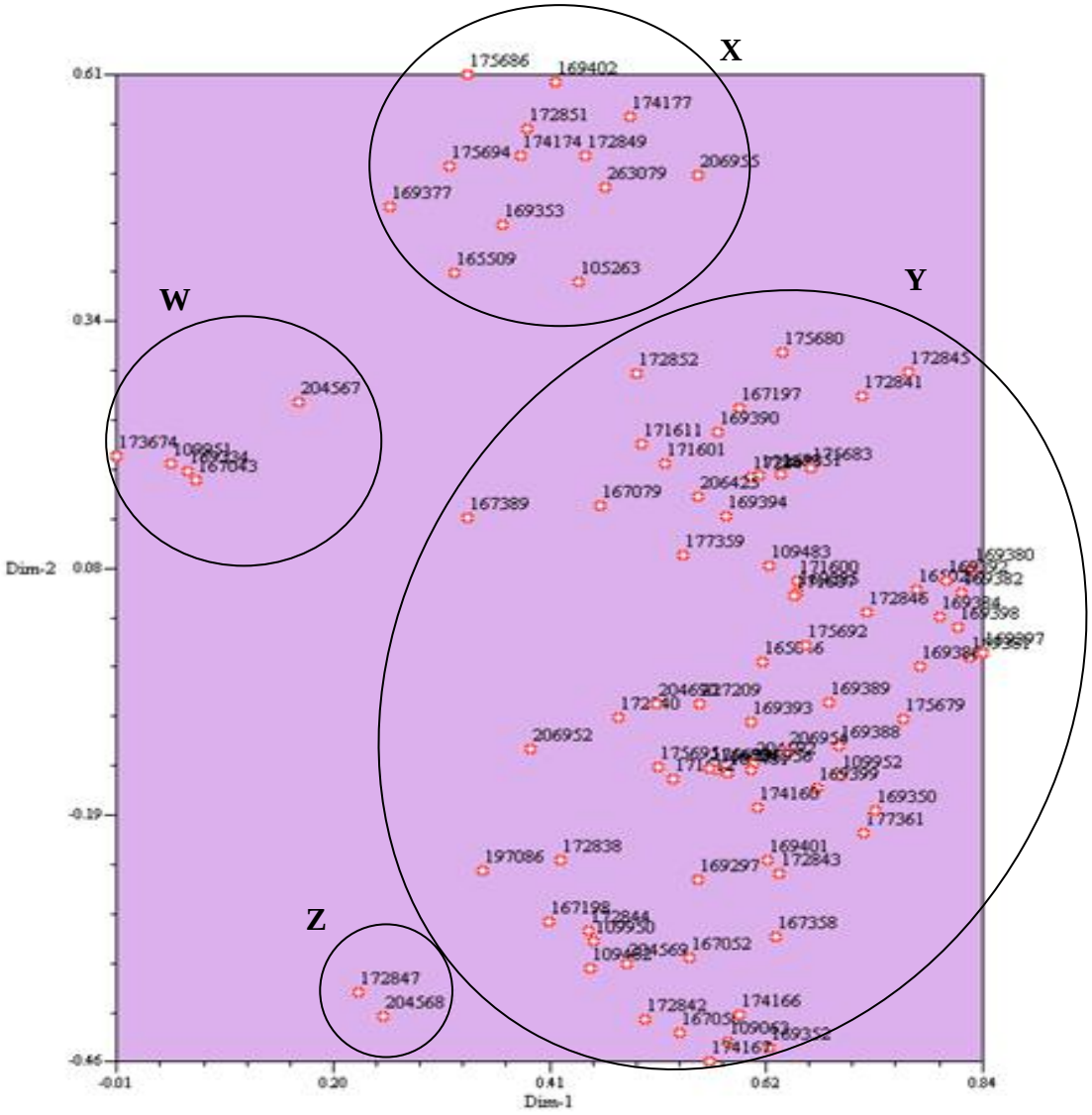

Figure 3.2. 2D Plot of 92 cucumber accessions.

