DETERMINATION OF GENETIC DIVERSITY AND ANTIOXIDANT CONTENT OF THE NATIONAL MELON (*Cucumis melo*) COLLECTION

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Hasan Özgür ŞIĞVA

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We approve the thesis of **Hasan Özgür ŞIĞVA**

_____
Assoc. Prof. Dr. Anne FRARY
Supervisor

_____
Assoc. Prof. Dr. Sami DOĞANLAR
Co-supervisor

_____
Assoc. Prof. Dr. Oğuz BAYRAKTAR
Co-Supervisor

_____
Prof. Dr. Ahmet YEMENİCİOĞLU
Committee Member

_____
Assist. Prof. Dr. Ahmet KOÇ
Committee Member

_____
Assist. Prof. Dr. Çağlar KARAKAYA
Committee Member

14 October 2008

_____
Prof. Dr. Semra ÜLKÜ
Head of the Biotechnology
and Bioengineering Programme

_____
Prof. Dr. Hasan BÖKE
Dean of the Graduate School of
Engineering and Science

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ABSTRACT

DETERMINATION OF GENETIC DIVERSITY AND ANTIOXIDANT CONTENT OF THE NATIONAL MELON (*Cucumis melo*) COLLECTION

In this study, characterization with 19 morphological criteria and two types of PCR-based molecular marker systems, Simple Sequence Repeats (SSRs, microsatellites) and Amplified Fragment Length Polymorphisims (AFLPs), were used to characterize genetic variability among 238 national melon (*Cucumis melo* L.) accessions and 12 different members of the *Cucurbitaceae* family.

According to morphological analysis, all accessions were vigourous. The accessions showed a great variety of fruit shape, 53 (38.4%) accessions were globular (round), 51 (37.0%) were eliptical, 15 (10.9%) were ovate, 7 (5.1%) oblate, 2 (1.4%) elongate and one accession was flattened. Another agronomically important criterion was predominant fruit skin colour. A total of 55 (39.9%) accessions had light yellow predominant skin colour, 34 (24.6%) were pale green, 29 (21.0%) were orange, 9 (6.5%) were cream, 6 (4.4%) were green, 4 (2.9%) were blackish-green and 1 (0.7%) was dark green.

In accordance with genetic characterization, a total of 345 polymorphic AFLP fragments (products of 10 AFLP primer combinations) and 93 SSR fragments (products of 12 SSR markers) were detected and used to calculate genetic distance using DICE matrix and UPGMA (Unweighted Pair Group Method) arithmetical averages. The average polymorphic AFLP fragments per combination was 34.5 and SSR fragments per marker was 7.75. The phylogenetic tree showed that groups were clearly separated by both marker systems. This study allowed the identification of the relationship between national melon accessions based on genetic similarity or differences.

Forty three melon accessions were also analyzed for total water soluble antioxidant and total phenolic compound activities.

ÖZET

ULUSAL KAVUN (Cucumis melo) KOLLEKSİYONLARINDAKİ GENETİK ÇEŞİTLİLİĞİN VE ANTİOKSİDANT İÇERİĞİNİN BELİRLENMESİ

Bu çalışmada, 238 adet ulusal kavun hattı ile 12 farklı kabakgiller üyesi arasındaki genetik çeşitliliğin bulunması amacıyla 19 farklı kriterde morfolojik karakterizasyon ile beraber iki tip PCR tabanlı moleküler markır sistemleri olan Simple Sequence Repeats (SSRs) ve Amplified Fragment Length Polymorphisims (AFLPs) kullanılmıştır.

Morfolojik analizleri sonucunda, hatlar meyve şekli bakımından büyük çeşitlilikler göstermektedir, 53 (38.41%) hat globular (küresel), 51 (36.96%) hat eliptik, 15 (10.87%) hat ovat, 7 (5.07%) hat oblat, 2 (1.45%) hat elongat, 1 (0.72%) hat düz şekillidir. Bir diğer önemli özellik ise birincil meyve kabuğu rengidir. 55 (39.86%) hat açık sarı, 34 (24.64%) hat soluk yeşil, 29 (21.02%) hat turuncu, 9 (6.52%) hat krem rengi, 6 (4.35%) hat yeşil, 4 (2.9%) hat siyah-yeşil ve 1 (0.72%) hat koyu yeşil renktedir.

Genetik karakterizasyonu sonucunda 306 adet polimorfik AFLP fragmentleri (10 adet AFLP primer kombinasyonundan) ve 93 adet SSR fragmentleri (12 adet SSR primerinden) elde edilmiş ve bu değerler genetik uzaklığın ölçümünde kullanılmıştır. AFLP primer kombinasyonu başıına ortalama 34.5, SSR primerleri başına ise 7.75 polimorfik fragment düşmektedir. 345 AFLP ve 93 SSR polimorfik fragmentleri ışığında, 238 adet ulusal kavun hattı ile 12 adet kabakgiller üyesi arasındaki genetik ağaç NTSYS-pc version 2.2 programında SHAN modülünde DICE katsayısı ve UPGMA metodu kullanılarak çizilmiş ve AFLP'de 10, SSR'da 21 adet grup kesin olarak ayrılmıştır. Bu çalışmada ulusal kavun hatları, genetik benzerlik ve farklılık ilkesine bağlı olarak saptanmıştır.

43 adet ulusal kavun hattında ABTS dekolorizasyon yöntemi kullanılarak suda çözünebilen toplam antioksidant ve toplam fenolik aktivitesi hesaplanmıştır.

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CHAPTER 1

INTRODUCTION

1.1. General Characteristics of *Cucumis melo* L

The genus *Cucumis* consists of 30 wild and cultivated species distributed around the world especially in Africa, tropical America and Southeast Asia. They are generally climbing or prostrate dicotyledonous plants that are located in tropical, subtropical and temperate climates (Salunkhe and Kadam 1998, Seçmen, et al. 2000, López-Sesé, et al. 2002, Decker-Walters, et al. 2002, Staub, et al. 2004). Of the species in the genus, *Cucumis melo* (2n = 2x = 24) has great importance because of its commercial value and is grown mostly for its fruits by farmers. The species plays a significant role in supplying fresh fruit vegetables used for salad, cooking, and dessert and it can also be candied. *C. melo* is one of the most diverse and highly polymorphic species in *Cucurbitaceae* (Danin-Poleg, et al. 2001, Oliver, et al., Decker-Walters, et al. 2002). Although the species is generally known as melon, it is also called sweet melon, muskmelon, casaba, and cantaloupe (Nayar and Singh 1998). It has been widely cultivated in different regions of the world by humans for ages on account of its delicious and nutritious fruits and edible seeds (Whitaker and Davis 1962). Immature fruits can also be used as a vegetable and roots are used as a medicinal compound (Walter and Walter 1997, Nayar and Singh 1998). Several historical and ethnobotanical records show that the Persians and the Egyptians cultivated melon in 2-3 BC (Akashi, et al. 2002). According to some Egyptian hieroglyphs, there were several types of cucurbits used as vegetables including melon and melon was known as *qishu* (Stepansky, et al. 1999). Moreover, the Chinese and the Japanese have been cultivating sweet and non-sweet types of melon since 2000 BC (Keng 1974, Akashi, et al. 2002).

The species *C. melo* is a member of the *Cucurbitaceae* family, *Cucurbitoideae* subfamily, *Melothrieae* tribe and *Cucumerinae* subtribe. There are a great variety of commercially important cultivated types around the world (Seçmen, et al. 2000,

Decker-Walters, et al. 2002). Especially Cantalupensis (Muskmelon) and Inodorus (Cassaba and Honeydew) are of commercial interest in Europe and the United States (Staub, et al. 2000). It is a morphologically diverse and outcrossing horticultural crop (Naudin 1859, Kirkbride 1993). In general, wild and cultivated types of *C. melo* are herbs or vine plants. They are annual or perennial, with or without a woody rootstock. They are commonly monoecious, yet in cultivated individuals rarely andromonoecious. Stems are generally procumbent and sulcate and stem hair is generally hispid, retrorse-strigose or rarely lanate. Petiole lengths vary from 1.5 to 20 cm. Generally, petioles are sulcate or not aculate. Leaf blade type can change from accession to accession but generally, it can be entire, trilobate, pentalobate or 3 to 5 palmately lobed. Male inflorescences are generally fasciculate or paniculate. In the male flower, pedicel is terete in cross section, 2.4 to 15 mm long and has no bracteols. Calyx lobes are linear or narrowly triangular in outline (rarely in the largest cultivated plants), narrowly acute at the apex. The corolla is infundibular, puberulent, pilose or glabrous outside and puberulent or glabrous inside. The corolla tube can be 0.8-2 mm long, pilose or glabrous. Corolla lobes are elliptic, broadly elliptic or broadly ovate in outline. Stamens are separating from the base of the hypanthium. Filament is terete or radially compressed in cross section. In the female flower, the pedicel is sulcate in outline and is generally 1.6-12 mm long. The lower 2/3 of the hypanthium is fused to the ovary. The fused portion of hypanthium is ellipsoid and 4-11 mm long and the upper 1/3 of the hypanthium is free from the ovary. The free portion of the hypanthium is generally 2.4-4.5 mm long. Calyx lobes are linear, triangular or narrowly elliptic in outline and narrowly acute at the apex. Corolla is puberulent or sparsely pilose. The corolla tube can be present or absent and it is generally puberulent and sparsely antrorse-strigose. Corolla lobes are broadly obovate, elliptic to broadly elliptic or ovate in outline. Staminoides are present and they can separate from the free portion of the hypanthium 0.8-2 mm above the ovary. In general, the stigma is 1.4-2.4 mm long and lobate. Melon fruits are not geocarpic, maturing above ground and readily visible. The pedicel is sulcate in cross section and 1.5-4 cm long. Fruits can be mono or bicolored with longitudinal stripes from base to apex. Normally, fruits are, green, red, yellow, white or brown with light green, yellow, brown, white or dark green longitudinal stripes. Their shapes can be ellipsoid, globose, cylindrical, ovoid or obovoid. Melon seeds have generally ovate or elliptic shape and generally they are 4-8 X 2.5-4 mm and 1-2 mm thick, unwinged or scarcely apically winged (Kirkbride 1993).

The species of *C. melo* was first described by Linné in 1753 (Stepansky, et al. 1999). After Linné, several authors tried to describe melon with respect to some morphological traits such as shape of leaves, vine, flower types, habitat and types of fruit (Stepansky, et al. 1999). According to Naudin (1859), there were 10 varieties of melon species and this study was the basis of all subsequent studies. After Naudin, Pangalo studied this subject in 1929. Pangalo worked on a live collection of 3000 specimens and presented a multi-level taxonomy based on the idea of a homologous series. According to this opinion, *C melo* species was divided into two homologous subspecioids: *cultus* (cultivated melon) and *agrestis* (wild type melon). After that, Jeffrey (1980) divided the species into two subspecies: subsp. *melo* and subsp. *agrestis* (Monforte, et al. 2003). These subspecies were defined based on the hairness of the ovary. Although, subspecies *melo* has spreading hairs, subspecies *agrestis* has appressed hairs (Kirkbride 1993). Additionally, Munger and Robinson studied and published a taxonomy in 1991. They proposed a simplified version of Naudin's taxonomy. They divided *C. melo* into 7 groups which included trinomial names, these are; *C. melo agrestis* Naud. (wild melon), *C. melo flexuosus* Naud. (snake melon), *C. melo conomon* Mak. (pickling melon, Chinese white cucumber), *C. melo cantalupensis* Naud. (cantaloupe or muskmelon), *C. melo inodorus* Naud. (winter melons, honeydew or casaba) *C. melo chito* (mango melon) and *dudaim* Naud. (queen's pocket melon) and *C. melo momordica* (phoot or snap melon) (Stepansky, et al. 1999). Of these groups, especially Cantalupensis and Inodorus are economically important around the world (McCreight, et al. 1993). Finally, Pitrat et al. (2000), divided melon into 16 cultivar groups: *conomon*, *makuwa*, *chinensis*, *acidulus* and *momordica* are within subsp. *agrestis*; *cantalupensis*, *reticulatus*, *adana*, *chandalak*, *ameri*, *inodorus*, *flexuosus*, *chate*, *tibish*, *dudaim* and *chito* are within subsp. *melo*.

1.2. Ecology and Dispersion of *Cucumis melo* L.

Melon cultivation requires a hot and dry climate and, therefore, is a warm season crop. The species requires an optimum temperature of 27-30 ^{0}C and its seed requires approximately 23-24 ^{0}C for germination and will not germinate at temperatures less than 18 ^{0}C. Although, light is not as important as temperature, it can affect plant color and fruit flavor. In addition, excess atmospheric humidity adversely affects sugar formation, texture and flavor (Salunkhe and Kadam 1998). Melon does not want too

much water, in general, 7-10 days is enough for watering. Excess watering causes a lack of sugar content in the fruits. Thus, high temperature, low atmospheric humidity, plenty of sunshine and low watering are essential for proper ripening and high quality fruits. Melon is very sensitive to low temperature and freezing.

A 100 g serving of melon fruit includes many important nutrients and vitamins. The nutrient and vitamin content of melon is presented in Table 1.1. Melon contains high amount of water, approximately 90% of its fruits. Moreover it contains primary metabolites (protein, lipid and carbohydrate), several significant vitamins (Vitamin A, Vitamin B_1, Vitamin B_2, Vitamin B_6, etc.) and several minerals (potassium, calcium, iron, magnesium and phosphorous). Its fruits have approximately 45 kcal energy per 100 g fruits (Salunkhe and Kadam 1998).

Table 1.1. Nutrient and vitamin composition of a 100 g serving of edible fresh melon fruit (Source: Salunkhe and Kadam 1998)

Compound	**Amount**
Water	87 g – 92 g
Protein	0,6 g - 1,2 g
Lipid	0,1 g - 0,2 g
Carbohydrate	6 g – 15 g
Total Sugar	7 g – 12 g
Total Carotene	0,05 mg -5,37 mg
Vitamin A	500 IU – 4200 IU
Vitamin B_1	0,06 mg
Vitamin B_2	0,02 mg
Vitamin B_6	0,01 mg
Niacin	0,4 mg - 0,9 mg
Vitamin C	6 mg - 60 mg
Panthothenic acid	0,13 mg – 0,21 mg
Potassium	130 mg - 330 mg
Calcium	5 mg - 18 mg
Iron	0,2 mg – 0,6 mg
Magnesium	8 mg - 17 mg
Phosphorous	7 mg - 57 mg
Fiber	300 mg - 600 mg
Energy	18 kcal - 53 kcal

The genus *Cucumis* originated in South Africa where a large amount of diversity of *cucumis* varieties is observed. A center of diversity of *C. sativus* is around the Himalaya mountains, this region is called the Indian center. In addition, the wild type of *C. melo* sp. *agrestis* originated from the south of the Sahara in eastern tropical Africa (Nakata, et al. 2005). According to some authors, the wild type of *C. melo* originated from south and east Africa (Mallick and Mausui 1986) and expanded from there around the world but unfortunately there is no obvious and accurate information about where the cultivatation of melon species started (Oliver, et al. 2001). Some authors believe that the wild type of melon originated from the Middle East and Asia (Nakata, et al. 2005) and it expanded from there to the rest of the world. In view of this information, Turkey has great importance for *C. melo* beause it is one of the secondary centers of its diversification and domestication in the world. Moreover, *C melo* sp. *melo*, *C. melo* sp. *flexuosus* and *C. sativus* are cultivated in many areas in Turkey (Seçmen, et al. 2000). According to FAO (Food and Agriculture Organization), approximately 11.480.900 Mt of melon were produced in Turkey in 2005. This number places Turkey as the eleventh major world producer of melon as shown in Table 1.2. China, India, Brazil and USA are very significant countries in terms of melon production in the world. These four countries produce approximately (195.383.229 mt/year) 53% of the melons around the world.

Table 1.2. Top 20 countries in respect of melon production in the world (Source: FAO 2005).

	Countries	**Mt/Year**		**Countries**	**Mt/Year**
01	China	87,055,600	11	**Turkey**	**11,480,900**
02	India	47,031,300	12	Uganda	10,567,650
03	Brazil	35,423,429	13	France	10,339,100
04	USA	25,872,900	14	Nigeria	9,127,000
05	Italy	19,203,132	15	Egypt	8,195,635
06	Spain	14,805,000	16	Thailand	8,138,000
07	Mexico	14,758,654	17	Colombia	7,840,222
08	Indonesia	13,776,664	18	Pakistan	7,753,423
09	Iran, Islamic Rep of	13,143,110	19	Ecuador	5,751,800
10	Philippines	12,452,620	20	South Africa	5,447,383

1.3. Importance of Plant Diversity

Many years ago, humans started cultivatation of several wild types of plants for different aims such as food, beverages, spices, herbs, oils, waxes, medicine, dyes and ornamentals. They chose desirable individuals from wild type plants and grew the next

generation in the field and, in this way, plant domestication was begun. During the domestication process, although plants gain several horticulturally-important traits like bigger fruit, desirable flavor and high production they can lose other traits like disease resistance and high secondary metabolite content. Thus, plants can lose some of the alleles related with horticulturally undesirable or nonselected traits. In this way, genetic diversity of cultivated plants can become narrower and may degrade into a dangerous condition for cultivated plants.. Thence, many researchers have been surveying plant germplasm for core collection establishment in order to protect the cultivated plants' gene pools. Recently, several researchers have shown that wild type alleles can be very important in order to improve horticultural and agronomic traits. For example, Bernacchi *et al*. (1998) defined eight quantitative trait loci in tomato that developed skin color in a population derived from the green-fruited wild type *Lycopersicum hirsutum* (Bernacchi, et al. 1998). In another study, Fulton *et al*. (1997) identified three QTL that improved fruit color in a population derived from an interspecific cross with another wild type, *Lycopersicum peruvianum* Miller. These type of studies demonstrate that a great variety of alleles are hidden in the genomes of wild types of plant.

All in all, the importance of plant diversity is to characterize the collection morphologically and genetically, establish a core collection by eliminating redundant accessions and identify lines that may be useful for melon breeding.

1.4. Morphological and Molecular Marker Systems

In breeding, mapping or surveys of genetic variation, there are two types of marker systems that are used for determination of variation in accessions or species: morphological and molecular. In morphological marker systems, visible phenotypic changes are observed, for example, morphological differences in leaves, flowers, fruits or seeds. On the other hand, difference at the gene, DNA or protein level are examined for molecular characterization. Surveys of plant species were limited in size until the discovery of molecular mapping. The primary difficulty with improving these surveys was the inability to incorporate many markers into a single stock to be used for genetic analysis. This inability occurred due to the deleterious effects of the expression of all mutant phenotypes in a single stock. Because normal DNA or protein molecules are used to score the genetic material, molecular markers are phenotypically neutral. This is a significant advantage compared to traditional morphological characterization. In view

of this information, morphological characterization is less reproducible than molecular characterization and also it cannot give accurate and clear information about plant accession or species, because, many plant morphologic traits can be changed by temporary environmental factors like temperature, light, lack of water or chemical structure of soil. On the other hand, molecular characterization is more reliable than morphological characterization since plant DNA structure is not affected by such factors.

1.4.1. Morphological Marker Systems

In morphological marker systems, several visible phenotypic characters can be used in order to define differentiation between plant accessions or species for breeding, mapping or genetic variation surveys. For example, morphological markers can code for visible phenotypic changes like orange fruit, dwarfism, leaf veins or anthocyanin production (Tanksley 1993). As a result, they are easily monitored. These characters are based on plant physiology and anatomy.

Many quantitative characters which are continuously variable are recorded on a 1-9 scale shown in Table 1.3. The authors of quantitative characters tables have occasionally defined only some selected states such as, 1, 3, 5, 7 and 9 or 3, 5 and 7 for such descriptors.

Table 1.3. Quantitative characters scored from 1 to 9 or very low to very high

1	Very low	**6**	Intermediate to high
2	Very low to low	**7**	High
3	Low	**8**	High to very high
4	Low to intermediate	**9**	Very high
5	Intermediate		

Morphological markers can be affected by environmental conditions, are limited in number and may appear during different stages of plant development. In addition, morphological markers can be affected by other morphological markers or morphological traits due to pleiotropic gene action (Tanksley 1993, Andersen, et al. 2003) In general, morphological markers are mutant forms of their wild types and they can interfere with plant health. Because the accumulation of multiple mutations may kill the plant, it is also difficult to get plants with multiple morphological markers.

However, they are very easy to study with Mendelian genetics tools because morphological markers permit the determination of the genotype of a particular locus from the morphology of progeny. (Tanksley 1993).

There are many criteria that can be used in morphological characterization such as plant branches, leaf shape, leaf lobes, leaf color, sex type, days to 50% flowering, flower color, fruit shape, fruit length, predominant fruit skin color, fruit surface, seed shape, seed size, and 100-seed weight (IPGRI 2003).

1.4.2. Molecular Marker Systems

In molecular marker systems , differences at the protein, gene and DNA sequence level that are not obvious by looking at the plants are used in order to define differentiation between plant accessions or species for breeding, mapping or genetic variation surveys (Wang, et al. 2004, Gostimsky, et al. 2005). Because normal DNA or protein molecules are used to score the genetic material, molecular markers are phenotypically neutral. This is a significant advantage compared to traditional morphological markers. In general, molecular markers can be used for several purposes. These uses are shown in Table 1.4.

1.4.2.1. Molecular Markers in *Cucumis melo*

Molecular markers are also known as genetic markers and are highly polymorphic nucleotide sequences located in the genome and their mutations may be detected via Polymerase Chain Reaction (PCR)-based techniques (Gostimsky, et al. 2005). Moreover, molecular markers have neutral phenotype (Jones, et al. 1997). This trait gives rise to a significant advantage compared to traditional phenotypic markers.

As a rule, there are fewer nucleotide sequences differences in the genome when the species are closely related to each other. One of the most important applications of molecular markers is the development of detailed chromosome maps of all living things. Another significant application is the improvement of the efficiency of conventional plant breeding via carrying out indirect selection with molecular markers. In addition to this, molecular markers can also be used for germplasm characterization, genetic diagnostics, characterization of transformants, studies of genome organization, and phylogenetic analysis (Jones 1997, Gupta, et al. 1999, Gostimsky, et al. 2005).

In recent years, in addition to morphological characterization based on morphology, cross compatibility or physiology, molecular markers have been used for studying genetic diversity and phylogenetic relationships in melon (Tanaka, et al. 2006). There are many surveys about the determination of melon genetic diversity with some molecular marker systems. In 1985, isozyme analysis was performed by Perl-Treves et al. who used 29 nuclear-coded enzymes in 21 Cucumis species. After Perl-Treves et al., Akashi, et al. (2002) published an isozyme study. Akashi et al. used five isozymes and 114 melon accessions. Neuhausen et al. (1992) worked on melon genetic diversity by using restriction fragment length polymorphism (RFLP) analysis. Garcia et al. (1998) were successfully used random polymorphic DNA (RAPD) anaysis in melon. They used to determine the genetic relationships among 32 breeding lines of melon belonging to seven varietal types and most of the breeding lines were Galia and Piel de Sapo genotypes. One year later, Stepansky et al. (1999) surveyed 54 melon accessions representing diverse genotypes from 23 countries. This technique was later used by Lopez-Sese et al. (2003), Nakata et al. (2003), Staub et al. (2004) and Tanaka et al. (2006). Simple sequence repeat (SSR, Microsatellites) analysis was used by Staub et al. (2000), Danin-Poleg et al. (2000), Chiba et al. (2003). Staub et al. used 46 melon accessions in two melon subspecies, subspecies *melo* (*Cantalupensis* and *Inodorus*) and *agrestis* (*Conomon* and *Flexuosus*). After this study, Danin-Poleg et al. (2000) surveyed 13 melon and 11 cucumber genotypes. Single-nucleotide polymorphism (SNP) analysis was used by Morales et al. (2004) and Szabó et al. (2005) used this technique. Morales et al. used 34 melon EST fragments between two distantly related melon genotypes, a group Inodorus, Piel de sapo, line T-111 and the Korean accession PI161375. Sequence characterized amplified region (SCAR) analysis was done by Noguera et al. (2005) and amplification fragment length polymorphism (AFLP) analysis was used by Garcia-Mas in 2000. Garcia-Mas et al. used 12 different primer combinations in order to determine the variation between six different melon genotypes.

Table 1.4. Uses of some types of molecular markers in molecular biology.

(Source: FAO/IAEA 2002)

Morphological	**Isozymes**	**RFLP**	**RAPD**	**STS/EST**
Genetic maps	Genetic maps	Comparative maps	Genetic maps	Fingerprinting
Alien gene introduction	Quality trait mapping	Framework maps	F1 identification	Varietal identification
Varietal identification	Varietal identification	Genetic maps	Varietal identification	Genetic maps
F1 identification	F1 identification	Breeding	Breeding	F1 identification
Novel phenotypes	Breeding	Varietal/line identification	Bulk segregant analysis	Gene tagging and identificati.
Breeding	Seed testing	Marker-assisted selection	Diversity studies	Bulk segregant analysis
		F1 identification	Marker-assisted selection	Diversity studies
		Diversity studies	Seed testing	Marker-assisted selection
		Novel allele detections	Map-based gene cloning	Novel allele detection
		Gene tagging		High-resolution mapping
		Bulk segregant analysis		Map-based cloning
		Map-based gene cloning		
SCAR	**CAPS**	**SSR**	**AFLP**	**SNP**
Framework mapping	Framework mapping	Fingerprinting	Fingerprinting	Genetic maps
Can be converted to allele-specific probes	Can be converted to allele-specific probes	Varietal/line identification	Very fast mapping	F1 identification
F_1 identification	F_1 identification	Framework/region specific	Region-specific marker satur.	Breeding
Gene tagging	Gene tagging	Genetic maps	Varietal identification	Gene tagging
Bulk segregant analysis	Bulk segregant analysis	F1 identification	Genetic maps	Alien gene introduction
Diversity studies	Diversity studies	Comparative mapping	F1 identification	Bulk segregant analysis
Marker-assisted selection	Marker-assisted selection	Breeding	Gene tagging	Diversity studies
Map-based cloning	Map-based cloning	Bulk segregant analysis	Breeding	Novel allele detections

These molecular markers can be broadly divided into four main groups based on their natural structure (Gupta 1999, Monforte 2004).

1. Protein-Based Molecular Marker Systems
2. Hybridization-Based Molecular Marker Systems
3. PCR-Based Molecular Marker Systems
4. Sequencing-Based Molecular Marker System

According to FAO/IAEA Division of Nuclear Techniques in Food and Agriculture an ideal genetic marker has some important traits:

1. They have no detrimental effect on phenotype
2. They are codominant in expression level
3. They are single copy
4. They are economical to use
5. They are highly polymorphic
6. They are easily assayed
7. They are multi-functional
8. They are highly available
9. They are genome-specific in nature especially when working with polyploids
10. They have the ability to be automated

Perhaps the most important criteria for morphological and molecular markers are polymorphism level, dominance condition, and ease of use with PCR-based markers being favored over other types. Comparison of various types of markers for these criteria is shown in Table 1.5.

Table 1.5. Comparison of some marker techniques in terms of being PCR-based or not, level of polymophism and dominance (Source: FAO/IAEA 2002).

Markers	**PCR-based**	**Polymorphism**	**Dominance**
Morphological	No	Low	Dominant-Recessive-Codominant
Isozyme	No	Low	Codominant
RFLP	No	Low-Medium	Codominant
RAPD	Yes	Medium-High	Dominant
STS/EST	Yes	High	Codominant-Dominant
SCAR	Yes	High	Codominant
CAPS	Yes	High	Codominant
SSR	Yes	High	Codominant
ISSR	Yes	High	Dominant
AFLP	Yes	High	Dominant

1.4.2.1.1. Protein-Based Molecular Marker Systems

DNA produces mRNA and mRNA produces protein. Different proteins, have different genome locations. An example of this type of molecular marker system is isozymes.

1.4.2.1.1.1. Isozymes

Isozymes, also known as isoenzymes, differ in amino acid sequence yet catalyze the same chemical reaction. They have different charge so they can be separated via electrophoresis (Zeidler 2000, Akashi, et al. 2002). Each isozyme has a specific role in the metabolic pathway and functions with other enzymes in the organization of cells. Isozymes generally exhibit tissue or cell specificity and molecular heterogenity of enzymes confers flexibility, versatility and precision on an organism in terms of metabolic functions (Zeidler 2000).

Isozymes have some advantages. For example; they are cheaper than other methods. Moreover, they are faster based on analysis methods and they also show co-dominant nature. On the other hand, isozymes have some disadvantages. For instance; they often have different protocols for each locus and also, they are sometimes difficult to interpret. The method of isozymes can be used for genetic maps, quality trait mapping, varietal/line identification (multiplexing of proteins or isozymes necessary),

F_1 identification, breeding, and seed testing (FAO/IAEA 2002). These uses are shown in Table 4.

1.4.2.1.2. Hybridization-Based Molecular Marker Systems

These molecular marker systems require hybridization between genomic DNA and a selected probe. Examples of this type of system are restriction fragment length polymorphisms (RFLPs) and oligonucleotide fingerprinting.

1.4.2.1.2.1. Restriction Fragment Length Polymorphisms (RFLPs)

Restriction fragment length polymorphism is a hybridization-based molecular marker system (Gupta, *et al*. 1999). RFLPs have some advantages and disadvantages. They have unlimited number of loci and also, they are codominant in nature. No sequence information is required for RFLP analysis. On the other hand, they are fairly expensive and a large quantity of DNA is needed to use this method. Besides, they have generally low levels of polymorphism. RFLPs can be used for determination of the approximate map location of any cloned piece of DNA. RFLPs can be used for comparative maps, framework maps, genetic maps, breeding, varietal/line identification (multiplexing of probes necessary), marker-assisted selection, F_1 identification, diversity studies, novel allele detections, gene tagging, bulk segregant analysis and map-based gene cloning (FAO/IAEA 2002).

In this method, DNA is cut into restriction fragments using restriction enzymes (such as EcoRI, EcoRV, MseI, HindIII, MvaI, BamHI, etc.) that only cut the genomic DNA molecules where there are specific and characteristic nucleotide sequences, termed recognition sequences or restriction sites. Many types of mutations cause single nucleotide or larger differences between individuals that create or destroy a restriction site in the genome which is then detected as a polymorphism by the RFLP marker (Jones, *et al*. 1997, Garcia-Mas, *et al*. 2000).

After using restriction enzymes, the technique of Southern hybridization is used in order to identify genomic DNA fragments. Genomic DNA fragments are separated via electrophoresis and are transferred to nylon or nitrocellulose membrane. Radioactively or chemiluminescently labeled probes are then hybridized to the genomic

DNA fragments. Hybridized DNA fragments can then be detected as visible bands on the filter (Jones, *et al*. 1997, Garcia-Mas, *et al*. 2000, Chang, *et al*. 2007).

1.4.2.1.3. PCR-Based Molecular Marker Systems

These molecular marker systems are the most used molecular marker systems due to their being reliable, easy and cheap. Random amplifed polymorphic DNAs (RAPDs), sequence characterized amplifed regions (SCARs), simple sequence repeats (SSRs) or microsatellites, sequence tagged sites (STS), amplifed fragment length polymorphisms (AFLPs), and cleaved amplifed polymorphic sequences (CAPS) are examples of these systems. PCR-based DNA marker systems can be divided into three groups:

1. Markers that are amplified using a single primer in PCR (RAPDs, SPARs, DAFs, AP-PCRs, SSR-anchored PCR *etc*.)
2. Markers that are selectively amplified with two primers in PCR (AFLPs)
3. Markers that are amplified using two primers in PCR that generally require genome sequences for the construction of specific primers (AMP-FLPs, STRs, SSRs, CAPS)

1.4.2.1.3.1. Random Amplified Polymorphic DNAs (RAPDs)

RAPD markers are generated via PCR amplification of genomic DNA using short random primers of between 8-12 nucleotides. Amplification products are separated by electrophoresis (on agarose or polyacrylamide gel). Results are defined as the presence or absence of certain bands (Stepansky, et al. 1998, Decker-Walters, et al. 2001, López-Sesé, et al. 2003, Mliki, et al. 2003).

The random amplified polymorphic DNA (RAPD) method is a very amenable, less expensive, technically simple and rapid marker technique (Stepansky, et al.1998, Mliki, et al. 2003, Gostimsky, et al.2005). Moreover, no sequence information is required for this method. RAPDs can detect more polymorphic loci than isozymes (Staub, et al., 1997). In general, RAPD primers yield 3 to 15 amplification products (Gostimsky, et al. 2005). Hence, the method of RAPD gives more polymorphic bands than isozymes and RFLP and, for this reason, may be preferred for some uses (Mliki, et al. 2003). On the other hand, RAPDs are highly sensitive to laboratory changes and

also, they are have low reproducibility within and between laboratories. Besides, they have dominant nature and generally give multiple loci.

This method can be used for genetic maps, F_1 identification, varietal/line identification (multiplexing of primers necessary), breeding, bulk segregant analysis, diversity studies, marker-assisted selection, seed testing, and map-based gene cloning (FAO/IAEA 2002).

1.4.2.1.3.2. Sequence Characterized Amplified Regions (SCARs)

Sequence characterized amplified region (SCAR) markers are originated from RAPD fragments yet identified with the use of long, specific primers (Gostimsky, et al. 2005). Polymorphic RAPD or ISSR fragments are used to obtain specific SCAR markers. These polymorphic fragments are cut out of the agarose gel, cloned and sequenced. After sequencing, the SCAR marker primers are selected for terminal regions of the fragments (Gostimsky, et al. 2005).

SCAR marker systems have some pros and cons. This system requires small DNA quantities and is highly reliable. Moreover, SCARs markers are species-specific markers. On the other hand, SCARs are very labour intensive and they have high development cost (FAO/IAEA 2002). They are shown in Table 4.

This molecular method is used for mapping , F_1 identification, gene tagging, bulk segregant analysis, diversity studies, marker-assisted selection, map-based cloning (FAO/IAEA 2002, Noguera, et al. 2005). They have a codominant nature.

1.4.2.1.3.3. Amplified Fragment Length Polymorphisms (AFLPs)

The amplified fragment length polymorphism (AFLP) marker system has great sensitivity and is effective in order to detect polymorphisms in genomic DNA. This method is related with selective amplification of restriction fragments from digestion of total cellular DNA (Blears, et al. 1998).

This method is divided into four steps:

1. Digestion of total genomic DNA
2. Ligation of adapters (pre-selective amplification)
3. Selective amplification
4. Visualization (gel analysis)

In general, two restriction endonucleases are used to generate restriction fragments in this method. Using two restriction endonucleases allows the specificity of this method because of the number of DNA fragments (Blears, et al. 1998). In addition to this, cutting with two endonucleases increases the number of different fingerprinting combinations. Commonly, *Eco*RI, *Mse*I, *Ase*I, *Hind*III, *Apa*I and *Pst*I endonucleases are used in AFLP. After digestion, restriction half-site specific adaptors are ligated to the restriction fragments. Then some of these fragments are selectively amplified with two PCR primers that have corresponding adaptor and restriction site specific sequences. AFLP primers for selective amplification consist of three types of DNA sequences. These are:

- 5' region complementary to the adapter
- 3' selective nucleotides
- restriction site sequence

Polyacrylamide gel separation and silver staining or sequencing are used for visualization of AFLP products (Blears 1998, Garcia-Mas 2000, Ferriol 2003, Zeid 2003).

The AFLP method is preferred for some of its traits. For example, only small quantities of DNA are required for success and no sequence information is required. Moreover, this method can be automated. On the other hand, they are dominant markers and they can be technically challenging (FAO/IAEA 2002).

The method of AFLP can be used for fingerprinting, very fast mapping, region-specific marker saturation, varietal identification, genetic maps F_1 identification, gene tagging, breeding, bulk segregant analysis, diversity studies, marker-assisted selection, high-resolution mapping and map-based gene cloning (FAO/IAEA 2002, Peters, et al. 2004).

1.4.2.1.3.4. Simple Sequence Repeats (SSRs) or Microsatellites

Simple sequence repeats (SSRs) are also known as microsatellites and are small, tandemly repeated segments of DNA (Danin-Poleg, et al. 2000, Chiba, et al. 2003, Garcia-Mas, et al. 2004, Ritschel, et al. 2004). SSRs, in general, have a high level of transferability to related species and for that reason, these markers are significantly valuable (Varshney, et al. 2005). They are among the most flexible types of genome sequences in plants (Wang, et al. 2004). Usually microsatellites are 2 to 5 bp in length and are repeated a number of times (Danin-Poleg, et al. 2001) with the most useful SSRs having the core motif repeated from 9 to 45 times. Some of the major core motifs that are using in the development of SSR markers for melon include TGA, GAT, CTT, GGA, AT and CT.

Expressed sequences tag (EST) collections are a very good source for SSR marker development (Morales, et al. 2003, Varshney, et al. 2005). Various software programs can be used for identification of SSRs including;

1. MISA (the Microsatellite)
2. SSRFinder
3. BuildSSR
4. SSRIT (SSR identification tool)
5. TRF (Tandem Repeat Finder)
6. TROL (Tandem Repeat Occurence Locator)
7. Sputnik

Trinucleotide repeats (TNRs) are the most common nucleotide repeats followed by dinucleotides (DNRs) and tetranucleotides (TTNRs) (Varshney, et al. 2005). For example, cereal species contain approximately 54-78% TNRs, 17-40% DNRs and 3-6% TTNRs in their ESTs (Varshney, et al. 2002) The abundance of trimeric SSRs in ESTs seems to be related to the fact that variations in trinucleotide repeat number do not cause frameshift mutations (Metzgar, et al. 2000).

SSR markers are preferred for some of their favorable traits. For example, they are hypervariable and codominant in nature. Moreover, they are very reproducible and highly informative. They are also locus-specific markers (Danin-Poleg, et al. 2001, FAO/IAEA 2002). Although they have some advantages, their development is time consuming for plant species and requires DNA sequence information (Wang, et al. 2004). The level of polymorphism detected by SSRs varies with method of sexual reproduction and has been reported in cereals as 57% for self-incompatible species, 39% for out-crossing species and 20% for self-pollinated species (Wang, et al. 2004).

The method of SSRs can be used for fingerprinting, varietal/line identification (multiplexing of primers necessary), framework/region specific mapping, genetic maps, F_1 identification, comparative mapping, breeding, bulk segregant analysis, diversity studies, novel allele detections, marker-assisted selection, high-resolution mapping, seed testing, map-based gene cloning and also they have provided detailed information about genetic structure and gene flow (FAO/IAEA 2002, Wang, et al. 2004).

1.4.2.1.3.5. Sequence-Related Amplified Polymorphism (SRAP)

The goal of the sequence related amplified polymorphism (SRAP) marker system is the amplification of open reading frames (ORFs) in the genome (Li, et al. 2001, Ferriol, et al. 2003). This molecular technique is based on a two-primer amplification (forward and reverse primers). These primers are generally 17 or 18 nucleotides long.

The SRAP marker system is a simple and efficient technique. They have a high ratio of codominance, give multiple bands and allow easy isolation for sequencing (Li, et al. 2001). On the other hand, this method is difficult in view of its complexity and optimization of the DNA digestion, ligation and amplification conditions for each step.

1.4.2.1.4. Sequencing-Based Molecular Marker System

Other types of DNA marker systems are based on sequencing. Single nucleotide polymorphisms (SNPs) can be given as an example of these systems.

1.4.2.1.4.1. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) have an extremely high level of polymorphism due to their high frequency of occurrence in the genome. Expressed sequences taq (EST) collections are very good source for SNP discovery (Morales, et al. 2003).

SNPs are generally used for genetic maps, F_1 identification, breeding, gene tagging, alien gene introduction, bulk segregant analysis, diversity studies, novel allele detections, marker-assisted selection, and high resolution mapping (FAO/IAEA 2002). Besides, they can be used to saturate genetic maps and this molecular method is also useful for association mapping of interesting traits (Morales, at al. 2003).

SNPs are highly robust in usage and their polymorphism levels are high. Moreover, they are very suitable for high throughput analysis, occur at high frequency and are easily automated (Morales, et al. 2003). On the other hand, they have very high developmental costs and they require sequence information. In addition, they can be technically challenging and they are less informative for population genetics studies (Morales, at al. 2003, FAO/IAEA 2002).

1.5. Expressed Sequenced Taqs (ESTs) and Unigenes

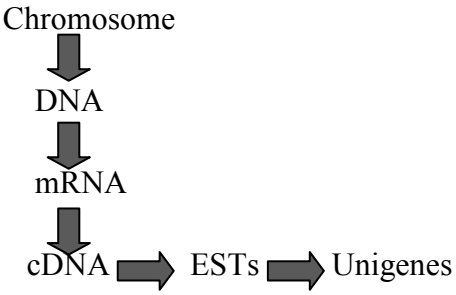
Expressed sequence tags (ESTs) are are small pieces of DNA sequence, generally, 200 to 500 nucleotides long that are generated by sequencing either one or both ends of an expressed gene that may or may not code for a protein (Varshney, et al. 2005, Clarke, et al. 2002, Aggarwal, et al. 2006). The identification of ESTs has proceeded quickly, with approximately 42 million ESTs now available in public databases like GenBank (Boguski, et al. 1993, Varshney, et al. 2005, Aggarwal, et al. 2006).

ESTs are produced by sequencing of cloned messenger RNA (mRNA). These clones are called cDNA (complementary DNA) and are single stranded DNA. cDNA is polymerized by the enzyme reverse transcriptase and it is complementary to mRNA (Varshney, et al. 2005). Two different types of ESTs can be obtained from cDNAs.

- 5’ ESTs
- 3’ ESTs

5' ESTs are obtained from the 5’ end of a transcript that usually codes for a protein. These regions tend to be conserved across species. 3’ ESTs are generated from the 3' end of a transcript, they are likely to fall within non-coding, or untranslated regions (UTRs), and therefore tend to exhibit less cross-species conservation than do coding sequences.

Researchers can map these ESTs to specific chromosome locations using physical mapping techniques, such as radiation hybrid mapping, FISH (Fluorescent In-Situ Hybridization) or several methods. For example, one of the most powerful mapping techniques is the Sequence Tagged Site (STS) mapping technique. An STS is a short DNA sequence that is easily recognizable and occurs only once in a genome or chromosome. The 3' ESTs serve as a common source of STSs because they provide the additional feature of corresponding to an expressed gene.



Unigenes are non redundant sets of gene oriented clusters. Genes could be expressed several times as mRNA. ESTs derived from this mRNAs may be redundant, thus, there may be several of the same or similar copies of the same EST in the database. Researchers search these ESTs and determine which are likely to correspond to the same gene. There are software programs for identifying unigenes available on the internet. One example is UniGene database from NCBI.

1.6. An Overview of Antioxidants

Reactive oxygen species are oxidants, compounds with a tendency to donate oxygen to other substances. Reactive oxygen species are free radicals, chemical species that have one or more unpaired electrons. Many free radicals are unstable and highly reactive. Some of the free radicals and other important oxidants found in living organisms are shown in Table 1.6. Several internal and external factors can trigger the production of free radicals. Internal factors that result in free radical production are xanthine oxidase, reactions involving iron and other transition metals, arachidonate

pathways, peroxisomes, inflammation, ischæmia/reperfusion. External factors that can generate free radicals include cigarette smoke, UV light, radiation, drugs, environmental pollutants and industrial solvents (Langseth, et al. 1995, Valko, et al. 2005).

Table 1.6. Some important reactive oxygen species in living organisms
(Source: Langseth, et al. 1995)

Free Radicals	**Nonradicals**
Hydroxyl radical (OH)	Hydrogen peroxide (H_2O_2)
Superoxide radical (O_2)	Singlet oxygen (O_2)
Nitric oxide radical (NO)	Hypochlorous acid (HOCl)
Lipid peroxyl radical (LOO)	Ozone (O_3)

Free radicals can cause damage because they can attack and damage proteins, lipids, nucleic acids, etc. They cause several types of cancer, artherosclerosis (from LDL damage), cell injury, cataracts, some neurological disorders, immunodeficiencies, heart disease and cell aging in animals (Langseth, et al. 1995). In addition, they cause membrane leakage, senescence, chlorophyll destruction, decreased photosynthesis and, as a result, decreased yield in plants. Antioxidants can interact with and stabilize free radicals and prevent free radical damage by donating H_2 or electrons to free radicals or reducing the rate of autoxidation of lipids. Antioxidants can be divided into two common groups. These are antioxidant enzymes and vitamins and phytochemicals.

1.6.1. Antioxidant Enzymes

Living cells are protected against oxidative stress via an interacting network of antioxidant enzymes. Although there are several antioxidant enzymes, there are three of them that are commonly known. One of the most known antioxidant enzymes is SOD (superoxide dismutase).The enzyme SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. It is one of the most important antioxidant defenses in nearly all cells exposed to oxygen (Langseth, et al. 1995, Valko, et al. 2005). The other is catalase. The enzyme catalase is a common enzyme found in living organisms. Its functions consist of catalyzing the decomposition of hydrogen peroxide to water and oxygen. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups which allow the enzyme to react with hydrogen peroxide (Langseth, et al. 1995, Valko, et al. 2005). Another antioxidant enzyme is gluthatione peroxidase. Glutathione peroxidase is the common name of an

enzyme family with peroxidase activity whose general biological role is to protect the organism from oxidative damage. The function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Langseth, et al. 1995, Valko, et al. 2005).

1.6.2. Vitamins and Phytochemicals

Vitamins and phytochemicals can be categorized in two groups: water-soluble and lipid-soluble antioxidants.

1.6.2.1. Water-Soluble Antioxidants

Ascorbic acid (vitamin C) is a water-soluble vitamin present in citrus fruits and juices, green peppers, cabbage, spinach, broccoli, kale, cantaloupe, kiwi, and strawberries. It is an electron donor and it donates hydrogen to lipid radicals (Langseth, et al. 1995, Valko, et al. 2005). Another important class of water-soluble antioxidants is phenolic compounds. Phenolic compounds is the largest category of phytochemicals. They are highly effective hydrogen donors. Phenolic compounds break oxidation chain reactions and flavonoids are the most important ones (Langseth 1995).

1.6.2.2. Lipid-Soluble Antioxidants

Vitamin E is d-alpha tocopherol, a lipid-soluble vitamin present in nuts, seeds, vegetable and fish oils, whole grains (especially, wheat germ), fortified cereals, and apricots. It is a hydrogen donor and the most efficient chain-breaking antioxidant. It prevents lipid peroxidation and LDL oxidation (Langseth, 1995 ; Valko, 2005). Another lipid-soluble antioxidant is carotenoids. Carotenoids are pigments that are located in plants and microorganisms. According to some surveys, carotenoids prevent or inhibit certain types of cancer, artherosclerosis, age-related muscular degeneration, and other diseases (Valko, 2005). β-carotene is a precursor to vitamin A (retinol) and a kind of carotenoid. It is present in liver, egg yolk, milk, butter, spinach, carrots, squash, broccoli, yams, tomato, cantaloupe, peaches, and grains. Because beta-carotene is converted to vitamin A by the body there is no set requirement for β-carotene. It is a lipid-soluble antioxidant and inhibits lipid peroxidation and oxidation of LDL (Langseth 1995, Palace 1998, Valko, 2005).

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

The 238 national melon accessions for this study were obtained from Aegean Agricultural Research Institute, Menemen, İzmir. A pedigree number was given to each accession in order to differentiate them from each other. The location of collection of these melon accessions in Turkey was also known. Other members of the *Cucurbitace* family such as *Luffa cylindirica*, *Luffa sicercia*, *Cucumis maxima*, *Momordica charantis*, *Cucumis pepo*, *Cucumis moschata*, *Cucumis melo* subsp. *flexuosus, Cucurbita pepo* convar. *turbaniformis* and *Cucurbita maxima* from Çukurova University were used as outgroups in order to compare them with national melon accessions and they were shown on Figure 2.1. A table of all Turkish melon accessions studied, is shown in Table 2.1. In this table, pedigree number, accession name and source of each Turkish melon accession is shown. Besides, 43 melon accessions from Yuksel Seeds Ltd.Co. were used for biochemical analysis.

Ten seeds of each accession were planted in torf, perlite and natural fertilizer mixture in seedling plates and germinated under optimal conditions for melon (20-24 ^{o}C, 16 h photoperiod, approximately 300 μmols.m^{2}s^{-1}) in a greenhouse at the İzmir Institute of Technology, Urla, İzmir, Türkiye in March-May 2007 for molecular characterization. In addition, the same seeds were planted in greenhouses of AARI (Aegean Agriculture Research Institute, Menemen, İzmir) for morphological characterization. After fruit development, heterogeneity within some accessions was apparent and for this reason, all heterogenous accessions were self pollinated and given new pedigree numbers.

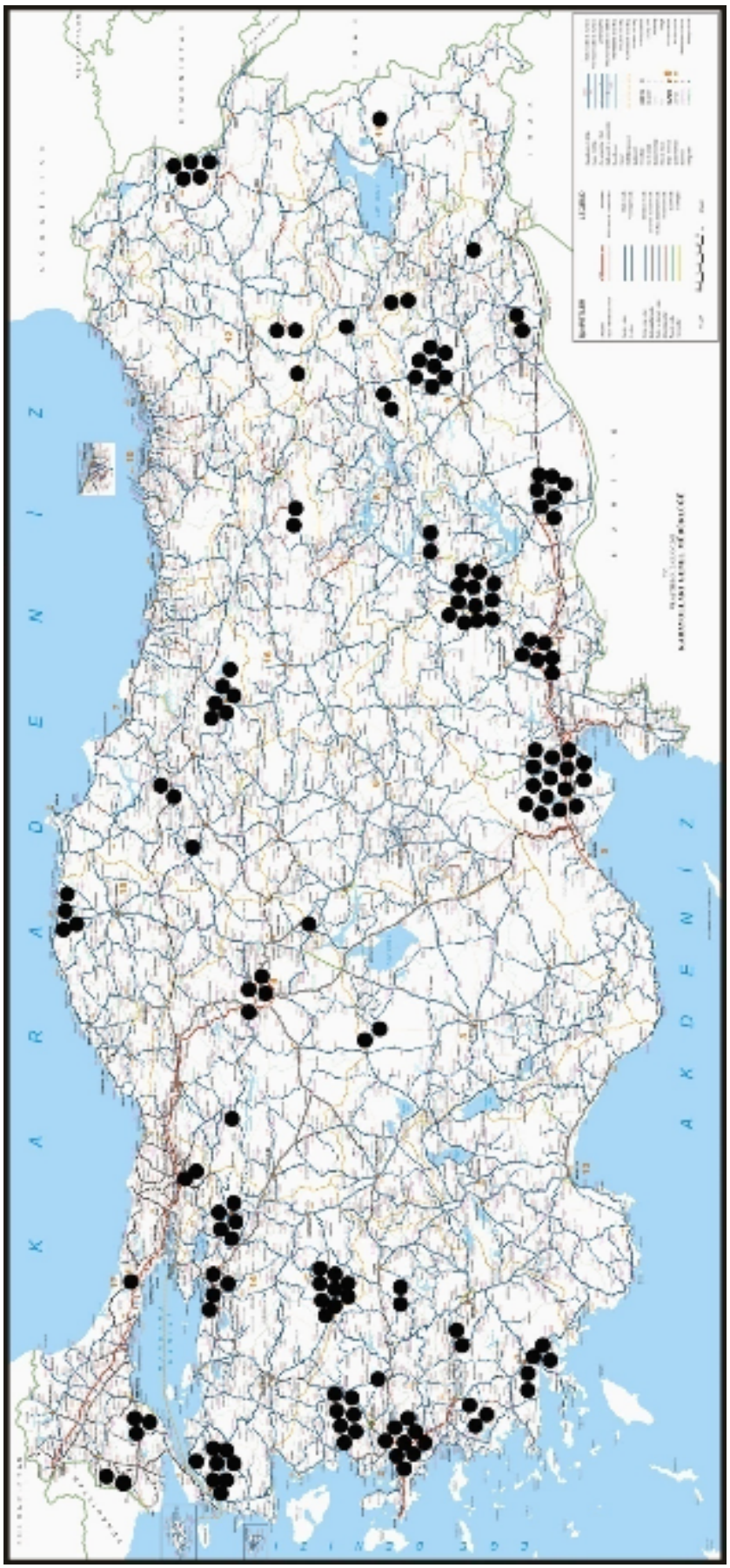


Figure 2.1. Location of the national melon accessions on Turkey map.

Table 2.1. Table of Turkish melon accessions from AARI and outgroups from Çukurova University with pedigree number, accession name, source name and collection location.

Pedigree Number	Accession Name	Source	Location
TR 33304	Cucumis melo sp melo L.	AARI	Edirne
TR 38116	Cucumis melo sp melo L.	AARI	Balıkesir
TR 38125	Cucumis melo sp melo L.	AARI	Balıkesir
TR 38479	Cucumis melo sp melo L.	AARI	Tekirdağ
TR 31586	Cucumis melo sp melo L.	AARI	Diyarbakır
TR 31588	Cucumis melo sp melo L.	AARI	Diyabakır
TR 31589	Cucumis melo sp melo L.	AARI	Diyabakır
TR 26762	Cucumis melo sp melo L.	AARI	Bilecik
TR 40365	Cucumis melo sp melo L.	AARI	Mardin
TR 40534	Cucumis melo sp melo L.	AARI	Elazığ
TR 45791	Cucumis melo sp melo L.	AARI	Erzurum
TR 45883	Cucumis melo sp melo L.	AARI	Kars
TR 46438	Cucumis melo sp melo L.	AARI	Tunceli
TR 46489	Cucumis melo sp melo L.	AARI	Sivas
TR 47776	Cucumis melo sp melo L.	AARI	Gaziantep
TR 47783	Cucumis melo sp melo L.	AARI	Gaziantep
TR 47793	Cucumis melo sp melo L.	AARI	Gaziantep
TR 47804	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47805	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47812	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47813	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47833	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47874	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 48527	Cucumis melo sp melo L.	AARI	Amasya
TR 48566	Cucumis melo sp melo L.	AARI	Tokat
TR 48567	Cucumis melo sp melo L.	AARI	Tokat
TR 48568	Cucumis melo sp melo L.	AARI	Tokat
TR 48671	Cucumis melo sp melo L.	AARI	Adıyaman
TR 26195	Cucumis melo sp melo L.	AARI	Konya
TR 31534	Cucumis melo sp melo L.	AARI	Amasya
TR 33380	Cucumis melo sp melo L.	AARI	Tekirdağ
TR 40280	Cucumis melo sp melo L.	AARI	Gaziantep
TR 40284	Cucumis melo sp melo L.	AARI	Gaziantep
TR 40514	Cucumis melo sp melo L.	AARI	Bingöl
TR 40530	Cucumis melo sp melo L.	AARI	Elazığ
TR 40559	Cucumis melo sp melo L.	AARI	Malatya
TR 40563	Cucumis melo sp melo L.	AARI	Malatya
TR 43744	Cucumis melo sp melo L.	AARI	Bursa
TR 47797	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47825	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47846	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47861	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 47884	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 47885	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 50719	Cucumis melo sp melo L.	AARI	Kars

(cont. on next page)

Table 2.1 (cont.) Table of Turkish melon accessions from AARI and outgroups from Çukurova University with pedigree number, accession name. source name and collection location.

Pedigree Number	Accession Name	Source	Location
TR 50728	Cucumis melo sp melo L.	AARI	Kars
TR 50747	Cucumis melo sp melo L.	AARI	Erzurum
TR 51531	Cucumis melo sp melo L.	AARI	Kastamonu
TR 51550	Cucumis melo sp melo L.	AARI	Kastamonu
TR 51561	Cucumis melo sp melo L.	AARI	Kastamonu
TR 51608	Cucumis melo sp melo L.	AARI	Kastamonu
TR 51676	Cucumis melo sp melo L.	AARI	Tokat
TR 51763	Cucumis melo sp melo L.	AARI	Sivas
TR 49882	Cucumis melo sp melo L.	AARI	Ankara
TR 38224	Cucumis melo sp melo L.	AARI	Edirne
TR 38484	Cucumis melo sp melo L.	AARI	Çanakkale
TR 39655	Cucumis melo sp melo L.	AARI	Kars
TR 39683	Cucumis melo sp melo L.	AARI	Siirt
TR 15776	Cucumis melo sp melo L.	AARI	Ankara
TR 40345	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 40350	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 40380	Cucumis melo sp melo L.	AARI	Diyarbakır
TR 40382	Cucumis melo sp melo L.	AARI	Diyarbakır
TR 40384	Cucumis melo sp melo L.	AARI	Diyarbakır
TR 40503	Cucumis melo sp melo L.	AARI	Van
TR 43015	Cucumis melo sp melo L.	AARI	Çanakkale
TR 43023	Cucumis melo sp melo L.	AARI	Çanakkale
TR 43105	Cucumis melo sp melo L.	AARI	Çanakkale
TR 43722	Cucumis melo sp melo L.	AARI	Bursa
TR 43749	Cucumis melo sp melo L.	AARI	Balıkesir
TR 45896	Cucumis melo sp melo L.	AARI	Kars
TR 46437	Cucumis melo sp melo L.	AARI	Erzincan
TR 47845	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47867	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 49591	Cucumis melo sp melo L.	AARI	İzmir
TR 35299	Cucumis melo sp melo L.	AARI	Mardin
TR 37394	Cucumis melo sp melo L.	AARI	Çorum
TR 40379	Cucumis melo sp melo L.	AARI	Diyarbakır
TR 43024	Cucumis melo sp melo L.	AARI	Çanakkale
TR 43041	Cucumis melo sp melo L.	AARI	Çanakkale
TR 43135	Cucumis melo sp melo L.	AARI	Çanakkale
TR 43263	Cucumis melo sp melo L.	AARI	İstanbul
TR 43265	Cucumis melo sp melo L.	AARI	Tekirdağ
TR 43746	Cucumis melo sp melo L.	AARI	Balıkesir
TR 43835	Cucumis melo sp melo L.	AARI	Balıkesir
TR 46503	Cucumis melo sp melo L.	AARI	Tokat
TR 47811	Cucumis melo sp melo L.	AARI	Adıyaman
TR 47822	Cucumis melo sp melo L.	AARI	Adıyaman
TR 48541	Cucumis melo sp melo L.	AARI	Tokat
TR 48611	Cucumis melo sp melo L.	AARI	Gaziantep

(cont. on next page)

Table 2.1 (cont.) Table of Turkish melon accessions from AARI and outgroups from Çukurova University with pedigree number, accession name, source name and collection location.