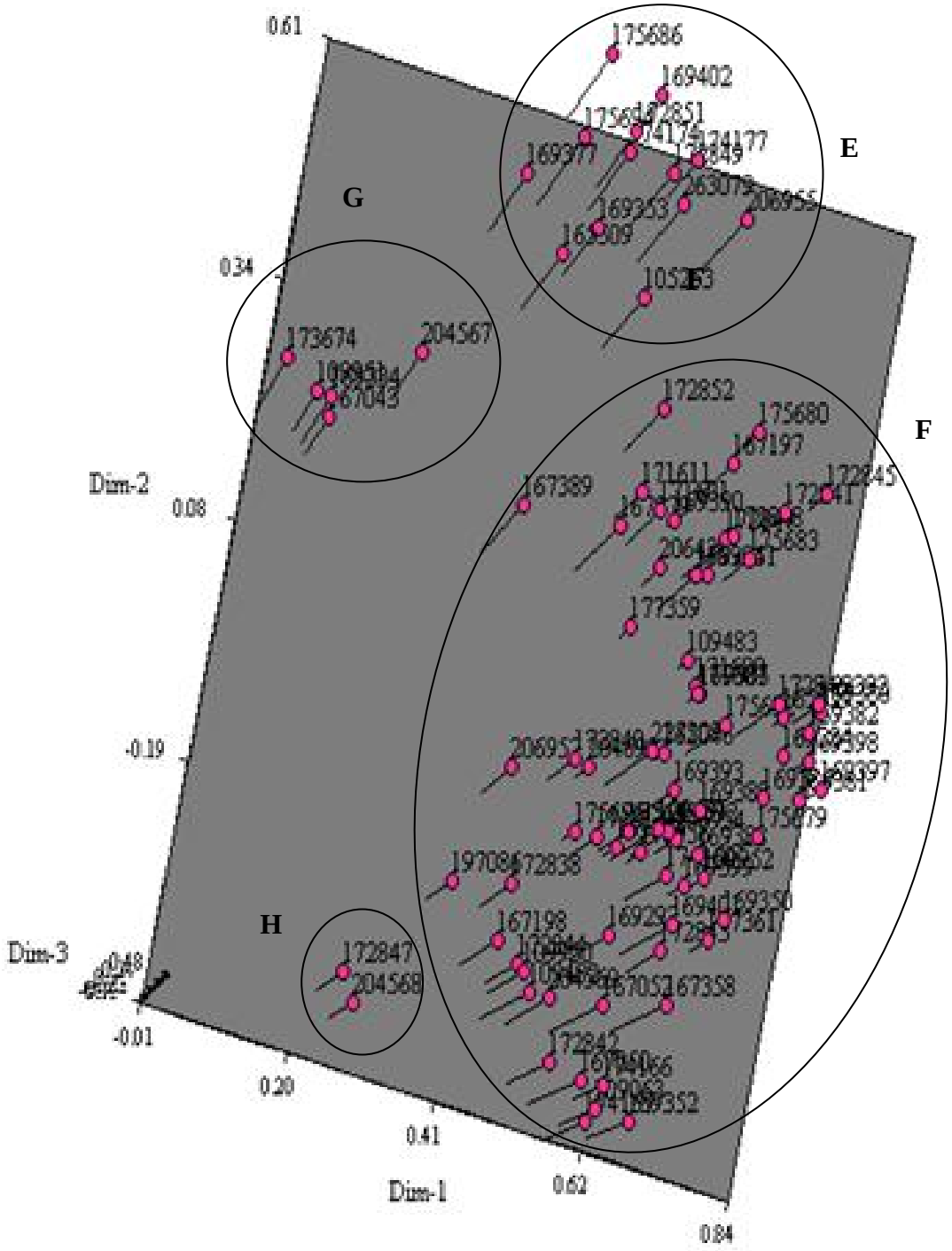

Figure 3.3. 3D Plot of 92 cucumber accessions.

In addition, we used the DARwin5 software program to draw genetic distance dendrograms. With this program, we drew another genetic distance dendrogram based on DICE matrix and UPGMA (Unweighted Pair Group Method) in the Hierarchical Clustering module and also we used the UnWeighted Neighbour-Joining method in Neighbour-Joining module. According to the genetic distance dendrogram of UPGMA, the correlation between the sample genotypic data and the dendrogram was very high. The dissimilarity values of the dendrogram varied between 0.73 and 0,93. The mimimum dissimilarity was seen between accessions PI109951 and PI167389 According to the DARwin5 UPGMA dendrogram, cucumber accessions clustered into 4 group; A, B, C, D (Figure 3.4.). These groups were composed of same accessions as seen in the dendrogram which was drawn with the UPGMA method using the NTSYS software program.