Pedigree Number	Accession Name	Source	Location
TR 48650	Cucumis melo sp melo L.	AARI	Şanlıurfa
TR 49583	Cucumis melo sp melo L.	AARI	İzmir
TR 57778	Cucumis melo sp melo L.	AARI	İzmir
TR 57780	Cucumis melo sp melo L.	AARI	İzmir
TR 57781	Cucumis melo sp melo L.	AARI	İzmir
TR 57782	Cucumis melo sp melo L.	AARI	İzmir
TR 57783	Cucumis melo sp melo L.	AARI	İzmir
TR 61566	Cucumis melo sp melo L.	AARI	Aydın
TR 61573	Cucumis melo sp melo L.	AARI	Aydın
TR 61626	Cucumis melo sp melo L.	AARI	Muğla
TR 61627	Cucumis melo sp melo L.	AARI	Muğla
TR 61659	Cucumis melo sp melo L.	AARI	Muğla
TR 61714	Cucumis melo sp melo L.	AARI	Muğla
TR 61812	Cucumis melo sp melo L.	AARI	Denizli
TR 61851	Cucumis melo sp melo L.	AARI	Denizli
TR 62023	Cucumis melo sp melo L.	AARI	İzmir
TR 62060	Cucumis melo sp melo L.	AARI	İzmir
TR 62474	Cucumis melo sp melo L.	AARI	Çanakkale
TR 66755	Cucumis melo sp melo L.	AARI	Sakarya
TR 68913	Cucumis melo sp melo L.	AARI	Konya
TR 68934	Cucumis melo sp melo L.	AARI	Manisa
TR 68940	Cucumis melo sp melo L.	AARI	Kütahya
TR 69022	Cucumis melo sp melo L.	AARI	Eskişehir
TR 69425	Cucumis melo sp melo L.	AARI	Ankara
TR 69428	Cucumis melo sp melo L.	AARI	Ankara
TR 69689	Cucumis melo sp melo L.	AARI	Balıkesir
TR 69895	Cucumis melo sp melo L.	AARI	Balıkesir
TR 71500	Cucumis melo sp melo L.	AARI	Tunceli
TR 71540	Cucumis melo sp melo L.	AARI	Kütahya
TR 71541	Cucumis melo sp melo L.	AARI	Kütahya
TR 71568	Cucumis melo sp melo L.	AARI	Kütahya
TR 71571	Cucumis melo sp melo L.	AARI	Kütahya
TR 71616	Cucumis melo sp melo L.	AARI	Bilecik
TR 64142	Cucumis melo sp melo L.	AARI	Kütahya
TR 64154	Cucumis melo sp melo L.	AARI	Bilecik
TR 64163	Cucumis melo sp melo L.	AARI	Bilecik
TR 66004	Cucumis melo sp melo L.	AARI	Kütahya
TR 66008	Cucumis melo sp melo L.	AARI	Kütahya
TR 66005	Cucumis melo sp melo L.	AARI	Kütahya
TR 66007	Cucumis melo sp melo L.	AARI	Bilecik
TR 66003	Cucumis melo sp melo L.	AARI	Bursa
TR 66002	Cucumis melo sp melo L.	AARI	Bursa
TR 61543	Cucumis melo sp melo L.	AARI	Aydın
TR 63230	Cucumis melo sp melo L.	AARI	Muğla
TR 66366	Cucumis melo sp melo L.	AARI	Uşak

(cont. on next page)

Table 2.1 (cont.) Table of Turkish melon accessions from AARI and outgroups from Çukurova University with pedigree number, accession name, source name and collection location.

Pedigree Number	Accession Name	Source	Location
TR 66362	Cucumis melo sp melo L.	AARI	Uşak
TR 69873	Cucumis melo sp melo L.	AARI	Kırşehir
06T773	Cucumis moschata	Çukurova University	Adana
06T774	Cucumis moschata	Çukurova University	Adana
06T775	Cucumis melo subsp. Flexuosus	Çukurova University	Adana
06T776	Cucumis melo subsp. Flexuosus	Çukurova University	Adana
06T777	Cucumis melo subsp. Flexuosus	Çukurova University	Adana
06T778	Luffa cylindirica	Çukurova University	Adana
06T779	Luffa sicercia	Çukurova University	Adana
06T780	Luffa sicercia	Çukurova University	Adana
06T781	Cucumis melo subsp. melo	Çukurova University	Adana
06T782	Cucumis melo subsp. melo	Çukurova University	Adana
06T783	Cucumis maxima	Çukurova University	Adana
06T784	Momordica charantis	Çukurova University	Adana
06T785	Cucumis pepo	Çukurova University	Adana
06T786	Cucurbita pepo convar. turbaniformis	Çukurova University	Adana
06T787	Cucurbita maxima	Çukurova University	Adana

2.2. Methods

2.2.1. DNA Extraction

Genomic DNA was extracted from fresh leaf tissue of each seedling at the 2-4 leaf stage with normal CTAB-DNA extraction protocol modified according to Fulton et al. (1995) and Promega Wizard Genomic DNA purification kit. The DNA was quantified on a Nanodrop ND-1000 spectrophotometer following the manufacturer's protocol. Nanodrop results (DNA concentrations (ng/µl), Abs 260/280 (nm) and Abs 260/230 (nm)) of all melon accessions are shown in Table 2.2. All genomic DNAs were stored in -20 ^{0}C temperature in TE buffer.

Table 2.2. Quantitation and quality check of melon accession DNA.

Accession Number	DNA Concentration (ng/ µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TR 15776-2	144.58	1.84	2.30
TR 15776-7	222.32	1.93	2.29
TR 2092-1	99.22	1.81	1.80
TR 2096-3	135.25	1.89	2.09
TR 2096-6	130.19	1.92	2.25
TR 26195-4	167.97	1.81	2.12
TR 26762-2	341.32	1.83	2.09
TR 31534-1	98.52	1.78	2.32
TR 31586-10	86.10	1.77	2.21
TR 31586-7	221.53	1.84	2.11
TR 31586-1	176.45	1.86	2.15
TR 31586-9	283.59	1.83	2.01
TR 31588-10	142.47	1.90	2.21
TR 31588-5	94.01	1.83	1.91
TR 31588-7	157.05	1.66	0.80
TR 31589-3	130.27	1.86	1.92
TR 31589-5	53.33	1.76	2.80
TR 31589-9	309.69	1.86	2.49
TR 33304-1	242.57	1.85	2.38
TR 33304-2	269.61	1.85	2.11
TR 33304-4	206.82	1.85	2.25
TR 33304-7	117.13	1.82	2.08
TR 33304-8	81.27	1.81	2.29
TR 33380-1	193.76	1.83	2.11
TR 33380-2	102.82	1.82	2.36
TR 35299-3	198.89	1.86	2.29
TR 35299-9	172.99	1.83	2.31
TR 37394-3	212.22	1.79	1.93
TR 37394-6	182.64	1.85	2.07
TR 38116-2	85.03	1.86	2.60
TR 38116-3	142.52	1.78	2.34
TR 38116-4	334.35	1.84	2.08
TR 38125-4	162.72	1.81	1.87
TR 38125-9	192.58	1.84	1.83
TR 38224-5	171.99	1.84	2.10
TR 38479-1	109.85	1.92	2.35
TR 38479-4	105.29	1.75	2.10
TR 38484-2	99.30	1.79	2.02
TR 38484-4	137.93	1.82	1.95
TR 38484-7	111.22	1.80	2.10
TR 39655-1	138.56	1.82	2.18
TR 39655-3	151.94	1.82	2.47
TR 39655-6	143.33	1.82	2.16
TR 39655-7	215.26	1.81	2.05
TR 39683-9	94.32	1.81	2.11
TR 40280-1	213.71	2.00	2.39

(cont. on next page)

Table 2.2 (cont.) Quantitation and quality check of melon accession DNA.

Accession Number	DNA Concentration (ng/ µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TR 40284-1	82.30	1.76	1.55
TR 40284-6	129.89	1.77	1.80
TR 40284-8	217.34	1.83	2.30
TR 40345-10	302.56	1.85	2.09
TR 40345-5	162.33	1.81	1.93
TR 40345-7	415.96	1.93	2.30
TR 40350-1	104.20	1.85	2.30
TR 40365-2	268.12	1.85	2.06
TR 40365-4	207.73	1.82	2.25
TR 40379-7	318.64	1.82	1.93
TR 40380-1	297.94	1.90	2.16
TR 40382-10	325.06	1.85	1.90
TR 40382-6	139.03	1.83	2.11
TR 40384-4	257.44	1.84	2.12
TR 40384-8	145.61	1.84	2.08
TR 40503-3	170.39	1.85	2.14
TR 40503-8	187.02	1.83	2.13
TR 40514-3	181.08	1.81	1.84
TR 40530-2	139.05	1.81	2.03
TR 40530-8	84.93	1.77	1.90
TR 40534-4	229.84	1.82	1.88
TR 40559-4	165.13	1.79	1.66
TR 40559-8	185.10	1.92	2.16
TR 40563-2	177.62	1.81	1.81
TR 40563-8	142.34	1.78	1.73
TR 43015-6	216.81	1.85	2.12
TR 43015-9	126.06	1.85	2.42
TR 43023-4	295.22	1.86	2.24
TR 43023-7	238.34	1.85	2.10
TR 43023-8	211.36	1.86	2.22
TR 43024-10	189.10	1.82	2.18
TR 43024-7	382.81	1.86	2.21
TR 43041-1	227.42	1.85	2.33
TR 43041-2	193.82	1.78	1.92
TR 43041-4	143.81	1.85	2.24
TR 43105-3	152.69	1.82	2.29
TR 43105-6	156.04	1.80	2.14
TR 43105-8	127.84	1.75	1.62
TR 43135-10	167.94	1.97	2.49
TR 43135-2	154.57	1.84	2.32
TR 43135-9	223.49	1.83	2.02
TR 43263-9	62.26	1.75	1.76
TR 43265-5	134.91	1.86	2.20
TR 43722-6	238.46	1.86	2.02
TR 43744-8	153.75	1.81	1.96
TR 43746-9	142.95	1.84	2.49
TR 43749-1	234.49	1.90	2.09

(cont. on next page)

Table 2.2 (cont.) Quantitation and quality check of melon accession DNA.

Accession Number	DNA Concentration (ng/ µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TR 43835-2	85.59	1.81	2.38
TR 43835-3	215.74	1.86	2.16
TR 45791-3	150.86	1.90	2.01
TR 45791-5	100.81	1.91	2.22
TR 45883-2	276.03	1.84	2.04
TR 45883-6	281.69	1.84	2.08
TR 45883-8	70.20	1.69	2.09
TR 45896-1	248.65	1.84	2.03
TR 45896-4	129.16	1.84	2.16
TR 45896-5	1188.20	2.10	2.27
TR 45896-7	204.42	1.85	2.23
TR 46437-2	192.63	1.91	2.19
TR 46437-4	136.34	1.80	1.88
TR 46437-5	130.50	1.81	1.98
TR 46437-8	143.52	1.78	2.02
TR 46438-9	98.62	1.86	2.02
TR 46489-1	118.61	1.89	2.35
TR 46489-2	98.17	1.82	2.29
TR 46489-5	99.96	1.87	1.99
TR 46489-9	64.98	1.75	2.95
TR 46503-2	272.61	1.90	2.25
TR 46503-4	337.43	1.86	2.14
TR 46503-5	212.74	1.85	2.08
TR 47776-5	216.69	1.81	2.06
TR 47776-9	164.86	1.79	2.38
TR 47783-1	172.71	1.77	2.26
TR 47783-4	114.02	1.89	2.29
TR 47793-1	268.23	1.85	2.25
TR 47797-1	149.86	1.81	1.75
TR 47797-6	140.93	1.80	1.63
TR 47797-9	173.10	1.81	1.88
TR 47804-3	178.45	1.84	2.19
TR 47805-8	346.26	1.84	2.09
TR 47805-9	184.02	1.83	2.00
TR 47811-2	107.13	1.83	2.40
TR 47811-4	113.55	1.80	1.94
TR 47812-3	186.19	1.90	2.17
TR 47812-5	301.54	1.85	2.30
TR 47813-2	168.89	1.89	2.08
TR 47813-7	149.25	1.87	2.08
TR 47822-3	70.20	1.79	1.95
TR 47822-5	195.64	1.83	1.99
TR 47822-6	119.43	1.82	1.92
TR 47825-2	183.14	1.80	1.84
TR 47825-5	185.14	1.81	1.93
TR 47825-7	190.05	1.81	1.87
TR 47825-8	100.75	1.78	1.81

(cont. on next page)

Table 2.2 (cont.) Quantitation and quality check of melon accession DNA.

Accession Number	DNA Concentration (ng/ µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TR 47825-9	235.00	1.82	1.89
TR 47833-5	171.90	1.81	2.09
TR 47833-9	206.24	1.82	1.94
TR 47845-2	178.28	1.81	1.96
TR 47845-5	81.64	1.82	1.80
TR 47845-7	203.72	1.84	1.96
TR 47846-1	212.89	1.82	1.78
TR 47846-4	316.19	1.82	1.85
TR 47861-4	195.66	1.81	2.03
TR 47861-7	267.83	1.86	1.76
TR 47867-1	343.30	1.85	2.04
TR 47867-4	243.40	1.82	1.99
TR 47867-5	236.48	1.82	2.04
TR 47867-7	212.10	1.81	2.10
TR 47874-4	213.22	1.82	1.73
TR 47874-6	224.25	1.82	2.26
TR 47874-8	144.72	1.86	1.81
TR 47884-6	165.44	1.78	1.82
TR 47884-7	163.11	1.79	1.81
TR 47885-2	278.47	1.82	1.94
TR 47885-6	158.74	1.82	1.93
TR 48527-2	95.13	1.73	1.85
TR 48527-5	91.57	1.83	2.10
TR 48541-1	159.06	1.78	1.85
TR 48541-3	129.03	1.77	1.63
TR 48566-5	94.04	1.83	2.20
TR 48567-1	129.73	1.80	2.09
TR 48611-3	206.16	1.97	2.13
TR 48650-10	109.04	1.87	2.15
TR 48650-2	92.35	1.84	1.99
TR 48650-7	81.55	1.90	1.91
TR 48671-5	192.98	1.80	1.94
TR 48671-6	107.24	1.79	2.21
TR 48671-7	231.64	1.83	2.09
TR 48671-8	213.84	1.82	2.04
TR 49583-1	148.49	1.90	2.36
TR 49583-3	106.11	1.87	2.06
TR 49591-10	538.61	2.05	2.26
TR 49591-3	167.85	1.85	2.25
TR 49882-2	80.97	1.78	2.03
TR 50719-2	141.98	1.79	2.02
TR 50719-5	159.70	1.77	1.93
TR 50728-7	204.56	1.82	1.99
TR 50747-3	120.40	1.81	2.11
TR 50747-4	142.90	1.82	2.11
TR 50747-6	141.11	1.84	2.22
TR 50747-8	292.32	1.93	2.31

(cont. on next page)

Table 2.2 (cont.) Quantitation and quality check of melon accession DNA.

Accession Number	DNA Concentration (ng/ µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TR 51531-3	212.33	1.82	2.10
TR 51531-5	134.25	1.96	2.68
TR 51531-9	170.81	2.02	2.55
TR 51550-2	165.15	1.82	2.27
TR 51550-3	128.09	1.79	2.28
TR 51550-5	197.10	1.84	2.17
TR 51561-2	235.82	1.82	1.90
TR 51561-3	262.70	1.84	2.10
TR 51608-2	296.16	1.85	2.16
TR 51608-6	88.17	1.77	1.97
TR 51676-2	86.41	1.80	1.98
TR 51676-7	110.11	1.81	2.05
TR 51763-1	96.78	1.79	1.84
TR 51763-2	146.97	1.84	2.02
TR 51763-9	79.45	1.78	2.02
TR 57778-7	141.87	1.90	2.13
TR 57780-2	124.89	1.88	1.94
TR 57781-3	112.02	1.96	2.54
TR 57782-4	84.38	1.84	2.11
TR 57783-7	113.48	1.88	1.96
TR 61543-6	104.43	1.91	2.20
TR 61566-5	86.82	1.95	2.09
TR 61573-3	125.84	1.86	2.22
TR 61626-5	103.35	1.88	2.01
TR 61627-10	91.17	1.86	2.04
TR 61627-3	104.59	1.91	2.03
TR 61659-1	102.79	1.88	2.03
TR 61659-2	51.11	1.72	1.97
TR 61714-4	71.74	1.79	2.01
TR 61714-8	101.32	1.85	2.57
TR 61812-3	73.58	1.85	2.07
TR 61851-2	95.44	1.89	2.03
TR 61851-6	124.49	1.87	2.05
TR 62023-2	108.41	1.91	2.27
TR 62060-10	51.97	1.82	2.02
TR 62474-3	134.15	1.84	2.05
TR 63230-5	72.61	1.76	2.03
TR 64142-2	99.84	1.85	2.35
TR 64142-5	159.02	1.88	2.13
TR 64154-2	83.17	1.88	2.32
TR 64154-7	55.12	1.80	2.00
TR 64163-6	73.00	1.76	1.61
TR 66002-4	111.39	1.91	2.69
TR 66003-2	140.97	1.92	2.64
TR 66003-4	115.31	1.97	2.57
TR 66004-10	47.97	1.71	3.39

(cont. on next page)

Table 2.2 (cont.) Quantitation and quality check of melon accession DNA.

Accession Number	DNA Concentration (ng/ µl)	Abs 260/280 (nm)	Abs 260/230 (nm)
TR 66004-8	62.87	1.82	2.03
TR 66005-1	62.89	1.86	2.15
TR 66005-3	54.76	1.80	1.81
TR 66005-8	84.73	1.85	3.86
TR 66007-7	157.62	1.94	2.70
TR 66008-10	92.43	1.82	1.87
TR 66008-8	56.58	1.74	3.86
TR 66362-9	105.76	1.85	2.04
TR 66366-1	81.90	1.85	2.30
TR 66366-2	117.64	1.90	2.11
TR 66755-3	103.41	1.84	2.04
TR 68913-2	206.32	1.96	2.24
TR 68913-3	66.09	1.85	2.11
TR 68934-5	126.21	1.87	2.14
TR 68934-7	54.90	1.86	2.37
TR 68940-10	105.53	1.86	1.99
TR 68940-9	92.25	1.86	2.05
TR 69022-1	37.62	1.84	1.88
TR 69425-1	60.03	1.72	1.88
TR 69425-4	61.85	1.81	3.18
TR 69425-8	101.44	1.81	2.09
TR 69428-1	53.69	1.82	2.54
TR 69428-2	101.49	1.85	2.14
TR 69689-9	98.55	1.91	2.62
TR 69873-7	116.74	1.86	2.33
TR 69873-9	150.11	1.89	2.03
TR 69895-1	53.63	1.89	2.45
TR 71500-5	69.61	1.85	2.17
TR 71540-4	66.94	1.85	2.08
TR 71540-5	77.44	1.94	2.36
TR 71541-1	88.00	1.80	2.23
TR 71541-4	103.77	1.90	1.97
TR 71568-2	49.82	1.89	2.86
TR 71571-1	101.78	1.79	1.98
TR 71571-3	100.79	1.82	1.90
TR 71616-1	92.02	1.81	2.00

2.2.2. Morphological Characterization

Nineteen criteria such as fruit shape, leaf colour, predominant fruit skin color, fruit length, etc. were used for determination of the morphological traits of 138 melon accessions from Aegean Agricultural Research Institute, Menemen, İzmir. Descriptors for Melon IPGRI (International Plant Genetic Resources Institute), 2003 was used for

morphological characterization. These 19 traits are shown in Table 2.3. Morphological criteria with IPGRI codes and number of samples shown in Appendix B.

Table 2.3. International Plant Genetic Resources Institute's Code Values for melons (Source: IPGRI, 2003)

Trait Number	IPGRI Code Value	Morphological Trait
01	7.1.8	Seedling Vigour
02	7.5.1	Leaf Shape
03	7.5.14	Leaf Color
04	7.6.1	Sex Type
05	7.6.14	Flower Colour
06	7.7.1	Fruit Shape
07	7.7.10	Predominant Fruit Skin Color
08	7.7.11	Secondary Fruit Skin Color
09	7.7.15	Secondary Skin Color Pattern
10	7.7.16	Fruit Surface
11	7.7.40	Main Colour of Flesh
12	7.7.43	Flesh Texture
13	7.7.45	Flesh Flavour
14	7.7.50	Flesh Thickness
15	7.7.50	Flesh Bitterness
16	7.8.1	Seed Size
17	8.1.8	Fruit Length
18	8.1.9	Fruit Width
19	8.1.10	Fruit Weight

2.2.3. SSR Marker Design Based on Unigenes

A total of 3522 melon unigenes were downloaded from the website of Cornell University melon EST database. All of these unigenes were used for determination of SSR primers on the website of PBC Public SSR Primer Discovery Input site (http://hornbill.cspp.latrobe.edu.au/cgi-binpub/ssrprimer/indexssr.pl). All unigenes were scanned in this software program and 428 SSR primers were found from these 3250 melon unigenes. When scanning these unigenes there were some criteria such as primer size (Min: 18-Max: 22), primer melting temperature Tm (Min: 50-Max:70), primer GC content % (Min: 50-Max:70), primer max complementarity (=8), and primer max 3' complementarity (=3) that were used. After finding SSR primers, the best 20 primers were chosen based on their repeat number (length), left TM, right TM and product size. These primers and associated SSRs are shown in Table 2.4.

All SSR markers designed from the Cornell University melon EST database are shown in Appendix A. with sequence ID, repeat type, repeat number, primer start base, primer end base, primer length, left primer melting temperature, right primer melting temperature and product size.

In addition, 15 published primers were chosen from Gonzalo et al.(2005). Finally, M13(-21) fluorescent labeled tail was added to both EST-designed and published SSR markers for sequencing and ordered from Integrated DNA Technologies, Inc. (Coralville, Iowa, USA).

2.2.4. SSR Analysis

PCR amplifications were carried out using four SSR markers (MU118, FR14G19, SSH6I23 and PH8C1) from melon EST libraries with fluorescent dye-labeled M13(-21) tail (5'-TGT AAA ACG ACG GCC AGT- 3) (Schuelke 2000, Bandelj et al. 2004) and 8 published SSR markers (CMCTN-5, CMCTN-86, CMGAN-25, CMCTN-35, CMAGN-68, CMGAN-80, TJ-10, TJ-27). The PCR mixture contained 0.75 µl of each reverse and FAM-labeled M13(-21) primer and 0.75 µl forward primer in a final 20 µl reaction volume with 2 µl 10X PCR buffer, 0.4 µl dNTPs, 0.4 µl AmpliTaq DNA polymerase, 13.95 µl sdH_2O and 50-100 ng template DNA. The amplification conditions were divided into two steps. The first step was related to the normal SSR protocol and the second step was related to the M13(-21) fluorescent labeled tail. This protocol was: 5 min initial denaturation at 94 ^oC, then 30 cycles of 30 sec of denaturing at 94 ^oC, 45 sec at 56 ^oC for annealing, and extension at 72 ^oC for 45 sec, followed by 8 cycles: 30 sec of heating at 94 ^oC, 45 sec for annealing of M13 flourescent labeled tail at 53 ^oC, 45 sec for extension at 72 ^oC and final extension at 72 ^oC for 10 min (Schuelke, 2000). After amplification, 27 µl SLS (Sample Loading Solution) and 0.5 µl size standard 400 were added to 3 µl PCR product and one drop mineral oil was added.

2.2.5. AFLP Analysis

In the first step, preselective amplification, total genomic melon DNA was double digested with the restriction enzymes *Eco*RI and *Mse*I following the protocol of Vos et al. (1995). According to this protocol, approximately 250 ng genomic DNA was combined with 2 µl EcoRI/MseI enzymes and 13 µl sterile distilled water in 96 well-plate and incubated 2 hours at 37 ^{0}C. After incubation, this mixture was incubated at 70 ^{0}C to inactivate the restriction enzymes. The products of digestion of genomic DNAs were ligated to half-site specific *Eco*RI and *Mse*I adapters with 24 µl adapter ligation solution and 1 µl T_4 DNA ligase and incubated 2 hours at 20 ^{0}C. After incubation, the products were diluted 1:10 (10 µl sample, 90 µl TE buffer) with TE buffer. After incubation the crucial step is preamplification reaction. For this, 40 µl Pre-amp mix, 5 µl 10X PCR buffer, 1 µl Taq DNA polymerase and 5 µl diluted ligated DNA . This PCR protocol was: 20 cycles; denaturing at 94 ^{o}C for 30 sec, 56 ^{o}C for annealing 1 min, and then extension at 72 ^{o}C for 1 min. PCR products were diluted 1:50 (3 µl PCR product, 147 µl TE buffer) with TE buffer.

In the next step, selective amplification, PCR products were amplified with 2µl 10X PCR buffer, 2.5 µl EcoRI primer, 1.5 µl MseI primer, 0.1 µl Taq DNA polymerase and 8.9 µl sdH_2O with touchdown PCR protocol: 2 cycles; 30 sec of denaturing at 94 ^{o}C, 56 ^{o}C for annealing 30 sec, and then extension at 72 ^{o}C for 1 min and 12 cycles; 30 sec for 94 ^{o}C, 30 sec for 65^{o}C . PCR products were diluted with sdH_2O 1:2 (7 µl PCR product and 14 µl sdH_2O) and these samples were diluted 1:10 (3 µl diluted PCR product and 27 SLS (Sample Loading Solution) and 0.5 µl Size Standard 600). Then 45 µl mineral oil was added to prevent evaporation of the products.

Table 2.4. General information about the designed SSR markers with repeat number, length, melting temperatures and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
MU151	trinucleotide	(TGA)4CGA(TGA)4	28	54.987	55.807	366
MU439	dinucleotide	(AT)4TT(AT)6	22	55.024	56.600	307
FR13F20	trinucleotide	(GAT)GAG(GAT)3GAG(GAT)2	38	55.401	54.696	329
PH16E11	tetranucleotide	(CAAA)2CAGA(CAAA)2	43	55.715	55.239	285
FR17O8	trinucleotide	(GGA)5TGAAGA(GGA)7	37	55.881	53.965	284
PH10E11	trinucleotide	(AGA)5C(AGA)2GAAGGA(GAA)3	24	56.119	54.923	137
PH14D12	pentanucleotide	(AAAAG)5	36	53.905	54.728	198
PH17H4	dinucleotide	(TC)18	22	54.961	54.657	357
PH19F12	pentanucleotide	(TTGGA)4	43	55.041	54.761	243
AF030382	dinucleotide	(CT)21	34	55.264	54.416	269
PH4B7	tetranucleotide	(TTTC)5TTAC(TTTC)2	21	55.435	55.037	387
PH8G11	dinucleotide	(CT)10	35	54.994	54.334	246

In the last step, visualization, the products were run on Sequencer CEQ 8800 (Beckman Coulter) and analysed with the fragment analysis software. The process of filtration for melon could be divided 3 steps.

1. Overlaying and Eliminating

    a. Overlaying size standard

    b. Eliminating samples with skewed standards.

Applying the Sample Filter Set: The parameters of the sample filtering set were those shown in Table 9.

Table 2.5. The parameters of sample filtering set in the process in AFLP for Sequencer CEQ 8800.

ID	Parameter Name	Operator	Value
1	Analysis outcome	≠	pass
2	avg current	>	13
3	avg current	<	6
4	low D1 SNR	=	yes
5	low D4 SNR	=	yes
6	number of peaks D4	<	10
7	current change	>	5
8	D4 fragments unsized	=	yes

3. Applying to Fragment Filter Set: The parameters of the fragment filtering set were as described below

The numerical data (present (1) and absent (0)) were obtained via the software program.

Table 2.6. The parameters of fragment filtering set in the process of AFLP in Sequencer SEQ 8800.

ID	Parameter Name	Operator	Value
1	fragment size not est	=	yes
2	peak height (rfu)	<	5000
3	peak height (rfu)	>	80000

In the AFLP process, 10 AFLP primer combinations were chosen from the study of Garcia-Mas et al. as follows; *Mse*I-CTC/*Eco*RI-AAC, *Mse*I-CTC/*Eco*RI-AAG, *Mse*I-

CTC/*Eco*RI-ACA, *Mse*I-CTA/*Eco*RI-ACG, *Mse*I-CTA/*Eco*RI-ACC, *Mse*I-CTA/*Eco*RI-ACT, *Mse*ICAT/*Eco*RI-AAG, *Mse*ICAT/*Eco*RI-ACA, *Mse*ICAC/*Eco*RI-AAC and *Mse*ICAC/*Eco*RI-AAG.

2.2.6. Data Analysis

In order to describe the genetic diversity of the national Turkish melon accessions, AFLP primer combinations and SSR markers data were used for matrix comparisons between both marker systems (Mantel 1967). Total melon genomic DNA fragments were scored as present (1) and absent (0) for both AFLP combinations and SSR markers to get numerical data. The distance matrice and dendrograms were constructed using NTSYS-pc version 2.2 (Numerical Taxonomy Multivariate Analysis System, Exeter Software, Setauket, N.Y.) software program.

The qualitative data were used to compute similarity coefficients using DICE (1945) similarty index. The DICE similarity index calculates the similarity between two samples *i*,*j* with the formula GS(*i*,*j*)=2a(2a+b+c) where GS(*i*,*j*) is the similarity coefficient between samples *i* and *j*, *a* is number of polymorphic bands shared between *i* and *j*, *b* is number of bands present in *i* and absent in *j* and *c* is number of bands present in *j* and absent in *i*. The similarity dendrogram was produced by clustering the similarity data with the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and the SAHN clustering program.

The quantitative data, on the other hand, were used to compute similarity coefficients using correlation matrix, after standardization. Similarity dendrograms were also produced by clustering the similarity data with the unweighted pair group method using arithmetic averages (UPGMA) and the SAHN clustering program.

Comparison among the data produced with the different types of markers was done using the Mantel test (1967), a randomization procedure that compares the correlation between two matrices with the correlation between one of these and randomizations of the other. Principal Component analysis (PCA) was measured and 2D and 3D plots were produced.

2.2.7. Determination of Total Water Soluble Antioxidant Contents

In order to determine the total water soluble antioxidant activity in national melon accessions, the ABTS decolorization method, modified by Re et al. (1999) was used.

According to this method, 200 g of ripe melon fruit was homogenized with 100 ml cold dH_2O for 2 min at low speed in a Waring blender equipped with a 1000 ml double walled stainless steel jar at $+4^oC$. Then, 10 g of this extract was taken from the homogenate and diluted with 15 ml cold distilled water. Homogenized melon samples were filtered through 4 layers of nylon cloth into two 15 ml falcon tubes. The filtrates were centrifuged at 3000 x g for 10 min at $+4^oC$ in a refrigerated centrifuge. After centrifugation, supernatants were merged into a single 50 ml falcon tube after filtration through 3 layers of nylon cloth to get a clear filtrate. Melon filtrate was kept on ice until it was used for measurement of total water soluble antioxidant activity. It was measured spectrophotometrically (Shimadzu, 1700 UV Visible Spectrophotometer, Japan) using the ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] . ABTS radical cation($ABTS^{\cdot+}$) is a free radical that absorbs at 734 nm. When this compound is reduced by antioxidant species its absorbance decreases. The ABTS radical cation stock solution was prepared by mixing 7mM ABTS with 2.45mM potassium persufate and was stored in the dark for 12-16 hours. Before use, the $ABTS^{\cdot+}$ stock solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to adjust its absorbance to 0.700 ($\pm$ 0.02) at 734 nm. Then 2.5, 5 and 7.5 µl aliquots of melon supernatant were mixed separately with 2 ml ABTS radical cation solution and decolorization of blue-green $ABTS^{\cdot+}$ solution was kinetically monitored at 734 nm for 6 min. Each assay was repeated to give three replicates for each aliquot volume. The results were calculated as area under the curve (AUC) and expressed as µmol Trolox/kg fresh weight of melon fruits. To calculate AUC, the percent inhibition/concentration values for the extracts and Trolox were plotted separately against the test periods (1, 3, 6 min) and the ratio of the areas of curves for extracts and Trolox was used to calculate the AUC value.

2.2.8. Determination of Total Phenolic Compounds

The total phenolic content of the some national melon accessions fruits was spectrophotometrically measured using Folin-Ciocalteau as a reactive reagent adapted

from the method of Singleton and Rossi (1965). In this procedure, gallic acid was used for generation of a standard curve. Homogenates were prepared by blending 200 ml cold distiled water with 100 g tomato sample for two min at low speed in a Waring blender at 4^{o}C. Then, 2.5 g homogenate was diluted with 20 ml cold distilled water and centrifuged at 3000 x g for 10 min at +4^{o}C in a refrigerated centrifuge. The clear supernatant was used for the determination of total phenolic content. In view of this, 2 ml of the supernatant was mixed with 10 ml 2 N (10%) Folin-Ciocalteu and incubated for 3 min, then 8 ml 0.7 M Na_2CO_3 was added. After 2 hours of incubation at room temperature, the absorbance of the reaction mixture was measured at 765 nm in a spectrophotometer (Shimadzu, 1700 UV Visible Spectrophotometer, Japan). There were three replicates for each sample. The total phenolic content was expressed as gallic acid equivalents (mg/kg fresh weight) based on a gallic acid standard curve.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Morphological Analysis

Morphological characterization of the 138 melon accessions grown during spring-summer 2007 indicated that many of the accessions were heterogeneous. This heterogeneity was most obvious when fruit morphology was examined. Figure 3.1. shows fruits from accession TR69022 (line 125).

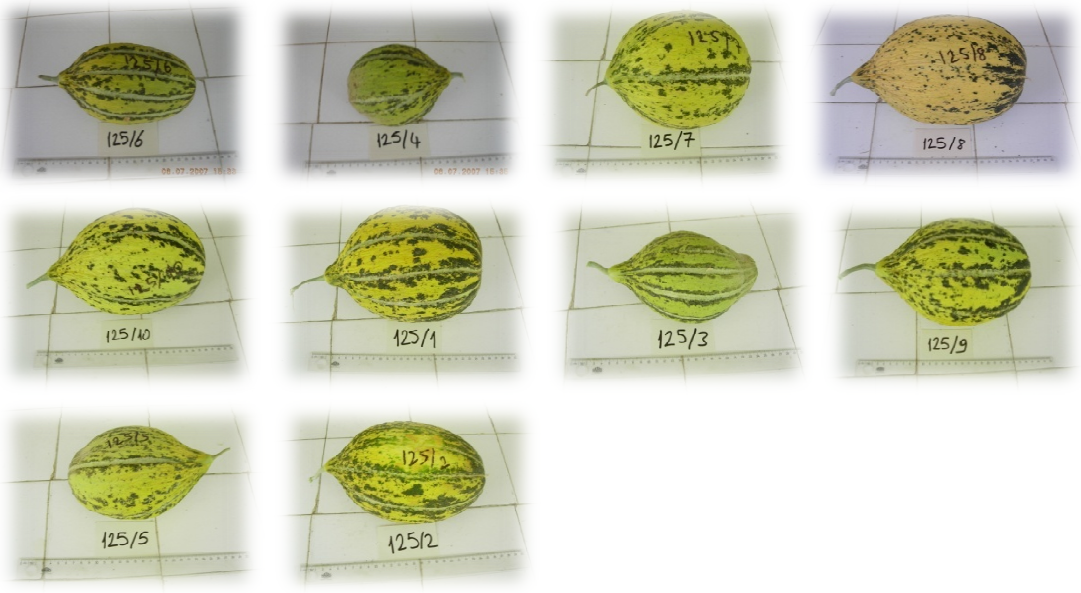


Figure 3.1. Fruits from the 10 individual plants grown for accession TR69022 showing homogeneous morphology.

As can be seen, the fruits from individual plants of this accession are all similar in size, shape and color indicating a certain degree of genetic homogeneity. In contrast, Figure 3.2. shows fruits from accession TR47874. In this accession, three distinct shape morphologies were seen: round, oval and elongated. These results indicated heterogeneity of this accession. Of the 138 accessions that were characterized, 53 (38%) were considered to be homogeneous while the rest were heterogeneous (62%).

Most of the heterogeneous accessions (52 lines, 61%) showed two different fruit morphologies, while 23 (27%) and 8 (9%) of the accessions had individuals showing three or four different morphologies, respectively. Two lines were extremely heterogeneous with 5 or 6 different fruit morphologies seen in the 10 individuals that were grown in the greenhouse. Table 3.1. indicates the number of fruit morphologies that were observed for each melon accession.

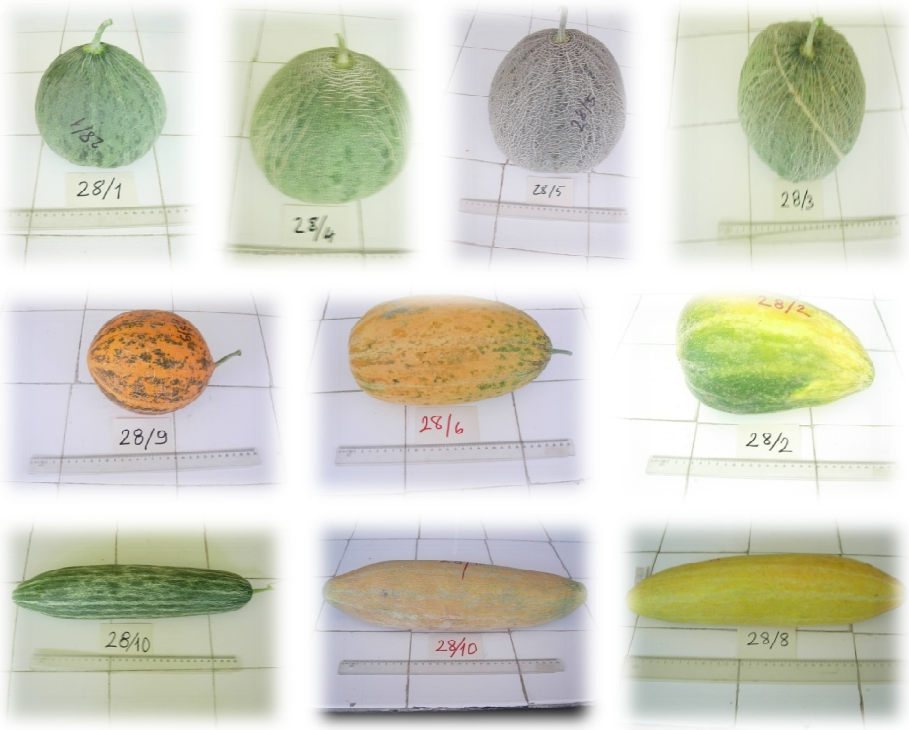


Figure 3.2. Fruits from the 10 individual plants grown for accession TR47874 showing heterogeneous fruit morphology.

In order to obtain more homogeneous materials for molecular genetic analysis, individual plants within each accession were self pollinated. Because it was impossible (and unnecessary) to perform genetic analysis on the progeny of all of these individuals (approximately 1380 plants), representatives of each accession were selected. If the accession was homogeneous, a single individual was selected as the representative of that accession. If the accession was heterogeneous the individuals were grouped based on morphology (as described above for TR47874) and one individual from each group was selected as representative. Thus, accession TR47874 was represented by three individuals (plant numbers 4, 6 and 8) for the molecular genetic analysis. Table 3.1. lists the individuals that were selected for genetic analysis for each melon accession. Thus, a total of 269 individuals were selected for genetic analysis.

Table 3.1. Number of different fruit morphologies for the melon accessions grown during spring-summer 2007. Also included are the identifying numbers for the individuals selected for molecular genetic analysis.

Accession Number	Number of Fruit Morphologies	Selected Individuals	Accession Number	Number of Fruit Morphologies	Selected Individuals
TR 15776	2	2,7	TR 47861	2	4,7
TR 2092	1	1	TR 47867	4	1,4,5,7
TR 2096	2	3,6	TR 47874	3	4,6,8
TR 26195	1	4	TR 47884	2	6,7
TR 26762	1	2	TR 47885	2	2,6
TR 31534	1	1	TR 48527	2	2,5
TR 31586	4	1,7,9,10	TR 48541	2	1,3
TR 31588	3	5,7,10	TR 48566	1	5
TR 31589	3	3,5,9	TR 48567	1	1
TR 33304	6	1,2,4,6,7,8	TR 48611	1	3
TR 33380	2	1,2	TR 48650	3	2,7,10
TR 35299	2	3,9	TR 48671	4	5,6,7,8
TR 37394	2	3,6	TR 49583	2	1,3
TR 38116	3	2,3,4	TR 49591	2	3,10
TR 38125	2	4,9	TR 49882	1	2
TR 38224	1	5	TR 50719	2	2,5
TR 38479	2	1,4	TR 50728	1	7
TR 38484	3	2,4,7	TR 50747	4	3,4,6,8
TR 39655	4	1,3,6,7	TR 51531	3	3,5,9
TR 39683	1	9	TR 51550	3	2,3,5
TR 40280	1	1	TR 51561	2	2,3
TR 40284	3	1,6,8	TR 51608	2	2,6
TR 40345	3	5,7,10	TR 51676	2	2,7
TR 40350	1	1	TR 51763	3	1,2,9
TR 40365	2	2,4	TR 57778	1	7
TR 40379	1	7	TR 57780	1	2
TR 40380	1	1	TR 57781	1	3
TR 40382	2	6,10	TR 57782	1	4
TR 40384	2	4,8	TR 57783	1	7
TR 40503	3	3,7,8	TR 61543	1	6
TR 40514	1	3	TR 61566	1	5
TR 40530	2	2,8	TR 61573	1	3
TR 40534	1	4	TR 61626	1	5
TR 40559	2	4,8	TR 61627	2	3,10
TR 40563	2	2,8	TR 61659	2	1,2
TR 43015	2	6,9	TR 61714	2	4,8

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Table 3.1 (cont.) Number of different fruit morphologies for the melon accessions grown during spring-summer 2007. Also included are the identifying numbers for the individuals selected for molecular genetic analysis.

Accession Number	Number of Fruit Morphologies	Selected Individuals	Accession Number	Number of Fruit Morphologies	Selected Individuals
TR 43023	3	4,7,8	TR 61812	1	3
TR 43024	2	7,10	TR 61851	2	2,6
TR 43041	3	1,2,4	TR 62023	1	2
TR 43105	3	3,6,8	TR 62060	1	10
TR 43135	3	2,9,10	TR 62474	1	3
TR 43263	1	9	TR 63230	1	5
TR 43265	1	5	TR 64142	2	2,5
TR 43722	1	6	TR 64154	2	2,7
TR 43744	1	8	TR 64163	1	6
TR 43746	1	9	TR 66002	1	4
TR 43749	1	1	TR 66003	2	2,4
TR 43835	2	2,3	TR 66004	2	8,10
TR 45791	2	3,5	TR 66005	3	1,3,8
TR 45883	3	2,6,8	TR 66007	1	7
TR 45896	4	1,4,5,7	TR 66008	2	8,10
TR 46437	4	2,4,5,8	TR 66362	1	9
TR 46438	1	9	TR 66366	2	1,2
TR 46489	4	1,2,5,9	TR 66755	1	3
TR 46503	3	2,4,5	TR 68913	2	2,3
TR 47776	2	5,9	TR 68934	2	5,7
TR 47783	2	1,4	TR 68940	2	9,10
TR 47793	1	1	TR 69022	1	1
TR 47797	3	1,6,9	TR 69425	3	1,4,8
TR 47804	1	3	TR 69428	2	1,2
TR 47805	2	8,9	TR 69689	1	9
TR 47811	2	2,4	TR 69873	2	7,9
TR 47812	2	3,5	TR 69895	1	1
TR 47813	2	2,7	TR 71500	1	5
TR 47822	3	3,5,6	TR 71540	2	4,5
TR 47825	5	2,5,7,8,9	TR 71541	2	1,4
TR 47833	2	5,9	TR 71568	1	2
TR 47845	3	2,5,7	TR 71571	2	1,3
TR 47846	2	1,4	TR 71616	1	1

Nineteen morphological criteria were used for characterization of the national melon accession. These criteria were chosen based on agronomical characters such as fuit shape, predominant fruit skin colour, fruit surface, fruit length, flesh thickness, main colour of flesh, flesh flavour, flesh bitterness, etc.

According to morphological analysis, all accessions were vigourous, nearly all accessions were pentalobate 119 (86.2%) and the others were entire 19 (13.8%). Leaf colour showed variation from light to dark green within the accessions, 67 (48.5%) accessions had green, 64 (46.4%) accessions had dark green and 7 (5.1%) accessions had light green leaves (Figure 3.3).

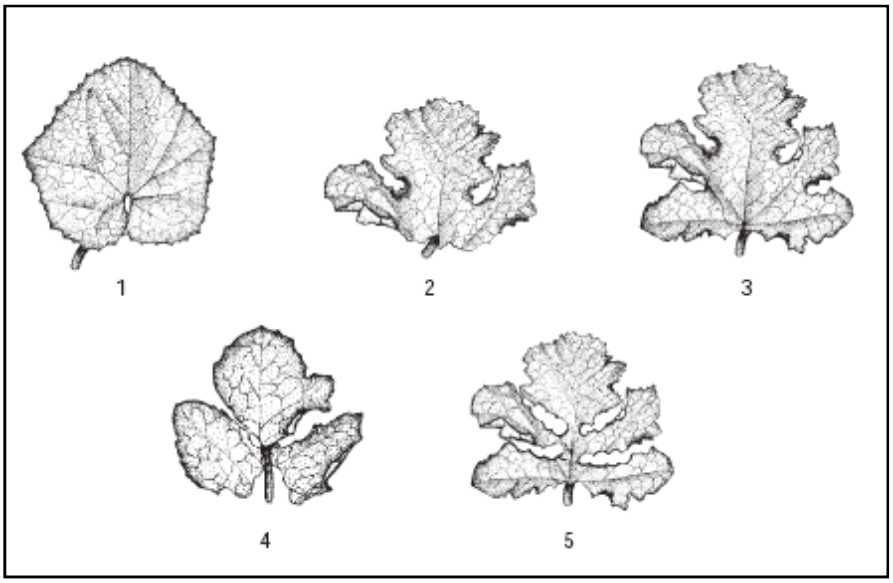

Figure 3.3. Types of melon leaf shape from IPGRI Descriptor for Melon

All accessions had yellow flower colour. The accessions showed a great variety of fruit shape, 53 (38.4%) accessions were globular (round), 51 (37.0%) accessions were eliptical, 15 (10.9%) were ovate, 7 (5.1%) oblate, 2 (1.4%) elongate and one accession was flattened (Figure 3.4).

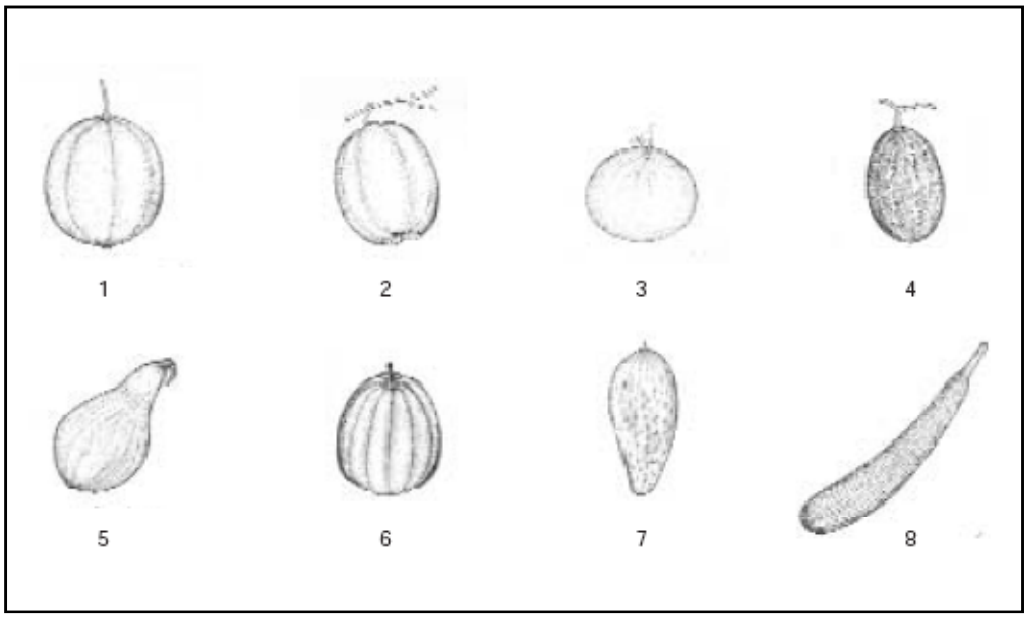

Figure 3.4. Types of melon fruit shape from IPGRI Descriptor for Melon

Another agronomically important criteria is flesh flavour. Most of accessions were intermediate 115 (83.3%), 13 (9.4%) accessions were inspid and 10 (7.2%) accessions were sweet. Fruit surface showed nine different kinds of traits within the accessions. A total of 44 (31.9%) accessions were shallowly wavy, 40 (29.0%) accessions were heavily corked/netted, 23 (16.7%) accessions were finely wrinkled, 12 (8.7%) accessions were smooth, 9 (6.5%) accessions had grainy surface, 6 (4.3%) accessions were lightly corked/netted, 2 (1.4%) accessions had sutures, 1 (0.7%) accession had numerous warts and 1 (0.72%) was deeply wrinkled. For predominant fruit skin colour, 55 (39.9%) accessions had light yellow predominant skin colour, 34 (24.6%) were pale green, 29 (21.0%) were orange, 9 (6.5%) were cream, 6 (4.3%) were green, 4 (2.9%) were blackish-green and 1 (0.7%) was dark green. All morphological data are shown in Table 3.2.

Table 3.2. Morphological characterization data for the national melon accessions.

Accession Number	7.1.8. Seedling Vigour	7.5.1. Leaf Shape	7.5.14. Leaf Colour	7.6.1. Sex Type	7.6.14. Flower Colour	7.7.1. Fruit Shape	7.7.10. Predominant FruitSkin Colour	7.7.11. Secondary Fruit Skin Colour	7.7.15. Secondary Skin Colour Pattern	7.7.16. Fruit Surface	7.7.40. Main Colour of Flesh	7.7.43. Flesh Texture	7.7.45. Flesh Flavour	7.7.50. Flesh Thickness	7.7.58. Flesh Bitterness	7.8.1. Seed Size	8.1.8. Fruit Length (cm)	8.1.9. Fruit Width (cm)	8.1.10. Fruit Weight (g)
TR 33304	7	1	2	2	3	1	3	3	3	5	3	6	5	23	3	4	26	16	9
TR 38116	7	3	3	2	3	4	2	7	2	3	3	5	5	25	3	4	21	18	9
TR 38125	7	3	2	2	3	2	2	4	1	5	1	5	7	32	3	4	20	17	8
TR 38479	7	3	2	2	3	1	2	7	2	5	3	5	5	27	3	4	16	16	7
TR 31586	7	1	2	2	3	5	5	6	2	9	2	1	3	18	3	4	19	16	8
TR 31588	7	3	2	2	3	4	4	6	2	1	3	1	3	16	3	3	16	13	5
TR 31589	7	3	2	2	3	1	3	5	3	5	3	5	5	35	3	4	19	19	9
TR 26762	7	3	2	2	3	6	3	4	2	8	4	5	5	17	3	3	16	13	5
TR 40365	7	3	2	1	3	4	4	6	3	9	4	5	5	27	3	4	23	17	9
TR 40534	7	3	3	2	3	4	4	4	1	8	7	5	7	31	3	4	20	14	8
TR 45791	7	3	3	2	3	4	8	5	1	9	6	3	5	30	3	3	25	15	8
TR 45883	7	3	3	2	3	5	7	7	0	9	3	5	5	29	3	4	22	17	8
TR 46438	7	3	2	2	3	5	2	8	2	1	3	1	3	15	3	3	15	13	4
TR 46489	7	3	3	2	3	4	2	6	2	5	7	5	5	30	3	4	20	17	8
TR 47776	7	3	3	2	3	4	4	6	1	9	3	5	5	25	3	4	16	14	6
TR 47783	7	3	3	2	3	1	2	7	2	3	3	5	7	23	3	3	16	14	5
TR 47793	7	3	3	2	3	1	2	7	2	5	3	5	5	24	3	3	16	15	7
TR 47804	7	1	2	2	3	3	4	6	1	9	3	3	5	26	3	4	17	17	8

(cont. on next page)

Table 3.2. (cont.) Morphological characterization data for the national melon accessions.

Accession Number	7.1.8. Seedling Vigour	7.5.1. Leaf Shape	7.5.14. Leaf Colour	7.6.1. Sex Type	7.6.14. Flower Colour	7.7.1. Fruit Shape	7.7.10. Predominant FruitSkin Colour	7.7.11. Secondary Fruit Skin Colour	7.7.15. Secondary Skin Colour Pattern	7.7.16. Fruit Surface	7.7.40. Main Colour of Flesh	7.7.43. Flesh Texture	7.7.45. Flesh Flavour	7.7.50. Flesh Thickness	7.7.58. Flesh Bitterness	7.8.1. Seed Size	8.1.8. Fruit Length (cm)	8.1.9. Fruit Width (cm)	8.1.10. Fruit Weight (g)
TR 47805	7	1	2	2	3	3	4	6	1	9	3	5	5	28	3	4	15	18	8
TR 47812	7	1	3	2	3	1	2	8	2	9	3	5	5	21	3	4	17	16	8
TR 47813	7	3	2	2	3	4	4	5	2	9	4	5	5	24	3	4	22	15	8
TR 47833	7	3	2	2	3	4	8	7	2	3	3	5	5	26	3	3	20	15	6
TR 47874	7	3	2	2	3	6	4	5	2	5	3	1	5	26	3	4	25	16	8
TR 48671	7	3	2	2	3	1	4	5	2	9	3	5	5	34	3	4	21	19	9
TR 48527	7	3	2	2	3	1	2	7	2	3	3	5	5	30	3	3	20	17	8
TR 48566	7	3	2	2	3	6	6	6	0	3	3	5	5	32	3	4	22	19	9
TR 48567	7	3	1	2	3	3	7	7	1	2	1	5	5	27	3	4	17	16	6
TR 40563	7	1	3	2	3	1	8	9	1	9	7	5	5	26	3	3	17	16	6
TR 43744	7	3	3	2	3	4	2	7	2	3	3	5	5	29	3	3	23	16	8
TR 47797	7	3	1	2	3	4	4	5	1	1	4	1	5	15	3	3	18	13	4
TR 47825	7	3	2	2	3	5	5	6	2	5	4	3	3	17	3	3	17	14	5
TR 47846	7	3	3	2	3	1	8	7	2	5	3	5	5	21	3	4	14	15	7
TR 47861	7	3	3	2	3	4	4	7	2	5	1	1	5	24	3	4	19	15	7
TR 47884	7	3	3	2	3	4	8	7	2	1	3	5	5	22	3	4	20	14	9
TR 47885	7	3	2	2	3	6	4	6	2	8	3	3	5	21	3	4	16	15	7
TR 50719	7	3	2	2	3	4	8	8	2	9	3	1	5	32	3	4	31	16	9

(cont. on next page)

Table 3.2. (cont.) Morphological characterization data for the national melon accessions.