According to the Neighbour-Joining dendrogram, the correlation between the genotypic data and the dendrogram was very high. The dissimilarity values of the dendrogram varied between 0.757 and 0.93. With regard to the dendrogram, cucumber accessions clustered into 5 groups and one accession did not cluster with the rest: U, W, X, Y, Z and accession 84 (Figure 3.5.). Group U was composed of 23 accessions and a minimum dissimilarity of 0,88 was determined between accessions PI167197 and PI167043 . Accessions in this group were the genetically most distant accessions. Group W was the largest group and was composed of 31 accessions. The minimum dissimilarity was 0,093 and determined between accessions PI172846 and PI204568. The accessions in this group were the genetically most related accessions. Group X was composed of 28 accessions and minimum dissimilarity was 0,05 which was determined between accessions PI169380 and PI169399. Group Y was composed of seven accessions and a minimum dissimilarity of 0,09 was seen between accessions PI169394 and PI206425. Group Z was the smallest group and composed of two accessions. The minimum dissimilarity was 0,04 and determined between accessions PI172848 and PI172852. The accessions in these group are shown in table 3.5.

There are only a few studies about characterization of genetic diversity of cucumber accessions. Determination of genetic diversity among 26 cucumber accessions collected from African countries and based on RAPD markers was carried out by Mliki and his friends (2003). They found that genetic distances varied between 0,41 and 0,97. Thess results indicated that African cucumber is likely to enhance the genetic diversity of cucumber. In our study, genetic distance varied between 0,16 and

0,99. According to this result, the range of genetic diversity available in the Turkish cucumber accessions is more than that of the African cucumber accessions. Therefore, Turkish cucumber has potential for broadening the genetic base of cucumber. In addition we used accessions throughout Turkey and also some accessions from different countries (Russia, Japan and India). However, clustering of the accessions based on gentic diversity did not correlate with origin of accession. This demonstrated that the amount of genetic diversity did not depend on accession collection site.

.

Figure 3.4. Genetic distance dendrogram of 92 cucumber accesions drawn by DARwin5 software program with UPGMA.