Accession Number	7.1.8. Seedling Vigour	7.5.1. Leaf Shape	7.5.14. Leaf Colour	7.6.1. Sex Type	7.6.14. Flower Colour	7.7.1. Fruit Shape	7.7.10. Predominant FruitSkin Colour	7.7.11. Secondary Fruit Skin Colour	7.7.15. Secondary Skin Colour Pattern	7.7.16. Fruit Surface	7.7.40. Main Colour of Flesh	7.7.43. Flesh Texture	7.7.45. Flesh Flavour	7.7.50. Flesh Thickness	7.7.58. Flesh Bitterness	7.8.1. Seed Size	8.1.8. Fruit Length (cm)	8.1.9. Fruit Width (cm)	8.1.10. Fruit Weight (g)
TR 49882	7	3	3	2	3	1	7	7	0	5	3	5	5	28	3	3	18	17	8
TR 38224	7	3	3	2	3	4	8	7	2	3	1	1	5	23	3	4	19	14	7
TR 50728	7	3	2	2	3	4	8	8	0	5	3	3	5	27	3	4	22	17	9
TR 50747	7	3	2	2	3	4	4	6	1	9	7	1	5	29	3	4	20	16	8
TR 51531	7	3	2	1	3	1	8	5	1	9	7	5	5	27	3	4	18	17	7
TR 51550	7	3	2	2	3	4	8	5	1	9	7	5	5	26	3	4	21	16	7
TR 51561	7	3	1	2	3	1	4	5	3	5	6	4	3	17	3	3	11	10	4
TR 51608	7	3	2	2	3	1	8	8	1	9	3	3	5	31	3	4	19	18	9
TR 51676	7	3	2	2	3	4	4	6	1	10	1	5	5	21	3	4	20	17	8
TR 51763	7	3	2	1	3	4	2	5	1	5	6	5	5	28	3	4	21	14	6
TR 40382	7	1	3	2	3	1	4	6	2	9	3	3	5	25	3	4	17	16	8
TR 40384	7	3	1	2	3	1	2	6	3	5	1	3	5	15	3	3	11	12	4
TR 40503	7	1	3	2	3	6	4	5	2	9	3	3	5	25	3	4	19	15	6
TR 43015	7	3	3	2	3	1	8	2	1	9	4	3	7	34	3	4	17	15	7
TR 43023	7	3	3	2	3	1	8	5	1	9	6	3	5	29	3	4	15	14	6
TR 43105	7	3	2	2	3	4	4	5	2	5	6	6	5	33	3	3	18	15	5
TR 43722	7	3	3	2	3	6	4	6	1	5	3	3	7	24	3	3	17	15	6
TR 43749	7	3	3	2	3	4	2	7	2	3	3	5	5	23	3	3	17	13	6

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Table 3.2. (cont.) Morphological characterization data for the national melon accessions.

Accession Number	7.1.8. Seedling Vigour	7.5.1. Leaf Shape	7.5.14. Leaf Colour	7.6.1. Sex Type	7.6.14. Flower Colour	7.7.1. Fruit Shape	7.7.10. Predominant FruitSkin Colour	7.7.11. Secondary Fruit Skin Colour	7.7.15. Secondary Skin Colour Pattern	7.7.16. Fruit Surface	7.7.40. Main Colour of Flesh	7.7.43. Flesh Texture	7.7.45. Flesh Flavour	7.7.50. Flesh Thickness	7.7.58. Flesh Bitterness	7.8.1. Seed Size	8.1.8. Fruit Length (cm)	8.1.9. Fruit Width (cm)	8.1.10. Fruit Weight (g)
TR 43041	7	3	3	2	3	1	4	6	1	9	3	5	5	27	3	4	15	15	6
TR 43135	7	3	3	2	3	1	2	7	2	5	3	5	5	28	3	4	19	16	6
TR 45896	7	3	2	2	3	4	2	5	1	9	6	5	5	26	3	4	20	13	7
TR 46437	7	3	2	2	3	4	2	6	1	10	7	1	5	27	3	3	20	15	7
TR 47845	7	3	3	2	3	4	8	4	1	9	3	5	5	25	3	4	17	15	7
TR 47867	7	1	2	2	3	1	4	6	2	9	3	5	5	23	3	4	18	15	6
TR 49591	7	3	3	2	3	1	2	7	2	3	3	5	5	23	3	4	17	16	6
TR 35299	7	3	2	2	3	5	8	5	2	9	3	1	3	23	3	4	22	18	9
TR 37394	7	3	2	2	3	4	4	5	1	7	7	5	5	26	3	4	23	16	7
TR 40379	7	1	2	2	3	4	3	4	1	9	1	1	5	21	3	4	23	14	6
TR 43024	7	3	3	2	3	6	2	6	2	5	3	5	5	31	3	4	18	17	7
TR 48611	7	3	3	2	3	4	8	5	1	9	3	5	7	25	3	3	18	15	7
TR 48650	7	3	2	2	3	6	4	6	2	5	6	5	3	15	3	3	15	13	6
TR 49583	7	3	3	2	3	4	2	7	2	2	3	5	7	23	3	3	19	13	6
TR 2092	7	3	2	2	3	3	8	6	2	8	4	1	5	20	3	4	15	18	5
TR 2096	7	3	3	2	3	4	3	3	0	5	1	5	5	23	3	4	22	15	6
TR 57778	7	3	3	2	3	1	8	7	2	5	4	5	5	27	3	4	17	17	7
TR 57780	7	3	3	2	3	1	2	7	2	5	3	5	5	26	3	4	18	18	8

(cont. on next page)

Table 3.2. (cont.) Morphological characterization data for the national melon accessions.

Accession Number	7.1.8. Seedling Vigour	7.5.1. Leaf Shape	7.5.14. Leaf Colour	7.6.1. Sex Type	7.6.14. Flower Colour	7.7.1. Fruit Shape	7.7.10. Predominant FruitSkin Colour	7.7.11. Secondary Fruit Skin Colour	7.7.15. Secondary Skin Colour Pattern	7.7.16. Fruit Surface	7.7.40. Main Colour of Flesh	7.7.43. Flesh Texture	7.7.45. Flesh Flavour	7.7.50. Flesh Thickness	7.7.58. Flesh Bitterness	7.8.1. Seed Size	8.1.8. Fruit Length (cm)	8.1.9. Fruit Width (cm)	8.1.10. Fruit Weight (g)
TR 61812	7	3	3	2	3	6	2	4	1	5	7	5	5	29	3	4	16	16	8
TR 57781	7	3	3	2	3	1	2	7	2	5	4	5	5	25	3	4	17	17	8
TR 57782	7	3	2	2	3	1	8	7	2	5	4	5	5	27	3	4	18	19	8
TR 57783	7	3	3	2	3	3	8	7	2	5	3	5	5	28	3	4	16	18	7
TR 61566	7	3	1	2	3	4	2	8	2	1	3	1	3	5	3	2	5	4	1
TR 61573	7	3	2	2	3	4	2	7	2	3	3	5	5	25	3	4	24	15	7
TR 61626	7	3	2	1	3	5	4	10	3	4	7	5	5	20	3	4	30	18	9
TR 61627	7	3	2	2	3	6	3	6	2	9	4	3	5	29	3	4	16	16	8
TR 61659	7	3	3	2	3	1	2	5	3	5	7	5	7	29	3	4	18	18	9
TR 61714	7	3	3	2	3	1	8	8	3	5	7	5	5	26	3	4	17	16	8
TR 69022	7	3	2	2	3	4	2	7	3	3	3	3	5	22	3	4	21	14	8
TR 69425	7	3	3	2	3	1	2	7	2	3	4	5	5	24	3	4	19	16	7
TR 69428	7	3	3	2	3	1	8	7	2	3	3	5	5	26	3	4	18	18	8
TR 69689	7	3	3	2	3	6	2	6	1	1	3	5	5	32	3	4	19	17	8
TR 69895	7	1	3	2	3	1	2	6	1	3	3	3	5	25	3	3	17	15	5
TR 71500	7	1	2	2	3	1	4	6	2	5	3	1	3	10	5	4	12	12	4
TR 71540	7	3	3	2	3	1	2	6	2	5	3	3	5	26	3	4	16	17	7
TR 71541	7	1	2	2	3	1	2	2	0	5	3	5	5	25	3	4	17	17	8

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Table 3.2. (cont.) Morphological characterization data for the national melon accessions.

Accession Number	7.1.8. Seedling Vigour	7.5.1. Leaf Shape	7.5.14. Leaf Colour	7.6.1. Sex Type	7.6.14. Flower Colour	7.7.1. Fruit Shape	7.7.10. Predominant FruitSkin Colour	7.7.11. Secondary Fruit Skin Colour	7.7.15. Secondary Skin Colour Pattern	7.7.16. Fruit Surface	7.7.40. Main Colour of Flesh	7.7.43. Flesh Texture	7.7.45. Flesh Flavour	7.7.50. Flesh Thickness	7.7.58. Flesh Bitterness	7.8.1. Seed Size	8.1.8. Fruit Length (cm)	8.1.9. Fruit Width (cm)	8.1.10. Fruit Weight (g)
TR 66005	7	3	2	2	3	1	2	2	0	5	3	3	5	29	3	4	14	16	6
TR 71568	7	3	2	2	3	4	5	7	2	3	1	5	5	18	3	4	20	14	6
TR 71571	7	3	3	2	3	1	5	5	2	5	4	5	5	22	3	4	15	17	8
TR 71616	7	3	2	2	3	1	8	4	1	9	4	5	5	30	3	3	15	14	7
TR 64142	7	3	2	2	3	4	2	7	2	3	3	5	5	22	3	4	23	13	7
TR 64154	7	3	2	2	3	4	2	2	0	1	3	5	5	32	3	3	17	15	6
TR 64163	7	3	3	2	3	3	8	4	1	9	6	2	5	26	3	4	14	19	6
TR 66004	7	3	2	2	3	6	2	6	1	9	6	5	5	26	3	3	17	15	6
TR 66008	7	3	3	2	3	1	4	6	1	3	3	5	5	25	3	4	17	15	7
TR 69873	7	3	1	2	3	1	2	4	2	5	3	3	5	24	3	4	17	14	6

3.2. Genetic Similarity

3.2.1. AFLP Results

Ten AFLP combinations (*Mse*I-CTC/*Eco*RI-AAC, *Mse*I-CTC/*Eco*RI-AAG, *Mse*I-CTC/*Eco*RI-ACA, *Mse*I-CTA/*Eco*RI-ACG, *Mse*I-CTA/*Eco*RI-ACC, *Mse*I-CTA/*Eco*RI-ACT, *Mse*ICAT/*Eco*RI-AAG, *Mse*ICAT/*Eco*RI-ACA, *Mse*ICAC/*Eco*RI-AAC and *Mse*ICAC/*Eco*RI-AAG) were used to assess genetic diversity among the 238 national melon accessions.

The characteristics of AFLP combinations are shown in Table 3.3. According to this table, all of these AFLP combinations were polymorphic and they provided 345 polymorphic bands and the number of polymorphic bands per combination was roughly 34.5. The combination of *Mse*ICAT/*Eco*RI-AAG gave the most polymorphic bands with 58 while the combination of *Mse*I-CTA/*Eco*RI-ACC gave the fewest polymorphic bands with 20. The distribution of polymorphic bands for each AFLP primer combinations is shown in Table 3.4.

These fragments were from 60 to 640 bp long because of the size standard 600.

Table 3.3. Characteristics of the AFLP combinations and the SSR markers used in analysing the genetic diversity of the national melon accessions

	Total number of primers	**Number of polymorphic primers**	**Percentage of polymorphic primers**	**Number of polymorphic bands**	**Number of polymorphic bands per total number of primers**
AFLP	10	10	100	345	34.5
SSR	12	12	100	93	7.75

Table 3.4. Number of polymorphic bands for each AFLP primer combination.

AFLP Combinations	**Number of Polymorphic Bands**
MseI-CTC/EcoRI-AAC	37
MseI-CTC/EcoRI-AAG	36
MseI-CTC/EcoRI-ACA	31
MseI-CTA/EcoRI-ACG	29
MseI-CTA/EcoRI-ACC	20
MseI-CTA/EcoRI-ACT	28
MseI-CAT/EcoRI-AAG	58
MseICAT/EcoRI-ACA	36
MseICAC/EcoRI-AAC	31
MseICAC/EcoRI-AAG	39

One of the AFLP patterns from primer combination *Mse*I-CTA/*Eco*RI-ACG is shown in Figure 3.5. for three melon samples.

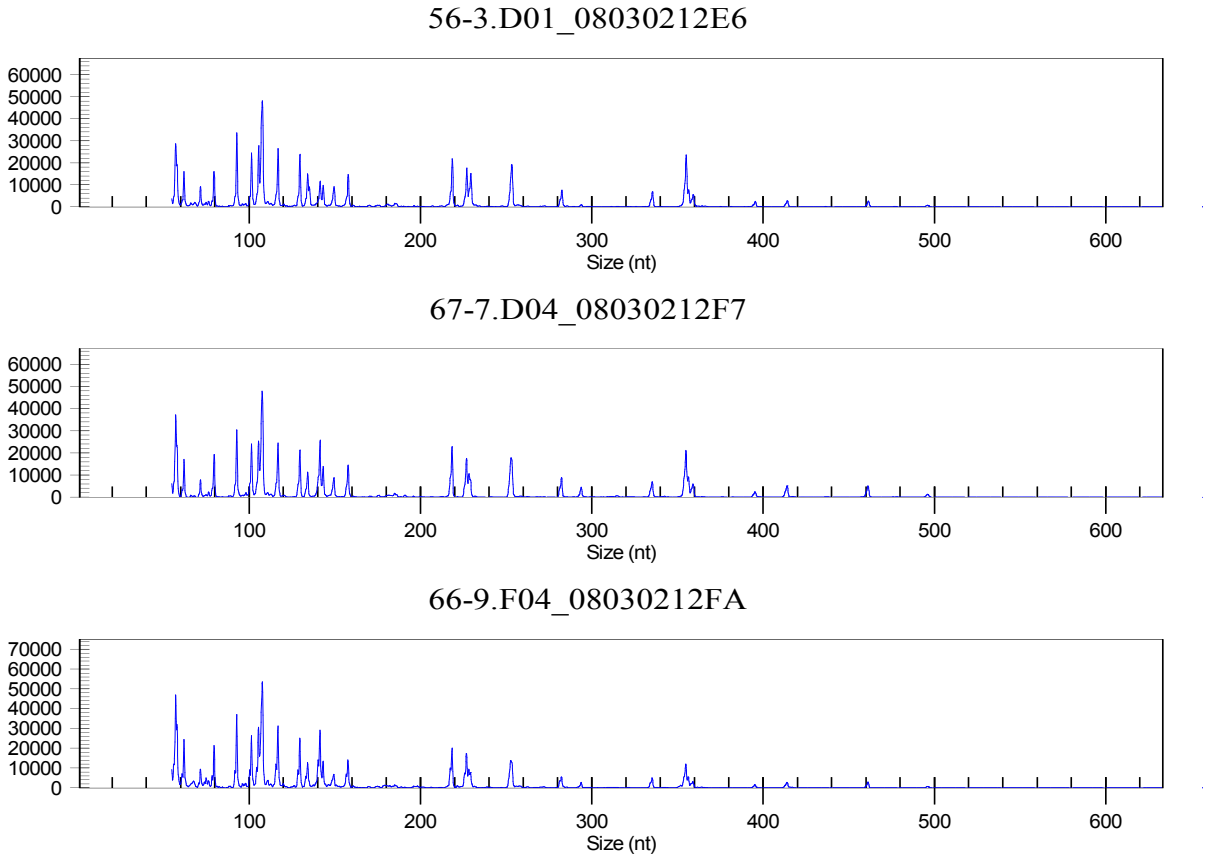

Figure 3.5. Three samples (56-3, 67-7 and 66-9) showing AFLP results obtained with the *Mse*I-CTA/*Eco*RI-ACG primer combination.

According to the AFLP results, a phylogenetic tree of the 238 national melon accessions and 12 outgroups was drawn based on DICE matrix and UPGMA (Unweighted Pair Group Method) arithmetical averages in SHAN module using the software programme NTSYS-pc version 2.2 (Figure 3.6.). Comparison among the data produced with the different markers was done using the Mantel test (1967). According to this test, the correlation between sample genotypic data and the dendrogram was high (0.91521). The dendrogram scale varied from 0.45 to 1.00 with a mean similarity of 0.73. At this value, the melon accessions fell into 10 groups (Figure 3.6.). The largest group, group A, contained the most lines with 188 accessions and a minimum similarity of ~0.75. Groups B and G had minimum similarities of 0.755 and 0.81, respectively, and contained 18 and 14 groups, respectively. Groups C and E contained 8 groups each with minimum similarities of 0.775. The remaining groups had 1 accession each. The outgroups clustered with a minimum similarity of approximately 0.45. Principal Component analysis (PCA) was performed and 2D and 3D plots were produced and are shown in Figure 3.7. and Figure 3.8.

The first, second and third axes explained 54, 8 and 5% of the total variance, respectively. Both of the multidimensional plots clearly show separation of the outgroups relative to the *Cucumis melo* accessions. In addition, the 2D plot shows that the melon accessions fell into two main clusters.

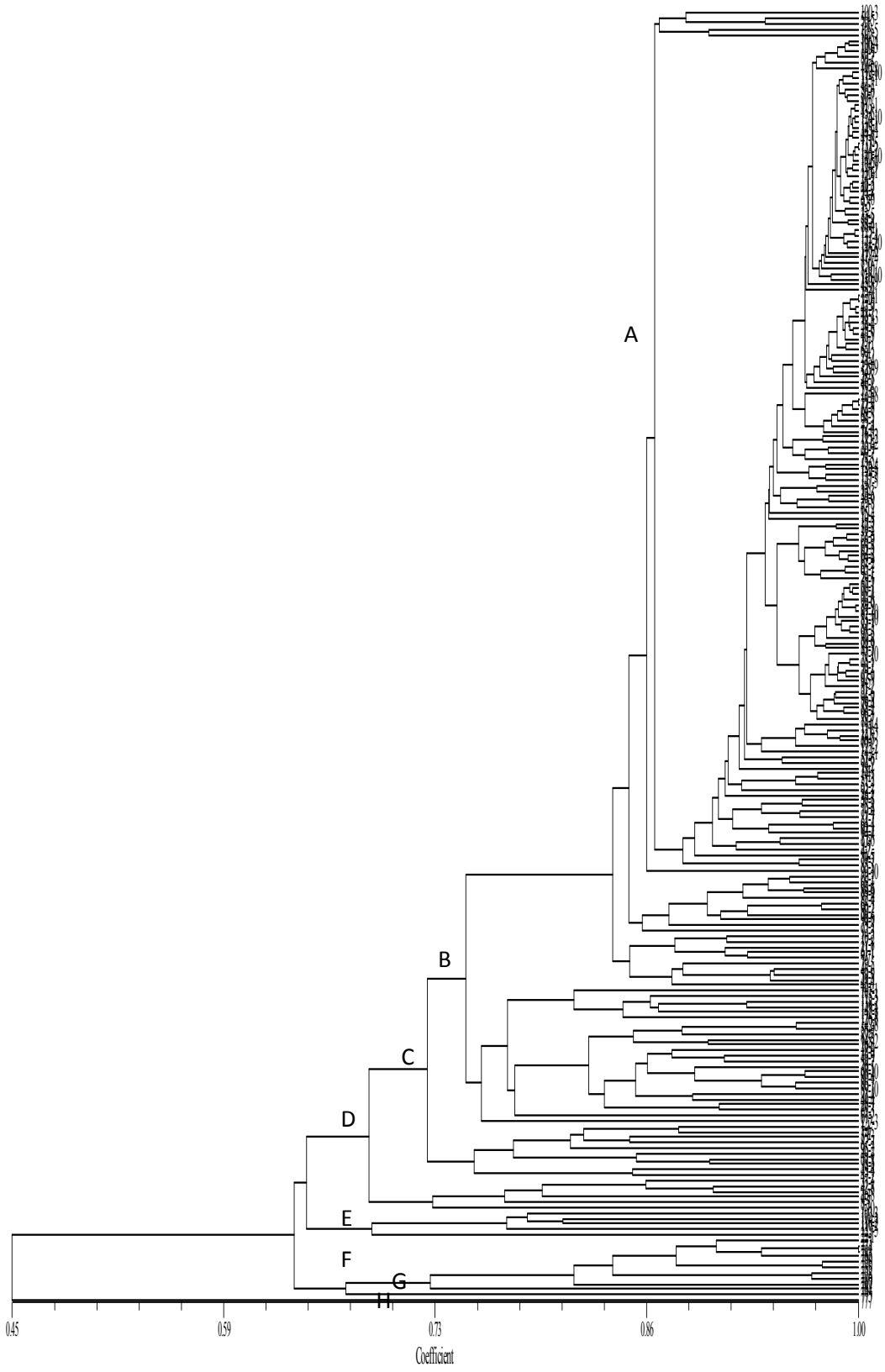

Figure 3.6. The phylogenetic tree based on AFLP results with 238 national melon accessions and 12 outgroups.

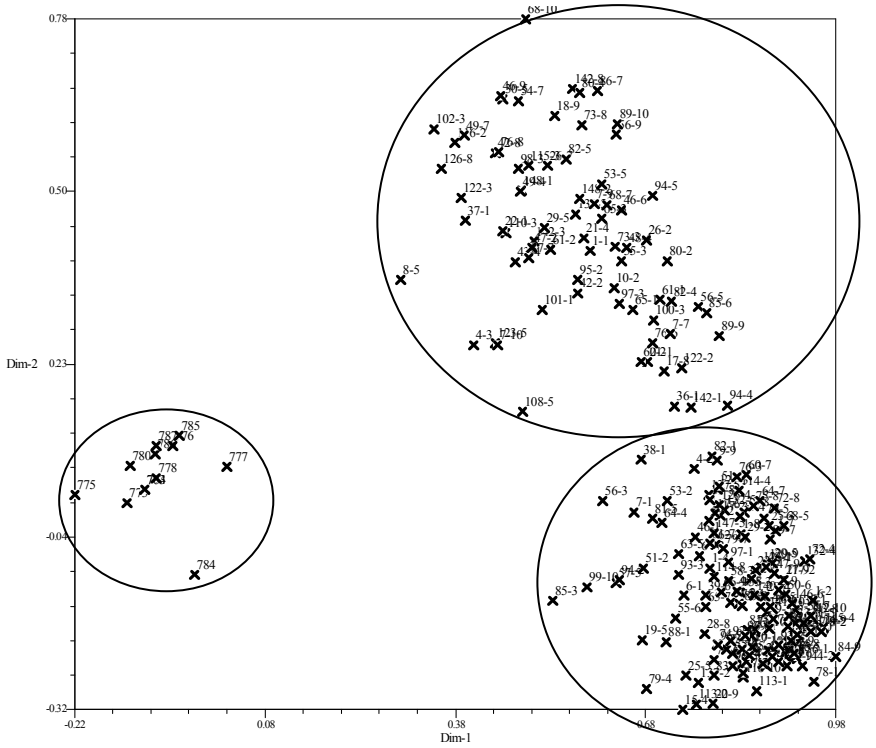

Figure 3.7. 2-D Plot of 238 national melon accessions and 12 outgroups based on AFLP results.

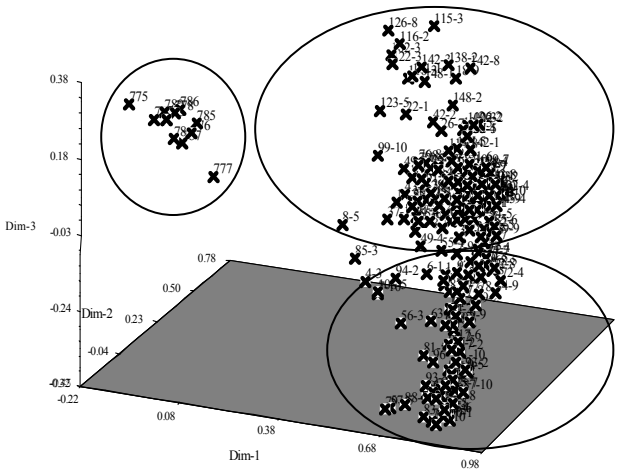

Figure 3.8. 3-D Plot of 238 national melon accessions and 12 outgroups based on AFLP results.

To see if clustering of the outgroups using AFLP data was consistent with their taxonomy, a phylogenetic tree and 2D plot were drawn for just these accessions, shown in Figure 3.9. and Figure 3.10, respectively. In general, these results show clustering of accessions of the same species as expected. For example, as can be seen in Figure 5, two component PCA shows clustering of the pairs of *Luffa*, *Cucumis moschata* and two accessions of *C. melo* subsp. *flexuosus*.

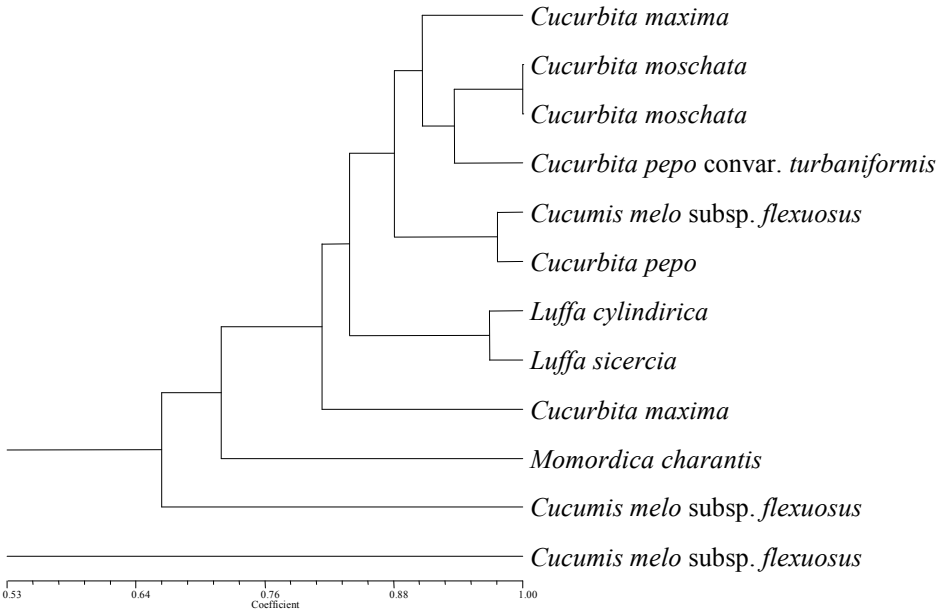

Figure 3.9. Phylogenetic tree of melon outgroups.

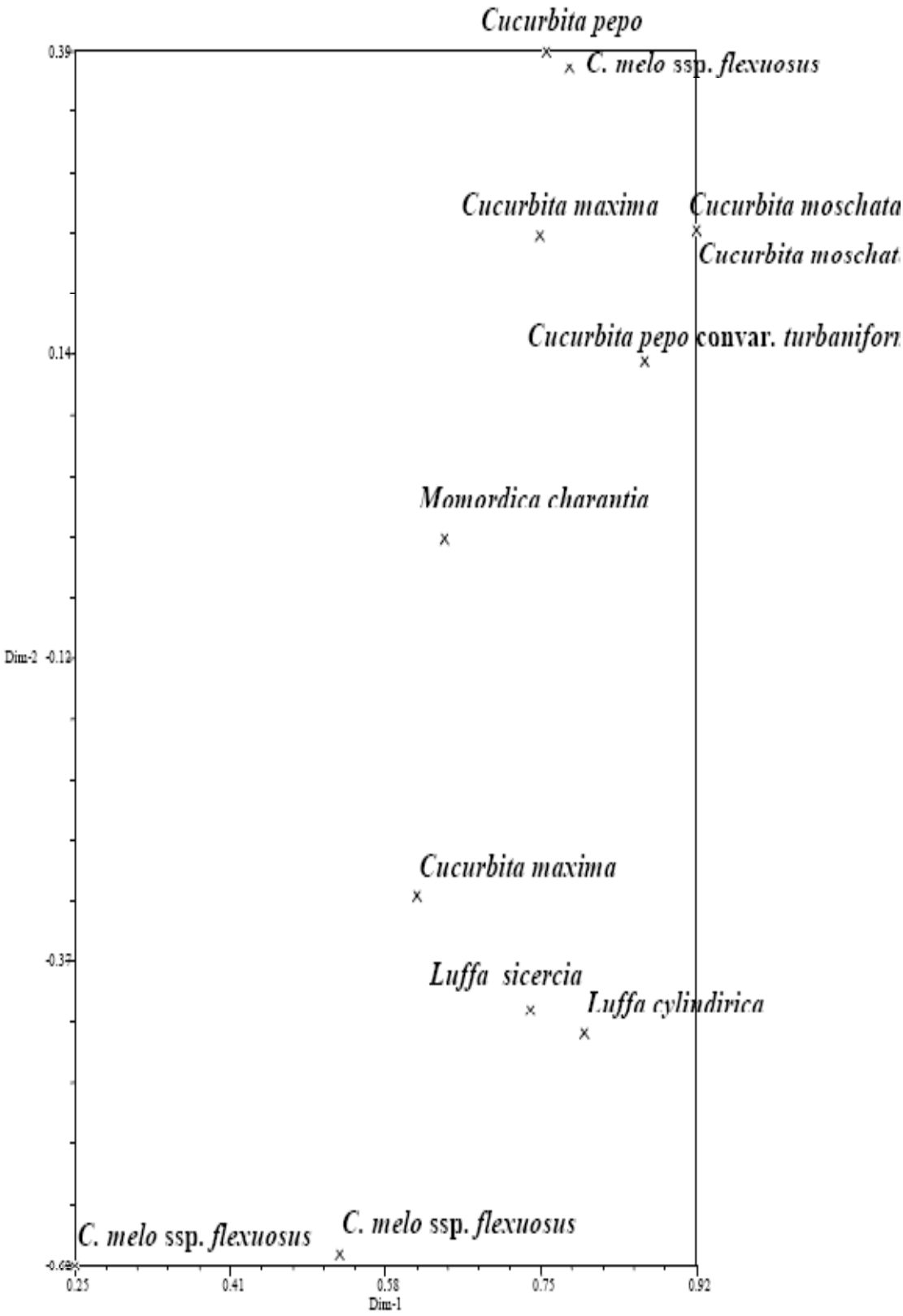

Figure 3.10. 2D plot of outgroups used for phylogenetic analysis of melon germplasm collection.

Because of the large number of samples which makes it difficult to distinguish individuals in the phylogenetic tree, a separate analysis was done using fewer accessions. For this analysis, single accessions were chosen to represent those melon lines with greater than 90% similarity. In this way, 54 accessions including the 12 outgroups were analyzed. This phylogenetic tree (r = 0.95451) showed separation of the melon accessions and outgroups at a genetic similarity of 55% (Figure 3.11.). The minimum similarity among melon accessions was 64%. 2D and 3D plots also show clustering of the outgroups away from *C. melo* species (Figure 3.12 and Figure 3.13.).

AFLP has also been used to study melon germplasm diversity by Garcia-Mas et al. (2000) and Ferriol et al. (2003). Garcia-Mas and co-workers only studied six genotypes but found that AFLP was more efficient for detecting polymorphism than RAPD or RFLP markers. In other work, both AFLP and SRAPs were used to study diversity and it was found that although AFLP effectively clustered genotypes based on subspecies, SRAPs gave clustering that was more consistent with morphological traits and were more polymorphic (Ferriol, et al. 2003).

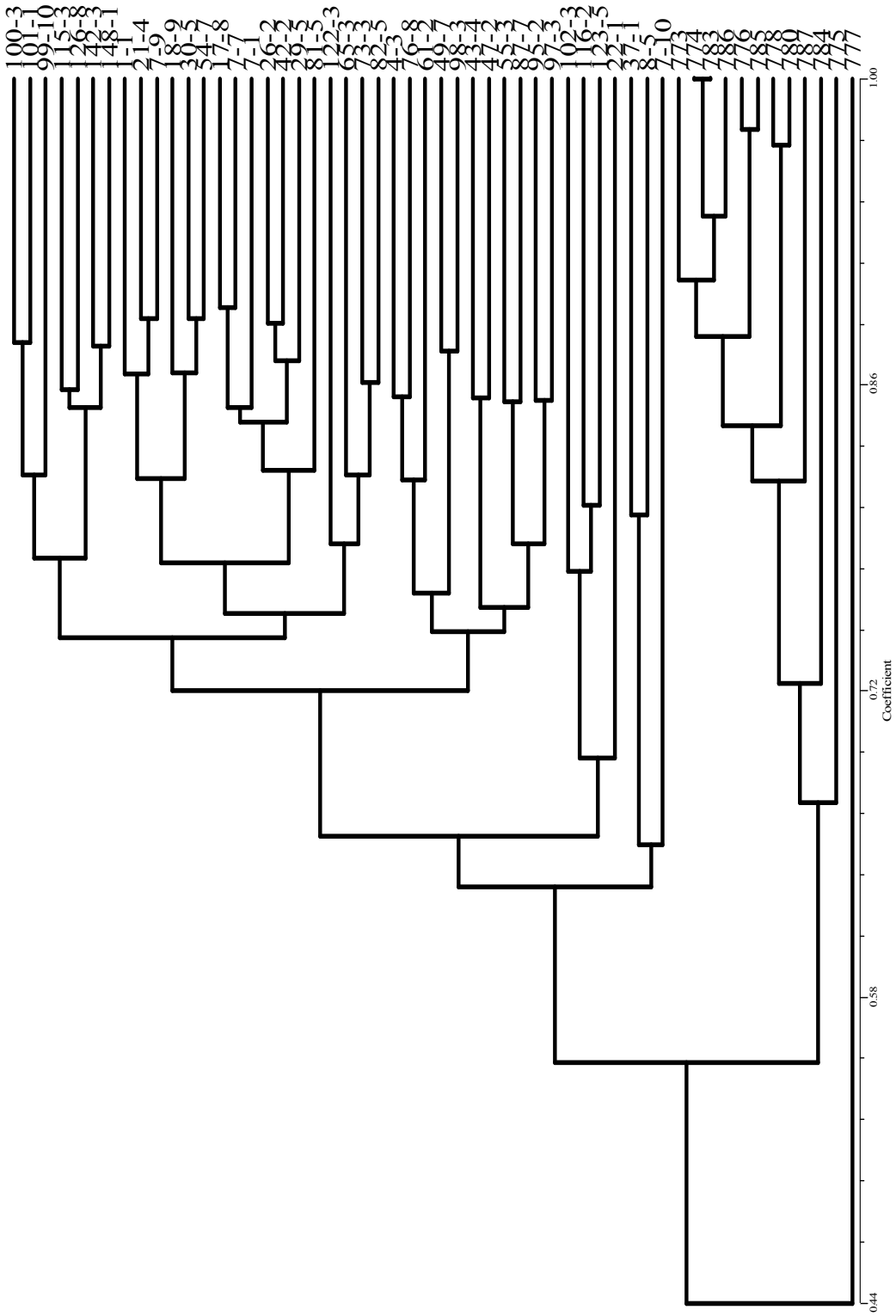

Figure 3.11. Phylogenetic tree of reduced number of melon accessions and outgroups.

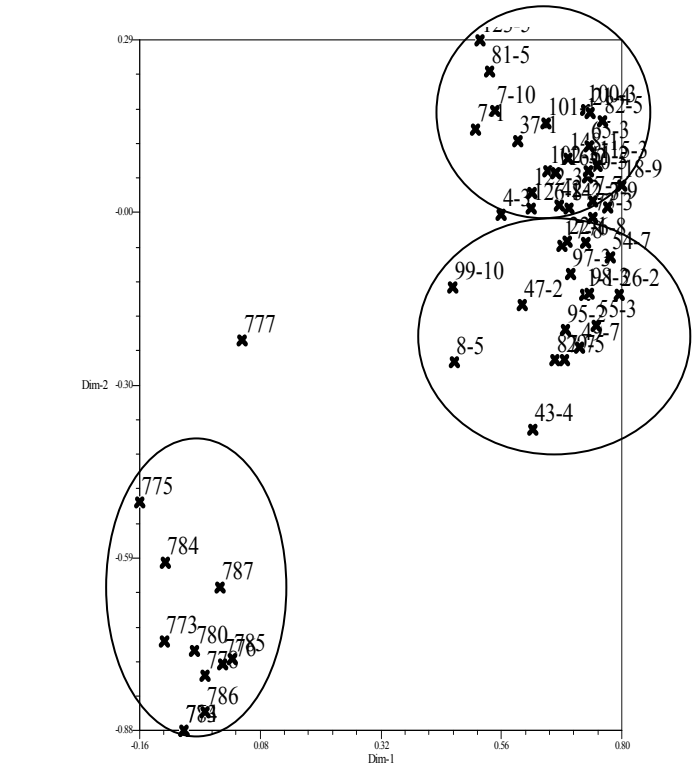

Figure 3.12. 2D plot of reduced data set.

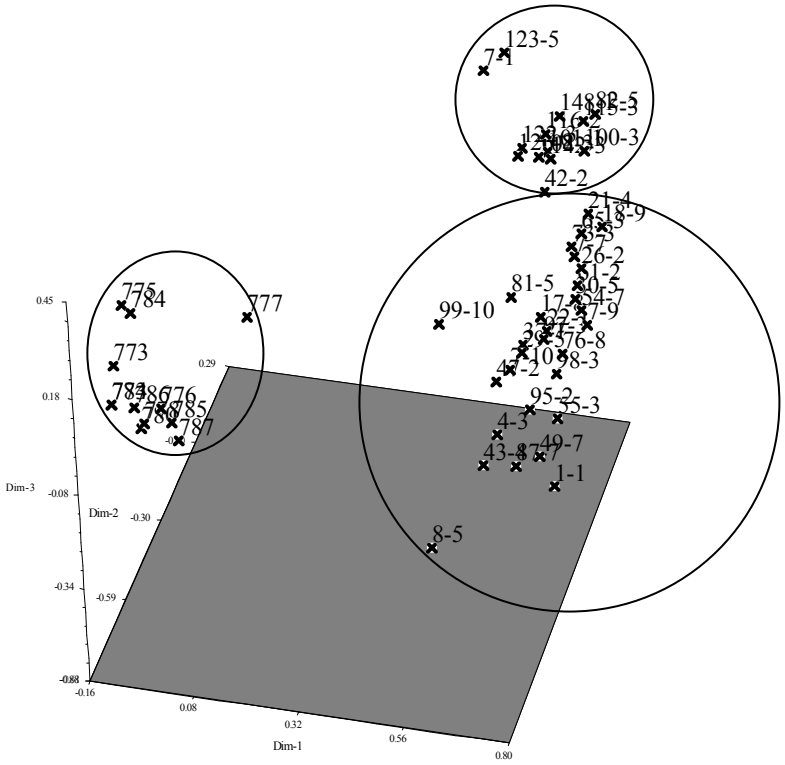

Figure 3.13. 3D plot of reduced data set.

3.2.2. SSR Marker Design Based on Unigenes Results

A total of 427 microsatellites were identified in 3522 melon unigenes that were downloaded from the Cornell University melon EST database. The PBC Public SSR Primer Discovery Input site was used for SSR detection and primer design. Of the 427 microsatellites that were identified, the most common repeat type was trinucleotide with 281 (65.8%) detected. The second most common type was dinucleotide with 65 (15.2%) detected, followed by pentanucleotide 48 (11.2%) and tetranucleotide 33 (7.7%) as shown in Table 3.5.

Table 3.5. Repeat type of microsatellite primers (dinucleotides, trinucleotides, tetranucleotides and pentanucleotides), number of identified SSRs and their percentages.

Repeat Type	**Number Identified**	**Percentage of Samples**
Dinucleotide	65	15.22
Trinucleotide	281	65.81
Tetranucleotide	33	7.73
Pentanucleotide	48	11.24
Total	427	100

The majority of dinucleotides consisted of TA/AT (33.8%) and TC/CT (32.3%) repeats (Table 3.6.). AG/GA (21.5%), TG/GT (7.7%) and AC/CA (4.6%) repeats were also identified. No GC/CG motifs were found in our designed microsatellites.

Table 3.6. Dinucleotide primers motif types, number of motifs and their percentages.

Motif Type	**Number of Detected**	**Percantage of Samples**
AT/TA	22	33.84
TC/CT	21	32.31
AG/GA	14	2.54
TG/GT	5	7.69
AC/CA	3	4.62
GC/CG	0	0

Similarly, among the loci with trinucleotide repeats, AGA/TCT (16.4%) was the most common motif followed by CTT/GAA (12.1%) and AAG/TTC (11.7%),

respectively (Table 3.7.). The remaining trinucleotide motifs each comprised less than 5% of the total.Total length of the microsatellite repeats ranged from 11 to 73 nucleotides with an average length of 17 nucleotides. The longest dinucleotide, trinucleotide, tetranucleotide and pentanucleotide in this study were: $(CT)_{21}$,42 nucleotides long (**AF030382**); $(AGA)_{24}$, 72 nucleotides long (**MU118**); $(TTTC)_5TTAC(TTTC)_2$, 32 nucleotides long (**PH4B7**); $(CAATC)_6$, 30 nucleotides long (**PH2E1**).

Table 3.7. Trinucleotide primers motif types, number of motifs and their percentages.

Motif Type	**Number of Detected**	**Percantage of Samples**
AGA/TCT	46	16.25
CTT/GAA	34	12.01
AAG/TTC	33	11.66
ACT/TGA	14	4.95
CTA/GAT	12	4.24
AGC/TCG	11	3.89
CCA/GGT	10	3.53
CCT/GGA	10	3.53
CCG/GCC	9	3.18
AAC/TTG	8	2.82
CAA/GTT	8	2.82
CTC/GAG	8	2.82
ATA/TAT	7	2.47
AGG/TCC	7	2.47
CAG/GTC	7	2.47
ACA/TGT	6	2.12

3.2.3. SSR Results

Twelve SSR markers were used for determination of genetic diversity of the 238 national melon accessions. The characteristics of these SSR primers are shown in Table 3.4. The SSR markers were chosen from some publications (CMCTN-5, CMCTN-86, CMGAN-25, CMCTN-35, CMAGN-68, CMGAN-80, TJ-10, TJ-27) and generated from melon EST library (MU118, FR14G19, SSH6I23 and PH8C1) in this study. Characteristics and some information about the published SSR markers were described by Katzir et al. (1996) and Danin-Poleg et al. (1997, 2000, 2001). The characteristics of SSR primers (from published and EST libraries) are shown in Table 3.8. All of these

SSR primers were polymorphic and provided 93 polymorphic bands. The number of polymorphic bands per primer was approximately 7.75.

The amplified fragments ranged in size from approximately 40 to 400 bp. One of the SSR patterns from CMCTN-5 is shown in Figure 3.14.

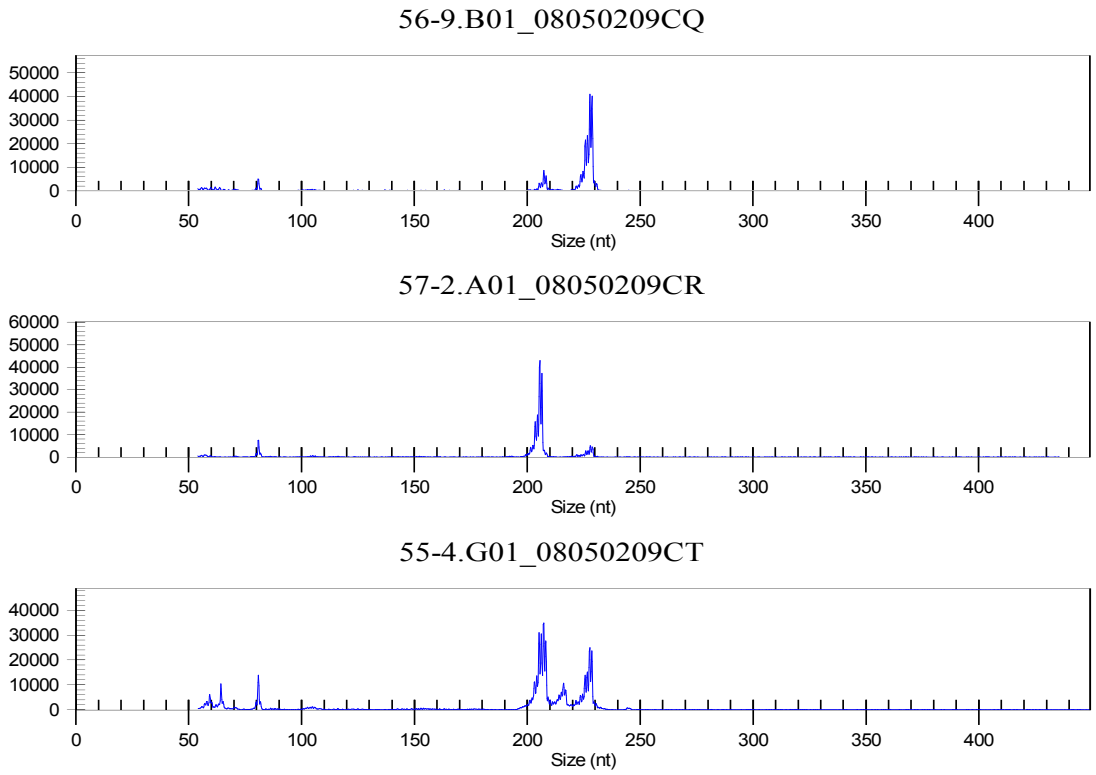

Figure 3.14. Three samples (56-9, 57-2 and 55-4) showing results obtained with the CMCTN-5 marker.

This value is higher than that obtained for other studies of melon diversity using SSRs. For example, López-Sesé et al. (2002) used 12 microsatellites, eight of which were polymorphic, to determine genetic variation of 15 Spanish melon accessions and they acquired 23 alleles (mean 2.9 alleles per locus). Monforte et al. (2003) used 18 SSR markers to study genetic diversity in 27 melon accessions and they detected 114 polymorphic locus (mean 6.3 alleles per locus). Nakata et al. (2005) used 12 SSR markers, in order to determine the diversity of 67 melon accessions from Japan and nine of them were polymorphic. They obtained 36 alleles (mean 4.0 alleles per locus). Szabó et al. (2005) used 20 SSR primers, eight of which were polymorphic, to determine the genetic variation of ancient melon accession and they obtained 40 alleles (mean 5

alleles per locus). As our work and the previous studies show, SSR (or microsatellite) markers show good polymorphism in melon and are highly effective for determination of genetic diversity in this crop.

According to the SSR results, a phylogenetic tree of the 238 national melon accessions was drawn based on DICE matrix and UPGMA (Unweighted Pair Group Method) arithmetical averages in SHAN module using the software programme NTSYS-pc version 2.2 (Figure 3.15.). Comparison among the data produced with the different markers was done using the Mantel test (1967). According to this test, the correlation between sample genotypic data and the dendrogram is in the acceptable values (0.80053). The dendrogram scale varied from 0.32 to 1.00 with a mean similarity of 0.66. At this value, the melon accessions fell into 7 groups (Figure 3.15.). The largest group, group A, contained the most lines with 132 accessions and a minimum similarity of ~0.50 and this group includes two subgroups group A1 which includes 13 accessions and group A2 includes 119. Group B is the second largest group and includes 86 accessions. Groups E and G had minimum similarities of 0.32 and 0.40, respectively, and contained 4 and 1 accessions, respectively. The remaining groups all had 1 accession each. The first, second and third axes for PCA explained 33, 13 and 8% of the total variance, respectively. Both plots show that the melon accessions fell into three main clusters. Overall the results show that SSRs are effective tools for phylogenetic analysis in melon.

In comparison with other studies of melon diversity in national germplasm, both our AFLP and SSR results show that Turkish melons are quite diverse. Staub et al. (2004) studied 24 RAPD primers in order to allow the genetic assesment of 17 Greek melon varieties. It was shown that the genetic distances (GD) were between 0.13 and 0.76 and the average GD was 0.46. López-Sesé et al. (2002) used 36 RAPD and 8 SSR primers over 15 Spanish melon accessions. The average GD was 0.42 and 0.29 for RAPD and SSR, respectively. In other work, López-Sesé et al. (2003) studied 34 RAPD primers in 125 Spanish melon accessions. According to their results, average GD was 0.36 over the Spanish melon accessions and the average GD was 0.46. Nakata et al. used 25 RAPD primers in order to determine the genetic diversity of 67 Japanese melon accessions. In this study, the GD between 0.42 and 0.89 and the average GD was 0.63. In view of these previous studies, Turkish melon accessions are more diverse than

Greece, Spanish and Japanese ones with an average GD of 0.73 and 0.66 for the AFLP and SSR analyses, respectively. These results stress the importance of conservation of this melon germplasm to maintain diversity in the crop and to provide material for future genetic improvements.

Table 3.8. Characteristics of SSR markers with forward and reverse primers and chromosomal locations.

	Marker Name	**Forward Primer**	**Reverse Primer**
1	**CMGAN25**	TAGCCAATGTGAAGGATGAACA	TGCAATTAGCCTCTTCTCTA
2	**CMCTN5**	TCACCTTAAAGTTTAGCCCC	AAAAATGCAATGAACTGAGCGC
3	**TJ10**	TACGAGGAAAACGCAAAATCA	TGAACGTGGACGACATTTTT
4	**CMGAN21**	TGCTGTAAAACGAAACGGAGA	CGATCTTCTTTATTCTTCGCC
5	**CMCTN35**	TCCAATAATGTAATCGTCTTGG	GTTCCAAACTTTCTACCAATCA
6	**CMCTN86**	TGTGACAGTTATCAAGGATGC	AAGGGAATGCATGTGGAC
7	**TJ27**	TAAGCGGAACAAGCTCATCTC	CAAAAGCATCAATTGCTTGAA
8	**CMAGN68**	TGGAAGGAAATTAGCATGCAC	GCCACTCTGTCTTTCTTCC
9	**CMGAN80**	TATATTGATTGCTGGGAAAGG	CTTTTTTGGCTTTATTGGGTC
10	**MU118**	TGTGTGCTGTACTCCTGAAA	CGGTTCTTTCTTCTCTTCCT
11	**SSH6I23**	CCGCTTCTTCTTCTTCTTCT	CTAGGACCGGAATCGTAATG
12	**FR14G19**	TCTTTGTCTACCACCAAACC	GTTTGAGAGGAGGAAGAGGT

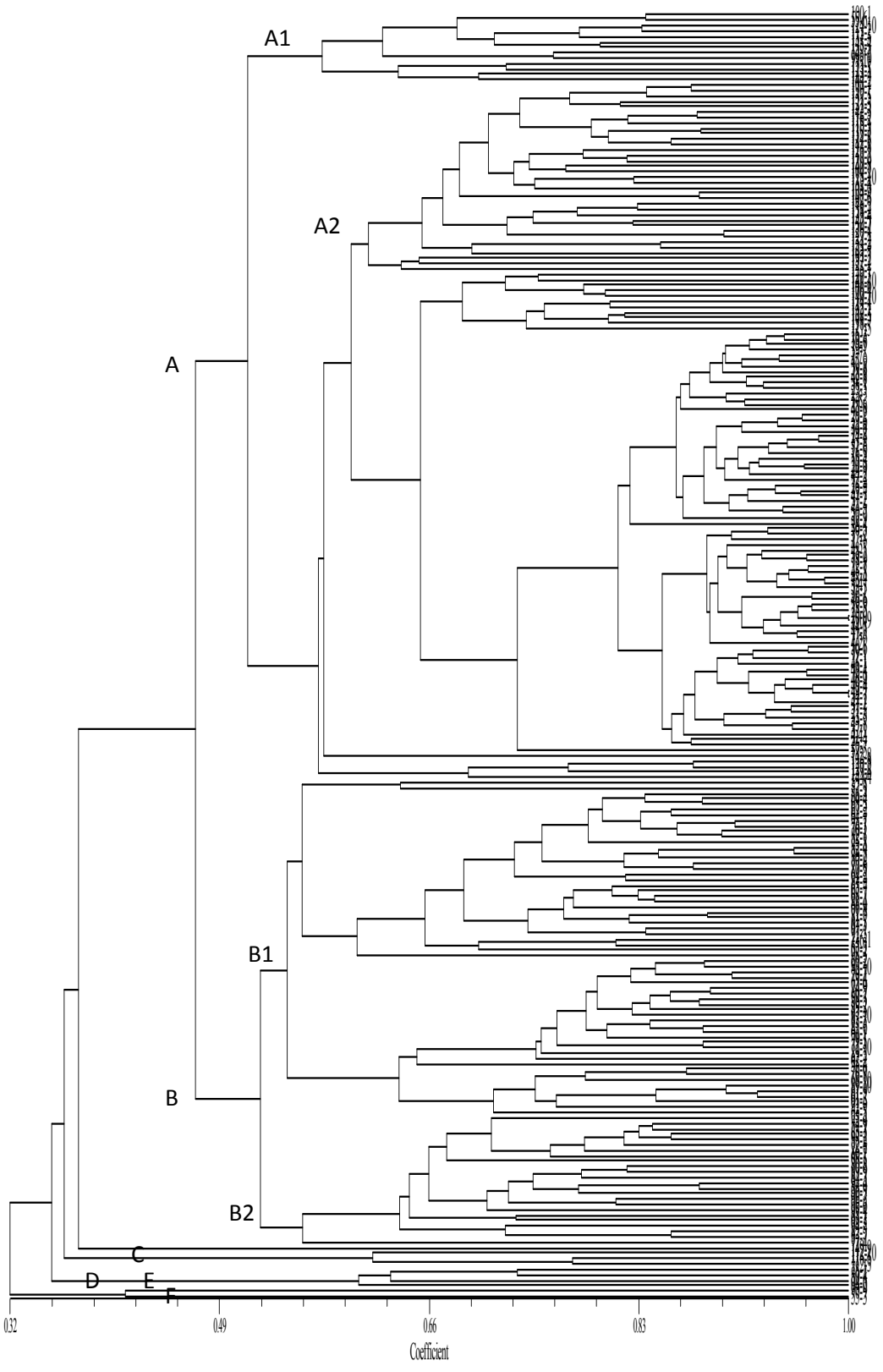

Figure 3.15. The phylogenetic tree based on SSR results with 238 melon accessions.

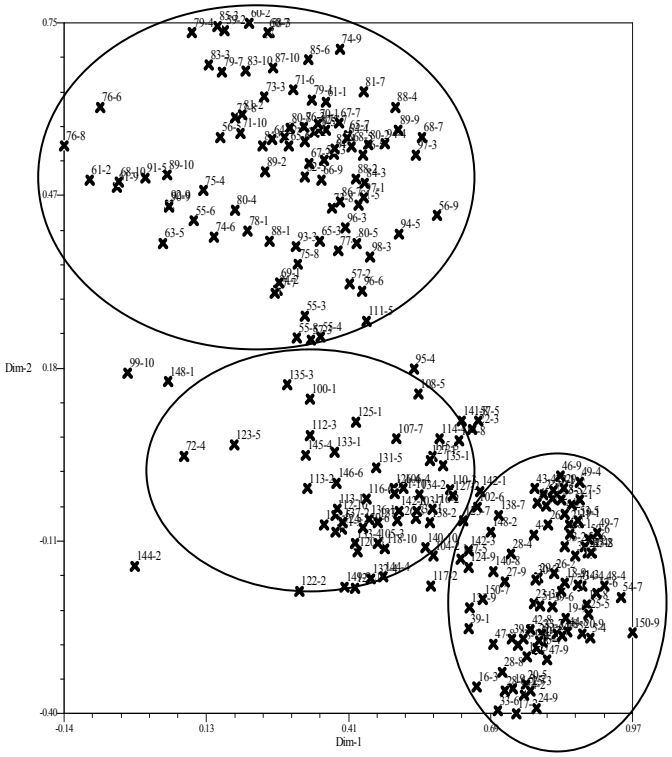

Figure 3.16. 2-D Plot of 238 national melon accessions based on SSR results.

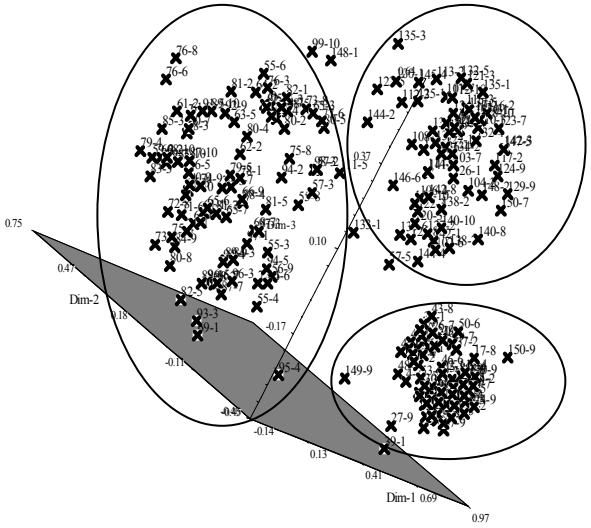

Figure 3.17. 3-D Plot of 238 national melon accessions based on SSR results.

3.3. Total Water Soluble Antioxidant Analysis

The 43 melon accessions which were used for measurement of total water soluble antioxidant and total phenolic compounds analysis are shown in Table 3.9. with their weight and flesh colour.

Table 3.9. Some national melon samples used for measuring total antioxidant, phenolic and Vitamin C contents.