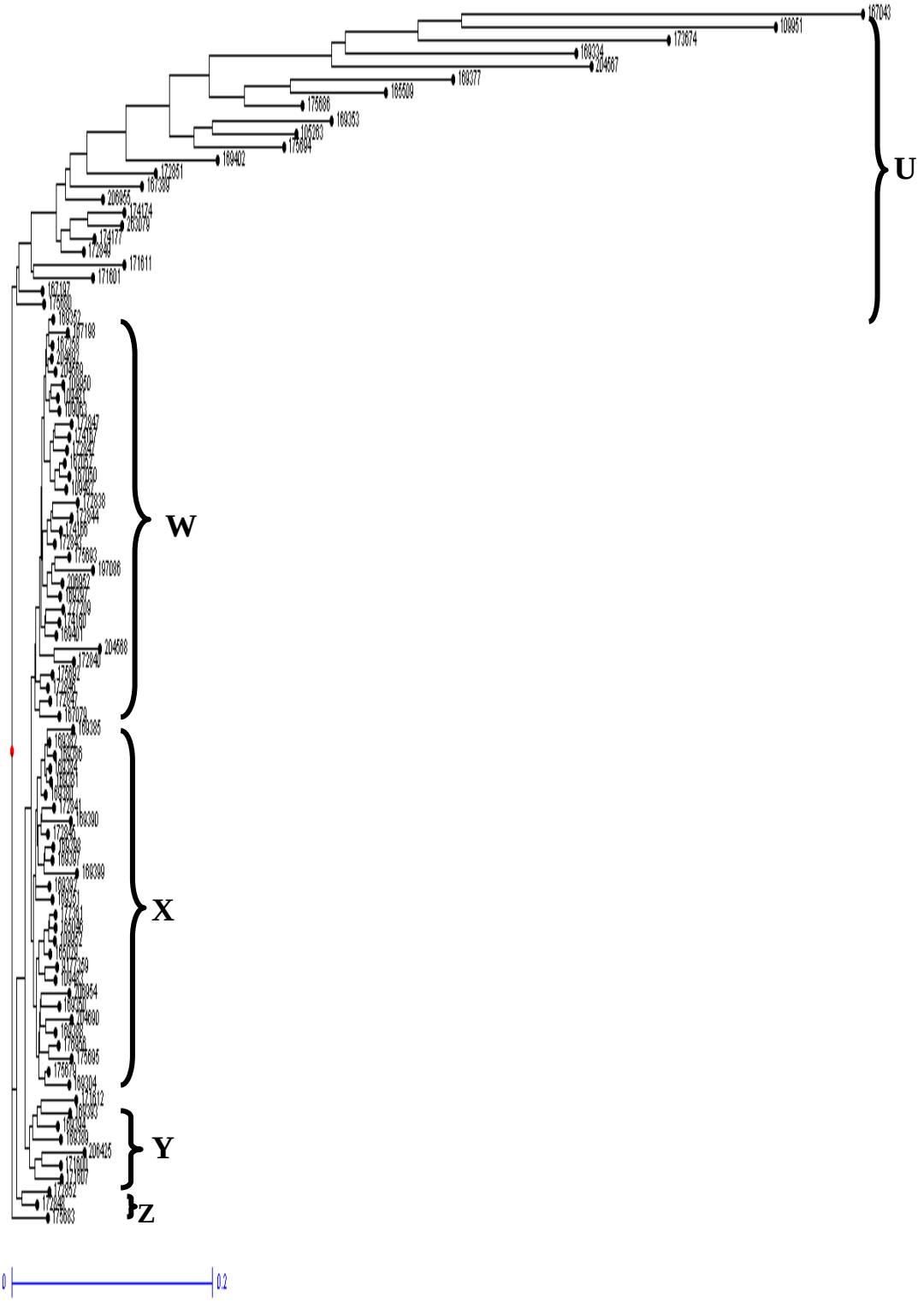

Figure 3.5. Genetic distance dendrogram of 92 cucumber accesions drawn by DARwin5 software program with Neighbour-Joining.

Table 3.5. Accessions of Groups in the genetic distance dendrogram based on DARwin software program with Neighbour-Joining.

Pedigree Number	**Location**	**Group Name**
PI167043	Hatay	U
PI109951	İstanbul	U
PI173674	Muş	U
PI169334	Manisa	U
PI204567	Yozgat	U
PI169377	Manisa	U
PI165509	India	U
PI175686	Türkiye	U
PI169353	Türkiye	U
PI105263	Türkiye	U
PI175694	Kayseri	U
PI169402	Hatay	U
PI172851	Malatya	U
PI167389	Adana	U
PI206955	Eskişehir	U
PI174174	Gaziantep	U
PI263679	Russia	U
PI174177	Malatya	U
PI172849	Maraş	U
PI171611	Trabzon	U
PI171601	Zonguldak	U
PI167197	İçel	U
PI175680	Balıkesir	U
PI169352	Türkiye	W
PI167198	İçel	W
PI167358	İçel	W
PI204692	Malatya	W
PI204569	Kayseri	W
PI109950	İstanbul	W
PI109481	Türkiye	W
PI109063	Türkiye	W
PI172847	Gaziantep	W
PI174167	Urfa	W
PI172842	Van	W
PI167052	Hatay	W

(Cont. on next page)

Table 3.5. (Cont.)

Pedigree Number	Location	Group Name
PI167050	Hatay	W
PI109482	Türkiye	W
PI172838	Urfa	W
PI172844	Urfa	W
PI174166	Urfa	W
PI172843	Mardin	W
PI175693	Kayseri	W
PI197086	India	W
PI206952	Trabzon	W
PI169237	Bursa	W
PI227209	Japan	W
PI174160	Kars	W
PI169401	Kocaeli	W
PI204568	Kayseri	W
PI172840	Kars	W
PI175692	Kırşehir	W
PI172846	Urfa	W
PI172847	Gaziantep	W
PI167079	Adana	W
PI169385	Kocaeli	X
PI169382	İstanbul	X
PI169386	Kırklareli	X
PI169384	İstanbul	X
PI169381	İstanbul	X
PI169380	İstanbul	X
PI172841	Van	X
PI169390	Çanakkale	X
PI172845	Urfa	X
PI169398	Kocaeli	X
PI169397	Bursa	X
PI169399	Kocaeli	X
PI169392	Çanakkale	X
PI169351	Çanakkale	X
PI177361	Çorum	X
PI165046	Ankara	X
PI165029	Yerli Hıyar	X

(Cont. on next page)

Table 3.5. (Cont.)

Pedigree Number	**Location**	**Group Name**
PI177359	Ankara	X
PI109483	Türkiye	X
PI206954	Giresun	X
PI169350	Çanakkale	X
PI204690	Malatya	X
PI169388	Çanakkale	X
PI177956	Konya	X
PI175695	Kayseri	X
PI175679	Sinop	X
PI169304	Manisa	X
PI171612	Trabzon	Y
PI169393	Çanakkale	Y
PI169394	Bursa	Y
PI169389	Edirne	Y
PI206425	Trabzon	Y
PI171600	Zonguldak	Y
PI171607	Tokat	Y
PI172852	Elazığ	Z
PI172848	Maraş	Z
PI175683	Erzincan	

CHAPTER 4

CONCLUSION

Cucumber (*Cucumis sativus L.*) is an very important vegetable crop because of its nutritional composition and its role in the economies of countries where it is grown. Its production is dispersed all over the world and throughout Turkey.