Genotype Number	Sample I Weight	Sample II Weight	Average Weight	Flesh Colour
1	2261	2658	2459,5	3
2	1676	1247	1461,5	3
5	1911	2558	2234,5	6
10	1803	1416	1609,5	4
12	2016	2084	2050	1
13	1362	2002	1682	1
15	1420	1607	1513,5	7
16	3634	3195	3414,5	6
18	2828	2356	2592	3
20	1374	1454	1414	3
21	1896	2292	2094	1
25	1441	1659	1550	2
26	1541	1756	1648,5	3
27	1719	1647	1683	3
29	1260	1226	1243	3
31	1311	1336	1323,5	2
35	2925	2383	2654	2
37	2505	3398	2951,5	3
38	1865	1994	1929,5	3
39	2085	2261	2173	6
40	1697	1365	1531	6
50	1703	1387	1545	6
54	1444	1555	1499,5	3
55	1895	2560	2227,5	3
56	2887	2542	2714,5	3
61	1692	1682	1687	3
62	2870	2050	2460	3
71	2128	1744	1936	6
74	1881	1611	1746	3
75	1663	1160	1411,5	1
76	1374	1914	1644	2

(cont. on next page)

Table 3.9. (cont.) Some national melon samples used for measuring total antioxidant, phenolic and Vitamin C contents.

Genotype Number	Sample I Weight	Sample II Weight	Average Weight	Flesh Colour
78	2363	2322	2342,5	1
79	3616	3918	3767	3
80	3361	1979	2670	3
84	2040	1967	2003,5	1
87	1962	1485	1723,5	3
88	2747	2393	2570	3
93	2122	1864	1993	3
94	1866	1603	1734,5	3
96	1439	1750	1594,5	4

Total water soluble antioxidant activity in the melon accessions ranged from 1027 to 4346 µM Trolox/kg. The least antioxidant capacity was seen in accession 21,and the highest antioxidant capacity was seen in accession number 5. The mean value for the population was 2747 ± 78 µM Trolox/kg. Distribution of total water soluble antioxidant capacity (µM Trolox/kg) in the 43 melon accessions is shown in Figure 3.18. This histogram shows that most melons had moderate activities.

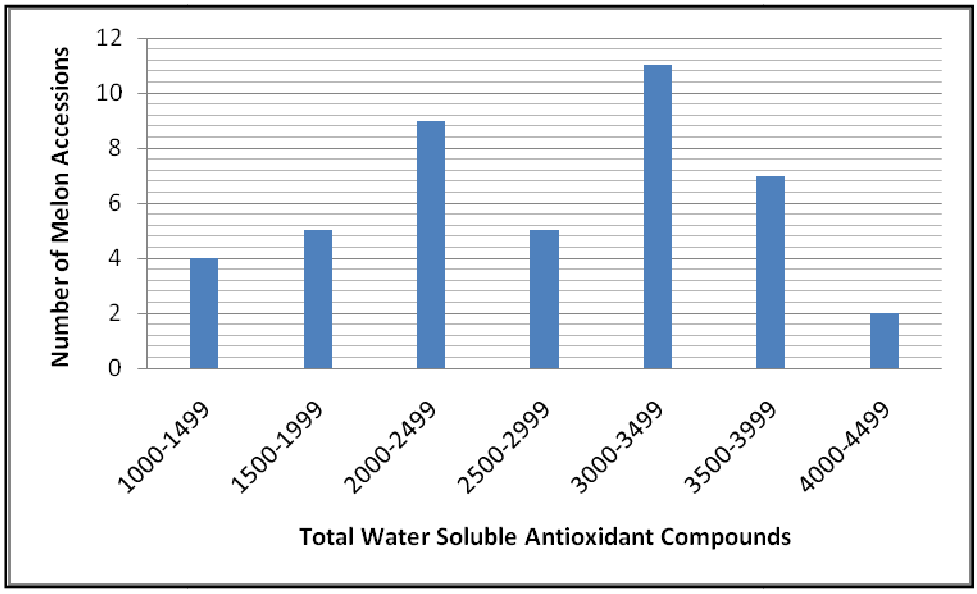

Figure 3.18. Distribution of total water soluble antioxidant contents (µM Trolox/kg) in the 43 melon accessions.

4.1.2. Total Phenolic Compound Analysis

The phenolic compound content (PHE) in the melon accessions ranged from 118 to 357 mg/kg. The lowest phenolic content was seen in accession 10, and the highest phenolic compound was seen in accession number 1. The mean value of total phenolic compounds for the population was 249 ± 8.5 mg/kg. Distribution of total phenolic compounds (mg/kg) within the 43 melon accessions is shown in Figure 3.19. Again, most of the accessions had moderate levels of phenolic compounds.

Overall, the antioxidant analysis of melon showed that diversity for both total antioxidant capacity and total phenolic content are present in the national melon germplasm. This diversity can be used for breeding of improved melon cultivars. Thus, the data generated here can be used to select promising materials for increased antioxidant activity in melon.

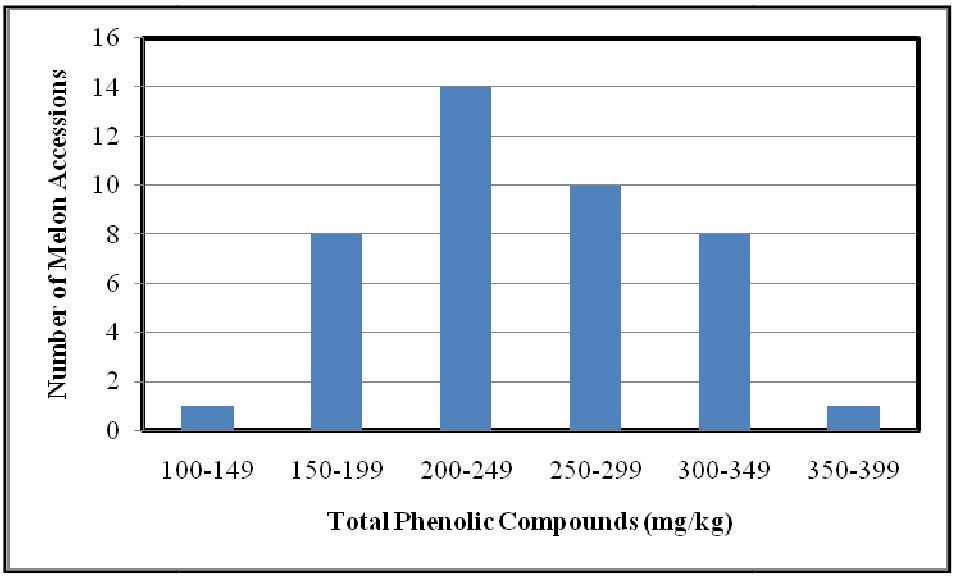

Figure 3.19. Distribution of total phenolic compounds (mg/kg) in the 45 melon accessions.

Moreover, our results agree with previous work with melon. Halvorsen et al. (2001) studied several berries, fruits and vegetables total anioxidant and phenolic compounds. In their study, cantaloupe melon had total antioxidant capacity of 1500 µmol Trolox/kg. Wu et al. (2004) studied common foods in the USA. They found thatcantaloupe melon had 3120 µmol Trolox/kg antioxidant activity and that honeydew melon had 2410 µmol Trolox/kg capacity. These values are similar to the values

obtained in our work. For phenolic content, Wu et al. (2004) found that cantaloupe melon had 1240 mg/kg and honeydew melon had 720 mg/kg phenolic compounds. These results areapproximately 5 fold higher than our results.

In comparison with other commonly consumed fruits that have been studied in our laboratory, the average melon total antioxidant and phenolic contents are lower than those for pepper and eggplant. According to Frary et al. (2008), Turkish pepper cultivars have a mean antioxidant content of 7500 µM Trolox/kg Thus, the average Turkish pepper antioxidant content is approximately 2.7 fold higher than melon. Turkish eggplant cultivars, on the other hand, have a mean of 4400 µM Trolox/kg (Ökmen, et al., in press). Thus the average Turkish eggplant antioxidant capacity is approximately 1.6 fold higher than for melon.

To our knowledge, this is the first study of antioxidant content in Turkish melon accessions.. Our work shows that significant genetic variation for antioxidant capacity and phenolic content exists in Turkish melon germplasm. Melon breeders may use this information to chose the most promising material for development of improved melon cultivars.

CONCLUSION

Melon (*Cucumis melo* L.) is a morphologically diverse, outcrossing horticultural crop of broad economic importance that produces over the world and Turkey. The main aims of our study was characterization of the national melon collection morphologically and genetically, establishing a core collection by eliminating redundant accessions and identifying lines that may be useful for melon breeding. In this aspect, 138 national melon accessions used for morphological characterization, 238 melon accessions used for molecular characterization and 43 melon accessions used for determination of total antioxidant (AOX) and total phenolic (PHE) contents. While, morphological characterization was carried out via visually scored, molecular characterization was carried out via 10 AFLP marker combinations and 12 SSR markers.

In this study, different *C. melo* L. accessions which were collected different location in Turkey, used for determining the differences based on their morphology, genome and biochemical compounds. According to morphological analysis, all accessions were really vigourous. The accessions showed a great variety of fruit shape, 53 (38.4%) accessions were globular (round), 51 (37.0%) were eliptical, 15 (10.9%) were ovate, 7 (5.1%) oblate, 2 (1.4%) elongate and one accession was flattened. Another agronomically important criterion was predominant fruit skin colour. A total of 55 (39.9%) accessions had light yellow predominant skin colour, 34 (24.6%) were pale green, 29 (21.0%) were orange, 9 (6.5%) were cream, 6 (4.4%) were green, 4 (2.9%) were blackish-green and 1 (0.7%) was dark green. In accordance with genetic characterization, a total of 345 polymorphic AFLP fragments (products of 10 AFLP primer combinations) and 93 SSR fragments (products of 12 SSR markers) were detected and used to calculate genetic distance using DICE matrix and UPGMA (Unweighted Pair Group Method) arithmetical averages. The average polymorphic AFLP fragments per combination was 34.5 and SSR fragments per marker was 7.75. The phylogenetic tree showed that groups were clearly separated by both marker systems. This study allowed the identification of the relationship between national melon accessions based on genetic similarity or differences. Forty three melon accessions were also analyzed for total water soluble antioxidant and total phenolic compound activities. Our results agree with previous work with melon. Halvorsen et al. (2001) studied several berries, fruits and vegetables total anioxidant and phenolic

compounds. In their study, cantaloupe melon had total antioxidant capacity of 1500 μmol Trolox/kg. Wu et al. (2004) studied common foods in the USA. They found thatcantaloupe melon had 3120 μmol Trolox/kg antioxidant activity and that honeydew melon had 2410 μmol Trolox/kg capacity. These values are similar to the values obtained in our work. For phenolic content, Wu et al. (2004) found that cantaloupe melon had 1240 mg/kg and honeydew melon had 720 mg/kg phenolic compounds. These results areapproximately 5 fold higher than our results. In comparison with other commonly consumed fruits that have been studied in our laboratory, the average melon total antioxidant and phenolic contents are lower than those for pepper and eggplant. According to Frary et al. (2008), Turkish pepper cultivars have a mean antioxidant content of 7500 μM Trolox/kg Thus, the average Turkish pepper antioxidant content is approximately 2.7 fold higher than melon. Turkish eggplant cultivars, on the other hand, have a mean of 4400 μM Trolox/kg (Ökmen, et al. in press). Thus the average Turkish eggplant antioxidant capacity is approximately 1.6 fold higher than for melon.

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APPENDIX A

ALL SSR PRIMERS from MELON UNIGENES

Table A-1. All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
MU1	dinucleotide	(AT)6	12	54.873	54.937	358
MU100	dinucleotide	(TC)10	21	56.324	55.013	374
MU102	dinucleotide	(TC)5	11	59.791	55.070	257
MU102	tetranucleotide	(TTG)3	14	54.728	53.992	399
MU109	trinucleotide	(GAA)4	13	55.190	54.952	156
MU109	trinucleotide	(GAA)4	12	54.952	55.033	349
MU109	trinucleotide	(GGA)6	19	54.952	55.033	349
MU113	trinucleotide	(TCT)5	17	54.779	54.970	310
MU118	trinucleotide	(AGA)24	73	55.277	55.332	313
MU128	trinucleotide	(AGA)4	12	54.817	62.062	192
MU131	trinucleotide	(GGT)4	14	55.181	55.118	338
MU150	trinucleotide	(AGC)5	16	54.974	55.030	374
MU151	trinucleotide	(TGA)4CGA(TGA)4	28	54.987	55.807	366
MU156	trinucleotide	(GGA)10	32	53.082	54.990	272
MU161	dinucleotide	(GA)9	19	55.013	54.797	343
MU161	dinucleotide	(AG)5	11	55.013	54.797	343
MU164	dinucleotide	(CT)4CC(CT)5	20	55.630	55.070	370
MU170	trinucleotide	(GAA)4	12	54.941	54.231	141
MU172	trinucleotide	(AGC)10	30	55.550	54.932	270
MU173	trinucleotide	(GAA)5	15	55.276	55.043	109
MU179	pentanucleotide	(CATCG)3	15	58.743	55.052	385
MU181	dinucleotide	(TC)14	29	54.282	54.913	394
MU186	trinucleotide	(TCT)4	14	55.364	54.901	296
MU190	tetranucleotide	(CTTT)4	16	55.288	55.152	185
MU193	trinucleotide	(ATG)4	12	55.120	55.100	320
MU193	dinucleotide	(TC)8	16	55.024	54.587	292
MU194	trinucleotide	(GAT)4	13	52.996	55.101	379
MU236	trinucleotide	(GCA)6	20	54.987	55.229	258
MU236	trinucleotide	(GAT)5	16	54.987	55.229	258
MU246	tetranucleotide	(GATG)3	13	55.020	55.027	311
MU256	trinucleotide	(GGC)5	15	55.033	54.873	237
MU259	trinucleotide	(CGG)4	14	55.372	57.641	171

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
MU260	dinucleotide	(TC)6	13	54.765	54.991	242
MU261	trinucleotide	(GTC)4	12	55.152	55.239	335
MU264	trinucleotide	(TCG)4	14	54.950	56.639	398
MU273	pentanucleotide	(TCTTC)3	17	60.656	55.159	231
MU276	trinucleotide	(CAG)6	18	55.386	55.028	389
MU277	trinucleotide	(CTA)4	14	54.967	55.038	334
MU29	trinucleotide	(GAT)4	13	55.120	58.697	194
MU294	trinucleotide	(TTC)4	12	55.289	54.959	265
MU316	dinucleotide	(TC)13	26	55.239	55.323	273
MU326	trinucleotide	(CTC)4	13	54.660	54.865	301
MU330	trinucleotide	(GAA)4	13	55.630	55.906	306
MU334	trinucleotide	(TGC)5	17	55.062	59.056	181
MU335	dinucleotide	(AT)10	20	64.605	55.070	323
MU340	pentanucleotide	(GAGGA)3	19	55.033	54.207	154
MU343	dinucleotide	(TG)5	11	54.899	55.331	222
MU345	trinucleotide	(TTG)6	20	54.971	54.959	224
MU345	dinucleotide	(CA)15	31	54.971	55.465	263
MU363	trinucleotide	(AAG)3AAA(AAG)2	19	54.994	56.360	125
MU370	tetranucleotide	(TTAT)3	14	55.298	55.383	335
MU374	trinucleotide	(GTT)4	13	55.078	55.644	235
MU38	trinucleotide	(CGA)5	16	55.013	55.120	336
MU403	trinucleotide	(TGC)4	14	55.003	54.941	380
MU411	pentanucleotide	(CTTTG)2	14	55.070	55.264	321
MU425	tetranucleotide	(GTGG)3	13	55.154	55.118	161
MU430	trinucleotide	(TCT)3	12	54.630	56.350	271
MU431	dinucleotide	(TA)6	12	54.814	55.339	259
MU439	dinucleotide	(AT)4TT(AT)6	22	55.024	56.600	307
MU451	pentanucleotide	(TTTC)2	14	54.983	54.914	144
MU472	tetranucleotide	(TATT)4	16	55.118	54.149	305
MU49	trinucleotide	(AAT)4	12	54.032	55.088	112
MU504	trinucleotide	(AAG)6	20	54.891	54.538	211
MU512	trinucleotide	(CTT)4	13	54.805	55.103	168
MU52	trinucleotide	(TCC)4	12	58.370	54.765	260
MU521	trinucleotide	(TCG)4	12	55.025	55.050	273
MU522	dinucleotide	(AG)6GG(AG)4	22	54.891	53.906	394
MU534	trinucleotide	(AGA)6GA(AGA)	25	54.979	54.997	255
MU534	trinucleotide	(AAG)5	15	54.979	54.997	255
MU534	trinucleotide	(AAG)5	15	54.979	54.997	255

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
MU538	trinucleotide	(GGT)4	12	55.027	55.013	192
MU541	tetranucleotide	(TTGT)4	13	55.014	55.003	211
MU543	dinucleotide	(AT)12	25	54.979	55.574	381
MU545	pentanucleotide	(TTTTC)2	14	54.971	59.127	362
MU55	dinucleotide	(TA)8	17	54.983	55.252	199
MU56	pentanucleotide	(AAACA)2	14	55.135	55.033	238
MU561	trinucleotide	(AAG)4	13	55.092	55.176	311
MU568	trinucleotide	(AGA)10	32	61.383	54.796	126
MU568	pentanucleotide	(TCTCA)3	15	55.135	55.050	327
MU569	trinucleotide	(TTC)8	24	55.027	55.140	243
MU577	trinucleotide	(GAA)11	33	55.216	55.070	247
MU59	trinucleotide	(TGA)4	12	55.290	55.057	379
MU590	trinucleotide	(GAT)8	24	53.911	54.866	308
MU590	pentanucleotide	(AAAAG)3	17	55.504	54.866	173
MU590	trinucleotide	(GGT)9	27	55.504	55.842	238
MU591	trinucleotide	(AGC)5	15	57.577	54.818	124
MU593	pentanucleotide	(TCCCT)2	14	56.920	54.380	271
MU6	trinucleotide	(TGA)4	12	54.873	55.047	301
MU604	trinucleotide	(TCA)4	12	55.240	54.932	395
MU619	pentanucleotide	(TTTGC)2	14	55.252	56.824	260
MU621	trinucleotide	(TCT)4	14	58.359	55.000	154
MU621	trinucleotide	(GGC)5	17	54.886	55.062	149
MU630	pentanucleotide	(TTTTA)2	14	54.913	54.931	208
MU634	trinucleotide	(AAC)6	18	54.982	55.141	311
MU637	trinucleotide	(GAG)5	16	55.016	54.997	293
MU638	trinucleotide	(ATG)4	13	57.062	57.515	254
MU648	dinucleotide	(TA)10	20	54.923	54.886	271
MU654	trinucleotide	(CTC)5	17	54.632	54.280	360
MU663	pentanucleotide	(TTTGG)6	34	55.100	55.022	332
MU668	trinucleotide	(AGC)5	15	55.414	54.941	183
MU668	trinucleotide	(GAA)4	12	55.264	55.510	284
MU669	dinucleotide	(TA)5	11	54.817	56.227	392
MU679	tetranucleotide	(AAAT)4	18	55.120	57.419	389
MU717	trinucleotide	(AGA)4	13	54.965	54.886	390
MU719	trinucleotide	(CAA)6	19	54.741	54.349	226
MU728	trinucleotide	(ACA)6	19	55.040	54.664	244
MU75	trinucleotide	(CCA)4	12	55.335	55.013	365
MU769	trinucleotide	(TTC)6	18	55.636	54.949	256

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
MU769	trinucleotide	(GGA)4	14	54.913	54.949	147
MU776	trinucleotide	(AGA)4	12	54.365	54.809	336
MU782	dinucleotide	(AT)13	26	54.097	55.156	229
MU794	dinucleotide	(AT)5	11	54.937	55.865	246
MU798	trinucleotide	(ACG)7	21	56.523	54.765	218
MU8	tetranucleotide	(TGTT)3	13	55.140	58.917	233
MU811	trinucleotide	(TAA)4	12	53.114	54.879	236
MU82	trinucleotide	(TCT)4	14	56.206	55.135	394
MU823	trinucleotide	(GGT)6	18	55.313	54.705	342
MU823	trinucleotide	(GTG)4	14	55.313	54.705	342
MU823	trinucleotide	(GGT)4	12	55.313	54.705	342
MU826	trinucleotide	(AAC)4	12	53.760	54.691	356
MU842	trinucleotide	(GAT)4	13	55.252	56.290	193
MU845	trinucleotide	(ATA)4	14	55.753	55.349	397
MU86	pentanucleotide	(TTCCT)5	29	57.464	54.946	117
MU89	trinucleotide	(CTT)5	16	55.024	54.770	373
SSH2K24	dinucleotide	(TG)8	17	55.944	55.670	114
SSH4L20	trinucleotide	(CAG)4	14	56.097	54.959	200
SSH4L22	tetranucleotide	(ATAC)3	14	57.225	56.710	101
SSH4O13	tetranucleotide	(ACAT)3	13	60.700	57.452	120
SSH6D16	tetranucleotide	(AGCA)4	17	55.383	54.959	145
SSH6D19	dinucleotide	(AG)5	11	55.500	57.514	203
SSH6E9	trinucleotide	(AGG)6	20	55.300	55.128	284
SSH6E9	trinucleotide	(CTC)6	18	55.168	57.698	243
SSH6G3	trinucleotide	(TGA)4	12	54.899	54.884	302
SSH6G3	trinucleotide	(TGA)4	12	54.899	54.884	302
SSH6H1	trinucleotide	(CCA)4	12	55.577	54.917	111
SSH6I22	dinucleotide	(TC)8	16	55.095	55.406	226
SSH6I23	trinucleotide	(CTT)9	28	54.176	54.873	302
SSH6I23	trinucleotide	(CAT)7CAA(CAT)2	32	55.649	57.188	160
SSH6I5	trinucleotide	(TCC)4	14	54.886	54.913	310
SSH6J20	pentanucleotide	(GAAAT)2	14	55.135	56.460	386
SSH9H11	trinucleotide	(TCT)4	12	55.083	54.803	160
SSH9H22	trinucleotide	(AAG)11	33	55.077	54.886	129
FR10D12	trinucleotide	(GAA)3GGA(GAA)2	19	54.899	55.539	297
FR10D12	trinucleotide	(GAC)7	23	54.899	55.539	297
FR10E10	trinucleotide	(GAA)4	12	55.002	55.042	170
FR10E8	tetranucleotide	(AACC)3	13	54.923	55.906	328

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
FR10F2	trinucleotide	(CCG)5	15	55.228	55.240	221
FR10G8	dinucleotide	(TC)5	11	53.674	53.785	237
FR10J13	trinucleotide	(CCT)3CCA(CCT)2	20	54.143	54.829	170
FR10J16	dinucleotide	(CT)9	18	53.182	55.504	282
FR10J21	trinucleotide	(CCA)6	19	55.580	55.192	170
FR10K21	trinucleotide	(AAG)4	12	54.936	55.872	119
FR10M21	trinucleotide	(CAG)4	14	54.727	55.897	162
FR10M21	trinucleotide	(ACA)4	12	54.727	55.897	162
FR10P14	trinucleotide	(AAG)4	13	54.647	55.584	221
FR10P14	trinucleotide	(GAA)6	20	55.478	54.189	348
FR11A2	trinucleotide	(CCT)4	14	54.994	55.573	280
FR11A2	trinucleotide	(TTG)4	13	54.994	56.374	334
FR11A20	pentanucleotide	(TTCTC)2	14	54.696	53.561	305
FR11H14	pentanucleotide	(CTTCC)3	16	55.303	54.961	171
FR11J18	trinucleotide	(CAA)4	12	55.215	54.848	171
FR11N15	dinucleotide	(AT)6	12	54.909	54.097	323
FR11P14	pentanucleotide	(AAAAG)3	19	56.459	55.228	228
FR12A1	trinucleotide	(TTG)4	14	54.924	55.092	250
FR12A22	pentanucleotide	(TATTT)4	21	55.184	55.886	163
FR12B18	trinucleotide	(AAG)9	27	53.962	55.468	193
FR12C7	trinucleotide	(GAT)4	14	54.642	54.971	228
FR12D22	trinucleotide	(GGC)4	12	55.297	55.040	332
FR12E8	trinucleotide	(AAG)4	12	54.913	54.587	188
FR12F12	trinucleotide	(GGT)4	12	54.915	56.099	235
FR12I11	trinucleotide	(GGA)5	15	55.377	55.173	327
FR12I13	trinucleotide	(TGT)4	13	55.176	55.147	274
FR12J12	trinucleotide	(TTC)9	28	54.994	55.852	136
FR12J13	trinucleotide	(AGA)4	12	55.998	55.000	368
FR12J20	dinucleotide	(AT)15	30	54.840	55.783	298
FR12L14	trinucleotide	(AGA)4	14	55.030	55.193	324
FR12M8	dinucleotide	(AG)6	13	55.793	55.778	339
FR12N13	trinucleotide	(CAC)4	12	54.971	55.057	288
FR13F20	trinucleotide	(GAT)GAG(GAT)3	38	55.401	54.696	329
FR13G13	trinucleotide	(GCA)4	12	55.057	60.945	245
FR13G3	tetranucleotide	(TTTA)3	15	55.458	54.987	165
FR13G3	trinucleotide	(GGA)5	15	55.458	54.657	211
FR13J1	trinucleotide	(GGA)5	15	54.937	54.722	128

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
FR13J10	trinucleotide	(TAA)5	16	54.809	55.070	237
FR13J11	pentanucleotide	(CTCCC)3	17	56.601	55.047	335
FR13L24	trinucleotide	(TTC)3TCC(TTC)4	24	54.954	54.097	223
FR13N10	trinucleotide	(GGT)5	15	54.994	55.240	396
FR13P1	dinucleotide	(GA)6	12	55.189	54.789	222
FR13P9	trinucleotide	(TCA)15	47	56.178	55.240	312
FR14B2	dinucleotide	(TG)5	11	54.045	55.161	101
FR14B2	dinucleotide	(TA)9	18	54.045	54.785	327
FR14B2	dinucleotide	(TC)10	21	55.372	55.192	287
FR14D15	dinucleotide	(TA)6	12	55.050	53.969	249
FR14D18	trinucleotide	(CTT)4	13	54.783	55.140	219
FR14D19	pentanucleotide	(ATTTC)3	15	53.923	55.024	226
FR14E9	trinucleotide	(CTT)4	12	54.913	55.276	346
FR14E9	pentanucleotide	(AAAAT)2	14	54.913	55.276	346
FR14G1	trinucleotide	(TGA)4	13	55.152	55.341	372
FR14G19	pentanucleotide	(CTCTT)3	17	55.120	54.976	352
FR14G19	trinucleotide	(GAC)9	27	55.120	54.976	352
FR14G2	trinucleotide	(AGA)4	14	55.123	55.152	176
FR14H10	trinucleotide	(CAA)8	24	54.375	55.474	356
FR14H10	trinucleotide	(CCT)5	15	55.474	57.482	166
FR14H23	trinucleotide	(TCT)5	15	55.013	55.103	281
FR14H5	trinucleotide	(CGG)5	16	54.803	55.045	216
FR14I9	tetranucleotide	(TTAT)3	14	54.239	55.941	346
FR14K5	trinucleotide	(GAA)6	20	55.264	55.103	372
FR14L1	trinucleotide	(CAG)5	17	54.937	54.937	295
FR14M14	trinucleotide	(CAA)4	12	54.968	55.031	144
FR14N19	trinucleotide	(TCG)4	12	54.913	54.928	398
FR14N24	pentanucleotide	(TGCCT)2	14	55.313	54.994	185
FR14O8	tetranucleotide	(TCCT)3	14	57.698	55.090	207
FR14P17	pentanucleotide	(TTTTC)2	14	54.611	54.440	370
FR15B22	trinucleotide	(TGA)4	13	55.057	54.327	170
FR15B8	trinucleotide	(GAA)5	16	53.689	56.726	373
FR15C21	dinucleotide	(TG)6	12	54.899	54.909	199
FR15C5	trinucleotide	(GAT)4	12	54.852	54.829	181
FR15E23	trinucleotide	(TCT)4	14	56.206	55.135	222
FR15G15	trinucleotide	(ACG)4	12	55.204	54.976	197
FR15G17	trinucleotide	(TCT)5	16	55.119	54.989	281
FR15G24	trinucleotide	(GCC)4	12	54.772	54.919	191