We aimed to characterize the amount of genetic diversity of Turkish cucumber accessions. To this end, we carried out the characterization of 92 Turkish cucumber accessions. According to the results, cucumber accessions clustered into 4 groups. These results may help in selection of accessions as breeding materials for new cultivars. For example if we want to obtain new cultivar with new mixes of alleles, we can choose the genetically most distinct accessions from our dendrogram and make crosses between these two accessions. In addition our study will be useful for managing and developing germplasm collections by eliminating redundant accessions. If we look at dendrogram group B, we see that it is composed of the genetically most related accessions. It may not be necessary to maintain all of these accessions in the germplasm collection. A selection of accessions from this cluster may be enough to represent the genetic diversity of the cluster. However it must be remembered, that SRAP or other molecular techniques are not sufficient for determining genetic diversity. Morphological traits of the cucumber accessions should be studied and this information used in conjunction with molecular data to maintain and manage germplasm collections. This study also demonstrated that the SRAP marker system is an efficient system for plant genetic diversity studies. In conclusion, this study was the first molecular-based study of the genetic diversity of Turkish cucumber accessions.

REFERENCES

Dijkhuizen, A., W.C. Kennard, M.J. Havey, and J.E. Staub. 2006. RFLP variability and genetic relationships in cultivated cucumber. *Euphytica* 90:79-89.

Gostimsky, S.A., Z.G. Kokaeva, and F.A. Konovalov. 2005. Studying Plant Genome Variation Using Molecular Markers. *Russian Journal of Genetics* 41: 480–492.

Kong, Q., C. XIANG, and Z. YU. 2006. Development of EST-SSRs in *Cucumis sativus* fromsequence database. *Molecular Ecology Notes* 6:1234-1236.

Li, G. and C.F. Quiros. 2001. Sequence- Related Amplified Polymorphism (SRAP), A New Marker System based on A Simple PCR Reaction. *Theor Appl Genet* 103: 455-461.

Mliki, A., J.E. Staub, S. Zhangyong, and A. Ghorbel. 2003. Genetic diversity in African cucumber (*Cucumis sativus* L.) provides potential for germplasm enhancement. *Genetic Resources and Crop Evolution* 50: 461–468.

Nam, Y.W., J.R. Lee, K.H. Song, M.K. Lee, M.D. Robbins, S.M. Chung, J.E. Staub, and H.B. Zhang. 2005. Construction of two BAC libraries from cucumber (*Cucumis sativus L.*) and identification of clones linked to yield component quantitative trait loci. Theor Appl Genet 111: 150–161.

Plader, W., W. Burza, and S. Malepszy. 2007. Cucumber. *Biotechnology in Agriculture and Forestry* 59: Transgenic Crops.

Prohens, J., and Fernando Nuez, eds. 2008. *Handbook of Plant breeding: Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae*. Valencia, Spain.Universisad Politecnica de Valencia. http://www.springer.com (accessed June, 2009).

Rao, V.R., and T. Hodgkin. 2002. Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture* 68: 1–19.

Sari, N., A. Tan, R. Yanmaz, H. Yetisir, A. Balkaya, I. Solma1, and L. Aykas. 2008. General status of cucurbit genetic resources in Turkey. *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008.*

Serquen, F. C., J. Bacher, and J.E. Staub.1997. Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativus* L.) using random-amplified polymorphic DNA markers. *Molecular Breeding* 3: 257–268.

Staub, J.E., F. C. Serquen, T. Horejsi, and Jin-feng Chen. 1999. Genetic diversity in cucumber (*Cucumis sativus* L.): IV. An evaluation of Chinese germplasm. *Genetic Resources and Crop Evolution* 46: 297–310.

Staub, J.E., M.D. Robbins, S.M. Chung, and Z. Sun. 2006. History and Application of Molecular Markers for Cucumber improvement. *Cucurbitaceae 2006*.

Tanksley, S.D., and S. R. McCouch. 1997. Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. *Science* 277

Wang, Y.H., T. Joobeur, R. A. Dean, and J.E. Staub. 2007. Cucurbits. *Genome Mapping and Molecular Breeding in Plants* 5: Vegetables.

Watcharawongpaiboon, N., and J. Chunwongse. 2008. Development and Characterization of Microsatellite Markers from an Enriched Genomic Library of Cucumber (*Cucumis sativus*). *Plant Breeding* 127:74-81.

Kong, Q., C. XIANG, and Z. YU. 2006. Development of EST-SSRs in *Cucumis sativus* fromsequence database. *Molecular Ecology Notes* 6:1234-1236.