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
FR15G24	trinucleotide	(TGC)4	13	54.772	54.919	191
FR15H5	trinucleotide	(ATC)6	18	60.414	54.315	109
FR15J5	trinucleotide	(CTG)4	12	57.396	55.013	342
FR15K24	trinucleotide	TTC)4	12	54.470	55.586	134
FR15L18	trinucleotide	(CTT)3CTC(CTT)CTC	40	54.667	55.171	175
FR15L21	trinucleotide	(CTC)4	13	56.381	55.024	220
FR15L8	trinucleotide	(GAA)4	12	64.724	55.140	174
FR15L8	trinucleotide	(GAA)4	14	55.332	55.140	117
FR15M13	trinucleotide	(AGC)4	12	54.959	57.999	355
FR15M21	trinucleotide	(AGA)4	13	54.979	54.950	166
FR15O18	tetranucleotide	(ATTC)3	15	55.084	54.437	356
FR15P21	tetranucleotide	(TTGC)5	21	57.292	55.252	185
FR16A10	trinucleotide	(TTC)9	29	55.216	54.500	220
FR16A20	trinucleotide	(CCG)4	14	55.103	55.075	211
FR16C17	dinucleotide	(AG)5	11	55.216	54.955	289
FR16D11	tetranucleotide	(TTTG)2TTTC(TTTG)2	23	55.501	55.449	347
FR16D11	trinucleotide	(TTG)6	18	55.823	56.123	371
FR16E19	trinucleotide	(TCC)7	21	55.573	55.011	370
FR16F6	trinucleotide	(AAG)4	12	55.205	54.586	217
FR16G10	trinucleotide	(AGA)6GA(AGA)	25	54.979	54.982	230
FR16G10	trinucleotide	(GAT)4	12	54.982	58.298	164
FR16G19	dinucleotide	(TA)10	20	55.157	55.024	290
FR16G7	tetranucleotide	(AGTC)3	13	54.551	55.047	333
FR16I18	trinucleotide	(TTG)4	12	55.000	55.112	290
FR16I3	trinucleotide	(TAA)4	13	56.180	55.327	185
FR16J10	tetranucleotide	(TTAG)3	15	54.989	54.324	119
FR16J21	trinucleotide	(CTT)6	19	54.974	54.891	207
FR16J21	trinucleotide	(TGA)4	13	54.891	55.176	292
FR16J4	trinucleotide	(ATT)4	14	55.111	55.159	393
FR16N20	trinucleotide	(GAA)20	61	55.443	55.468	296
FR17A10	trinucleotide	(ATG)4	12	54.806	54.994	289
FR17B20	trinucleotide	(TCC)4	12	55.312	55.002	168
FR17C13	trinucleotide	(TAT)4	13	55.190	58.909	303
FR17E16	trinucleotide	(TCT)6	19	54.908	55.181	227
FR17E6	trinucleotide	(AAG)4	12	55.881	60.312	104
FR17F15	trinucleotide	(CGG)5	15	55.090	54.751	174
FR17G14	trinucleotide	(GAA)4	13	55.060	54.426	206

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
FR17G24	trinucleotide	(TCT)4	12	54.997	55.033	337
FR17I1	trinucleotide	(AAG)5	16	54.923	55.052	366
FR17I20	tetranucleotide	(CTTC)3	13	55.039	55.033	285
FR17I20	pentanucleotide	(TTTTC)4	22	55.033	55.027	363
FR17I24	trinucleotide	(CAC)4	12	55.090	55.203	399
FR17J20	dinucleotide	(AT)6	13	55.265	56.884	209
FR17J20	dinucleotide	(AG)7	14	55.265	56.884	209
FR17J23	trinucleotide	(GGC)6	20	55.228	55.539	187
FR17K24	dinucleotide	(AT)7	14	54.971	56.196	290
FR17O7	trinucleotide	(CGA)4	14	55.178	54.886	370
FR17O8	trinucleotide	(GCT)4	14	54.913	54.829	284
FR17O8	trinucleotide	(GGA)5TGAAGAGGA	43	54.913	54.829	284
FR18A4	trinucleotide	(CAG)4	12	55.299	55.493	151
FR18C17	dinucleotide	(AG)5AA(AG)2AA(AG)	35	54.994	54.334	185
FR18D20	trinucleotide	(AGA)7	22	55.406	54.660	107
FR18E12	trinucleotide	(AGC)5	16	54.974	55.276	147
FR18F15	trinucleotide	(ATT)6	19	55.070	55.449	174
FR18F20	dinucleotide	(GA)11	23	55.844	55.024	332
FR18J9	dinucleotide	(TC)6	12	55.424	55.169	303
FR18K21	trinucleotide	(TCT)10	32	55.463	55.066	331
FR18L15	trinucleotide	(GGC)4	14	54.913	58.001	166
FR18L16	dinucleotide	(CT)4TT(CT)4	18	60.134	54.967	371
FR18M12	trinucleotide	(TTC)7	22	55.383	56.581	347
FR18P24	trinucleotide	(GAG)4	12	54.974	55.020	256
PH12C1	trinucleotide	(TTA)4	14	55.590	58.920	394
PH2B11	trinucleotide	(AGA)5	16	54.636	54.362	349
PH2B11	trinucleotide	(AGA)4	12	54.636	54.362	349
PH2B11	trinucleotide	(AGA)6	19	54.636	54.362	349
PH2E1	pentanucleotide	(CAATC)6	34	54.946	55.080	242
PH2E10	dinucleotide	(TC)7	15	55.215	61.277	218
PH2E10	dinucleotide	(AT)5	11	55.215	61.277	218
PH2H9	trinucleotide	(AAG)4	12	55.203	55.043	252
PH3B4	dinucleotide	(AG)11	23	53.962	55.888	373
PH3B6	tetranucleotide	(TTTC)4	19	55.027	53.869	250
PH3B8	trinucleotide	(GAA)6	18	54.765	56.576	246
PH3F4	trinucleotide	(TGA)4	12	54.937	56.187	160

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
PH3F10	dinucleotide	(CT)5	11	55.887	54.865	390
PH3H5	trinucleotide	(GAA)4	12	56.434	53.629	367
PH3H6	trinucleotide	(ACC)4	12	55.313	54.982	361
PH3H12	dinucleotide	(AT)6	12	54.848	55.057	219
PH4B7	tetranucleotide	(TTTC)5TTAC(TTTC)2	34	55.264	54.416	387
PH4B7	pentanucleotide	(TATTT)2	14	55.264	54.416	387
PH4D12	tetranucleotide	(TTTG)3	15	55.491	56.326	341
PH4E2	pentanucleotide	(AAAGA)4	24	54.350	55.145	244
PH4E4	trinucleotide	(TTC)4	14	55.951	55.015	327
PH4E4	trinucleotide	(TTA)4	14	55.951	55.015	327
PH4E12	pentanucleotide	(AAAAC)3	19	53.122	54.842	208
PH1D2	trinucleotide	(CAT)4	12	54.765	55.075	237
PH5A1	trinucleotide	(TCT)6	18	54.691	54.765	141
PH5A1	trinucleotide	(TTC)4	13	54.691	57.483	185
PH5B5	dinucleotide	(AG)7	14	57.577	56.285	265
PH5C11	pentanucleotide	(GAAGT)5	26	54.952	54.905	390
PH5C11	trinucleotide	(TAT)4	12	54.952	54.905	390
PH5D7	trinucleotide	(CAA)7	22	55.103	55.218	203
PH5D8	pentanucleotide	(AAAAC)2	14	54.965	54.824	161
PH1D7	trinucleotide	(ACA)8	25	54.990	54.959	279
PH5G5	pentanucleotide	(TTTTC)3	14	55.491	55.057	179
PH6B1	trinucleotide	(GAA)6	18	54.440	54.971	237
PH1E6	trinucleotide	(AAG)4	12	55.189	55.089	272
PH6G1	trinucleotide	(AAG)5	17	54.417	54.885	262
PH7B3	trinucleotide	(CTG)4	13	54.828	54.899	280
PH7E3	trinucleotide	(TCT)6	18	52.295	55.118	269
PH1F3	trinucleotide	(CTG)4	14	55.203	55.070	391
PH7G8	trinucleotide	(TCT)3TT(TCT)2	18	55.014	57.639	237
PH7H9	pentanucleotide	(ATATG)3	16	54.953	55.018	295
PH7H12	pentanucleotide	(TTTTC)3	16	58.773	54.842	139
PH7H12	dinucleotide	(CT)10	20	58.773	54.842	139
PH7H12	dinucleotide	(AC)6AT(AC)7	29	58.773	54.691	353
PH7H12	pentanucleotide	(AAAAC)2	14	54.842	54.691	234
PH7H12	trinucleotide	(ATC)5	16	54.842	54.691	234
PH8C1	trinucleotide	(CTT)11	34	55.070	54.952	243
PH8E7	trinucleotide	(AGA)4	12	55.103	58.414	368
PH8E7	trinucleotide	(TGA)4	12	55.103	58.414	368

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
PH8F4	trinucleotide	(TCG)4	14	55.018	57.871	127
PH8F8	pentanucleotide	(ATATG)3	16	54.953	55.018	373
PH8F10	trinucleotide	(ATA)4GTA(ATA)2	23	55.024	55.083	280
PH8G11	dinucleotide	(CT)10	21	55.435	55.037	246
PH1G4	tetranucleotide	(ATTT)6	27	56.915	52.947	161
PH1G4	trinucleotide	(TAT)4	13	52.947	54.765	282
PH1G4	dinucleotide	(AT)8	17	52.947	54.765	282
PH9A10	tetranucleotide	(TAAT)3	15	55.414	55.007	384
PH9H7	trinucleotide	(AGA)4	14	55.476	54.944	286
PH10A9	trinucleotide	(TTC)6	19	56.195	55.135	341
PH10B11	trinucleotide	(TCG)5	17	54.913	55.118	251
PH10E11	trinucleotide	(AGA)5	16	53.237	57.451	107
PH10E11	trinucleotide	(AGA)5C(AGA)2GAAG	37	55.881	53.965	137
PH10E11	trinucleotide	(AGA)4	12	55.881	53.965	137
PH10G1	trinucleotide	(TAG)4	12	55.313	54.685	323
PH10G7	trinucleotide	(CAC)4	13	60.582	55.077	161
PH10G7	trinucleotide	(GAA)4	13	55.406	55.070	364
PH10G7	dinucleotide	(GT)6	12	55.077	55.070	319
PH11B6	trinucleotide	(AAT)4	12	55.903	55.465	105
PH11B11	trinucleotide	(ACA)4	13	55.015	55.123	220
PH11B11	trinucleotide	(CAA)5	17	55.070	56.025	208
PH13E7	trinucleotide	(AAG)4	12	55.372	54.959	229
PH14E8	trinucleotide	(TGG)5	17	66.038	55.394	327
PH14E8	trinucleotide	(TCT)4	13	54.946	56.627	396
PH14G7	dinucleotide	(TA)6	13	55.007	54.937	332
PH14G8	pentanucleotide	(TGATA)2	14	54.817	65.225	281
PH14H1	dinucleotide	(TC)6	13	60.134	54.937	393
PH14H9	dinucleotide	(CT)9	18	55.332	55.341	181
PH15B5	tetranucleotide	(GCAA)3	13	57.765	54.937	137
PH15C5	trinucleotide	(AGA)4	12	54.796	53.709	214
PH15E3	pentanucleotide	(GGGGC)3	17	57.104	54.979	196
PH15E3	trinucleotide	(TCT)4	13	54.595	54.979	138
PH15F3	trinucleotide	(GAG)4	12	54.994	54.190	395
PH15H10	trinucleotide	(TCT)7	23	56.761	55.128	371
PH16A7	pentanucleotide	(TTTTC)3	19	55.776	55.001	241
PH16A7	tetranucleotide	(CCAA)3	15	55.776	55.001	241
PH16E11	tetranucleotide	(CAAA)2CAGA(CAAA	20	55.715	55.239	285
PH16H5	dinucleotide	(GA)15	30	63.878	54.416	194

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Table A-1. (cont.) All SSR primers from Melon unigenes with sequence ID, repeat type, repeat number, length, left TM, right TM and product size.

Sequence ID	Repeat Type	Repeat Number	Length	Left TM	Right TM	Product Size
PH17D7	trinucleotide	(TTC)11	33	54.937	59.720	209
PH17F4	trinucleotide	(ACA)5	16	56.377	55.152	169
PH17G11	pentanucleotide	(TTTTC)2	14	54.573	54.411	163
PH17H4	dinucleotide	(TC)18	36	53.905	54.728	357
PH17H4	tetranucleotide	(TGTT)3	13	55.016	54.728	346
PH17H4	trinucleotide	(AGA)4	16	55.016	54.728	346
PH17H9	pentanucleotide	(TCTTT)2	30	54.699	55.043	236
PH18A4	tetranucleotide	(TTTC)3	13	55.463	54.003	148
H18D3	dinucleotide	(GA)6	13	54.586	55.334	211
PH18G1	trinucleotide	(GAA)5	17	54.719	55.877	160
PH18G1	trinucleotide	(TGA)4	12	54.719	60.469	279
PH18G5	trinucleotide	(CAT)4	14	55.070	55.174	254
PH18G5	trinucleotide	(CAA)5	15	55.070	55.468	312
PH18H4	trinucleotide	(AGA)4	13	54.994	54.783	255
PH19F7	trinucleotide	(ATC)5	16	56.844	55.190	129
PH19F12	pentanucleotide	(TTGGA)4	22	54.961	54.657	243
PH19H12	trinucleotide	(GAA)4	12	55.020	56.824	212
PH19H12	trinucleotide	(TGA)4	12	55.020	56.824	212
PH20B8	trinucleotide	(TCA)4	12	54.721	54.827	385
AB125974	trinucleotide	(GCT)4	12	55.083	55.033	342
AF030382	trinucleotide	(CGT)6	19	54.678	55.060	361
AF030382	dinucleotide	(CT)21	43	55.041	54.761	269
AF030384	trinucleotide	(TTC)4	13	55.033	55.055	239
AF062466	trinucleotide	(ACT)4	14	54.939	55.194	280
AF062466	pentanucleotide	(TGTAA)3	16	55.127	55.449	351
AF081953	dinucleotide	(TA)11	23	55.481	55.414	157
AF081953	tetranucleotide	(TTCT)3	13	55.481	58.187	171
AY288911	trinucleotide	(TTC)4	12	56.647	54.913	153
X76130	trinucleotide	(TCT)4	13	54.346	59.549	184

Appendix B

MORPHOLOGICAL CRITERIA

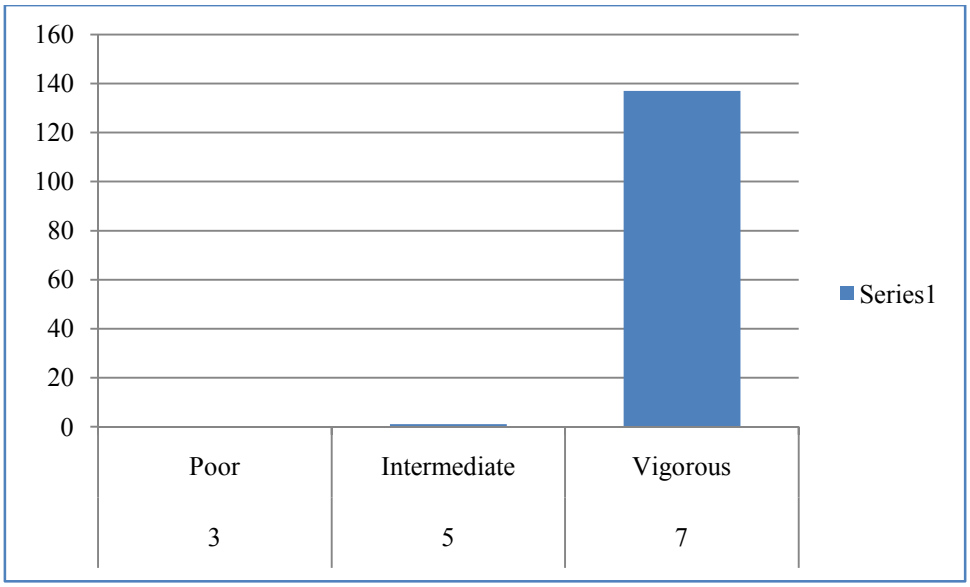

Figure B-1. Seedling Vigour

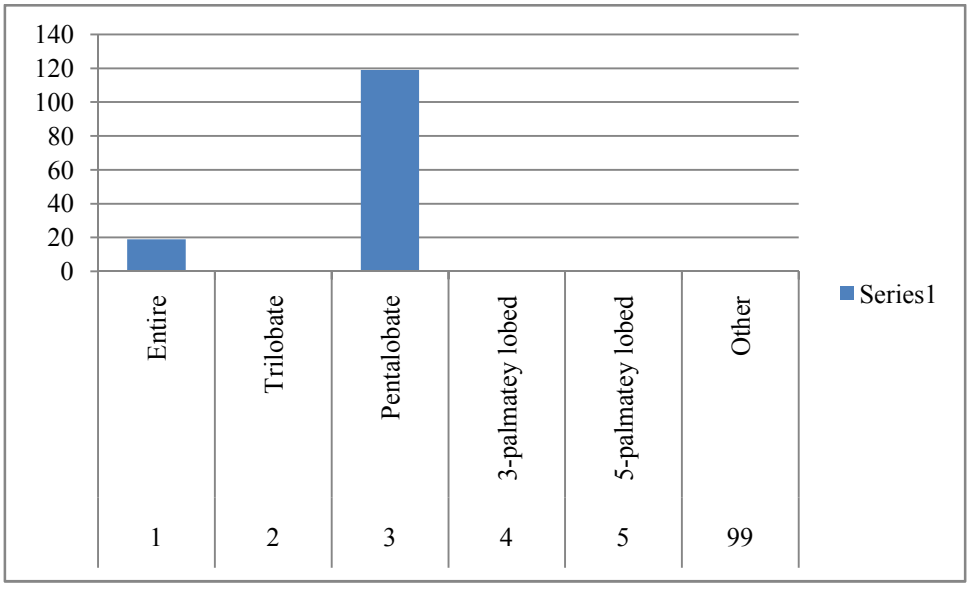

Figure B-2. Leaf Shape

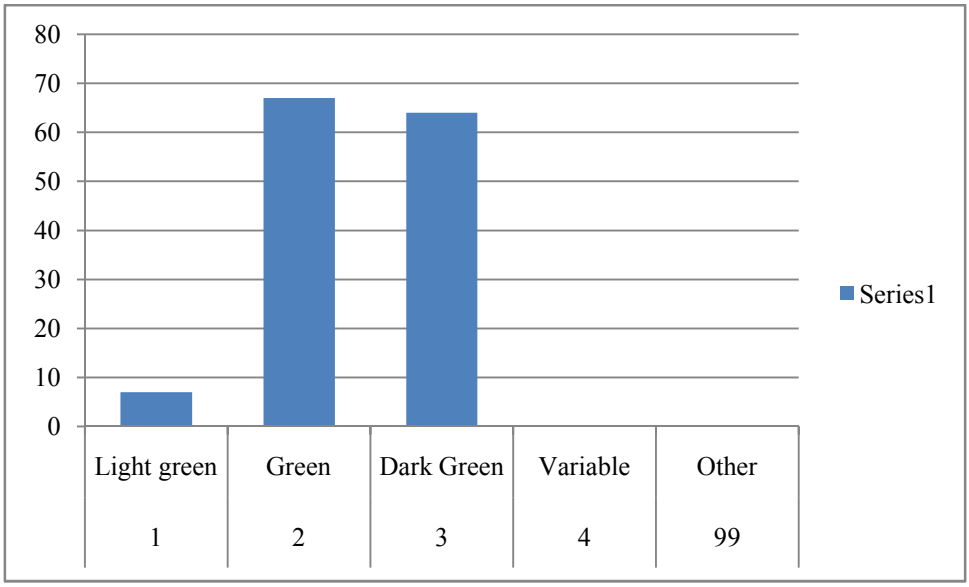

Figure B-3. Leaf Colour

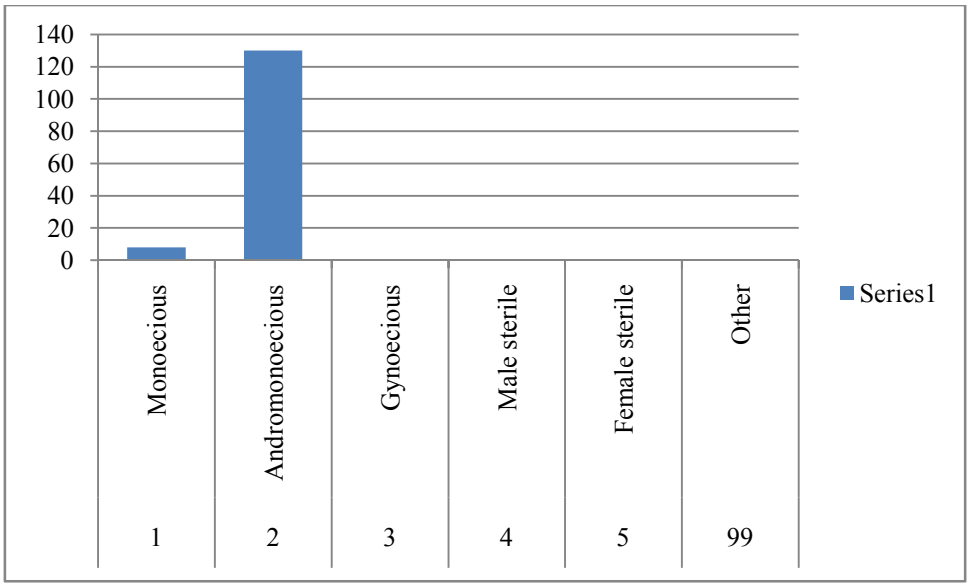

Figure B-4. Sex Type

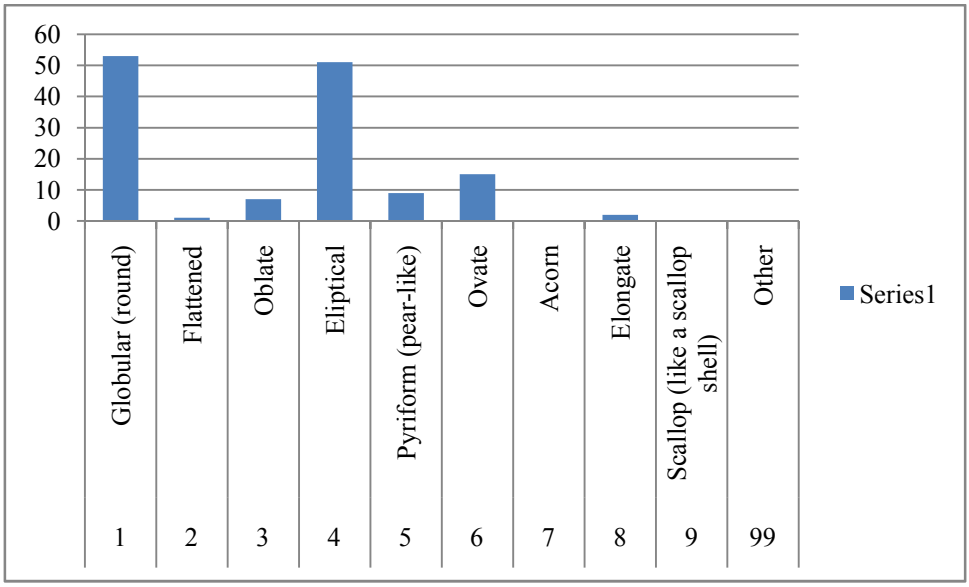

Figure B-5. Fruit shape

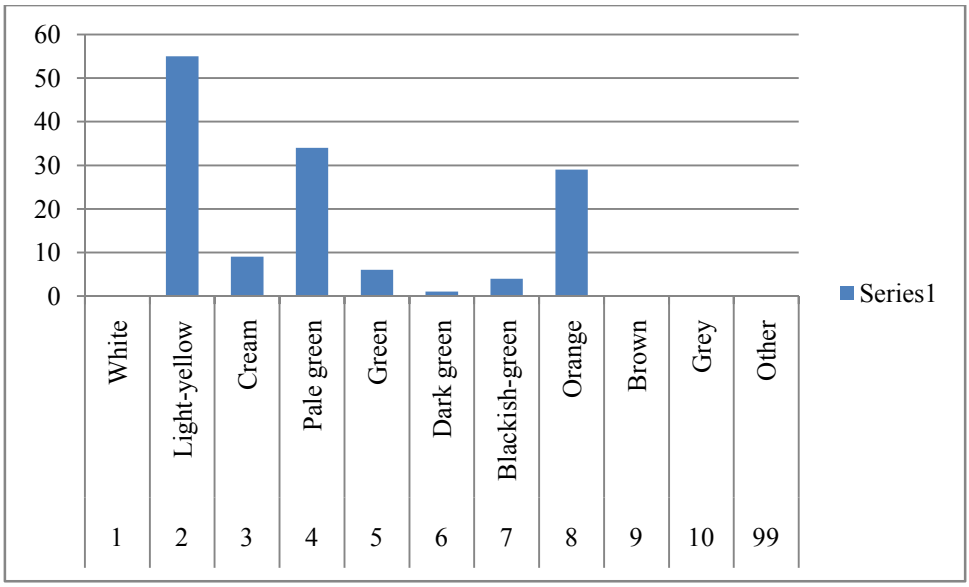

Figure B-6. Predominant Fruit Skin Color

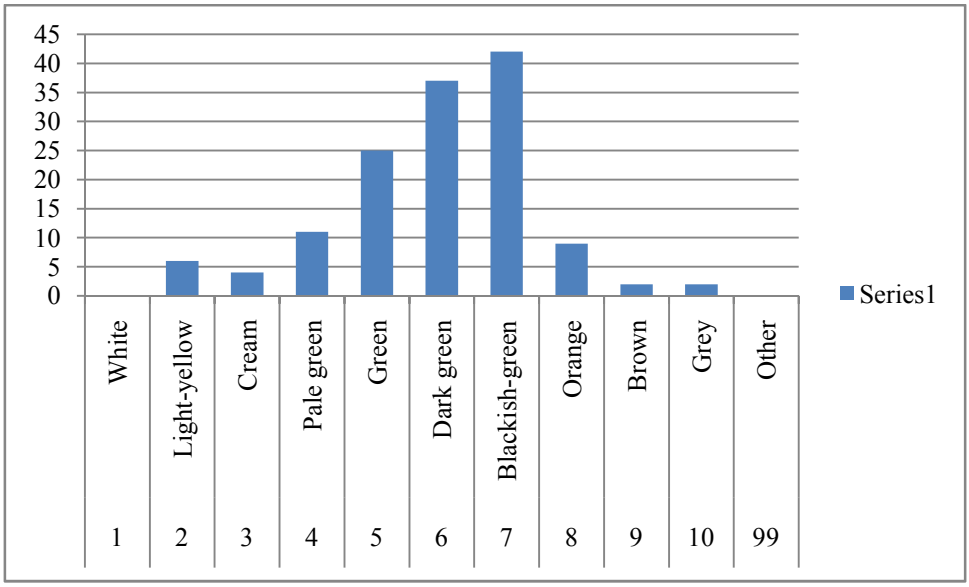

Figure B-7. Secondary Fruit Skin Colour

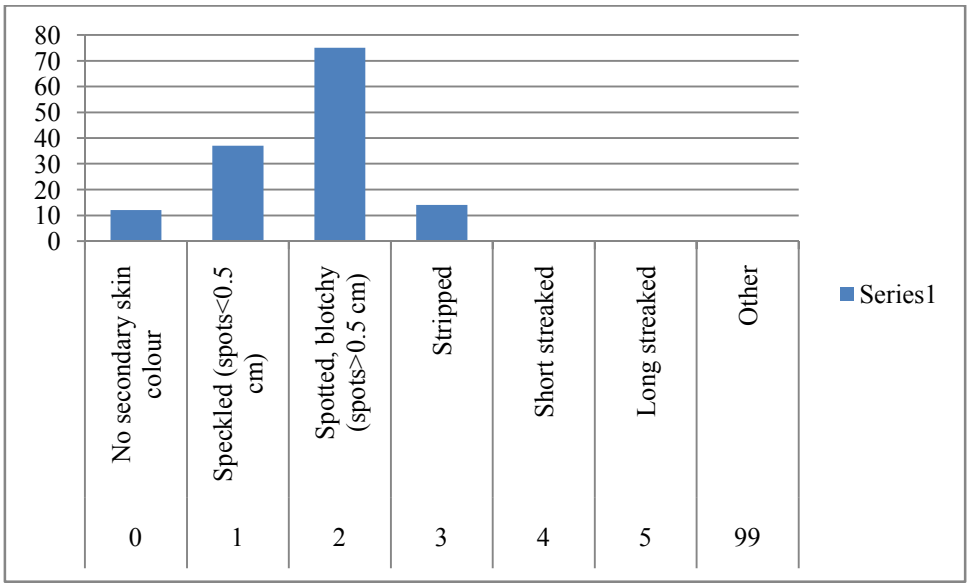

Figure B-8. Secondary Skin Color Pattern

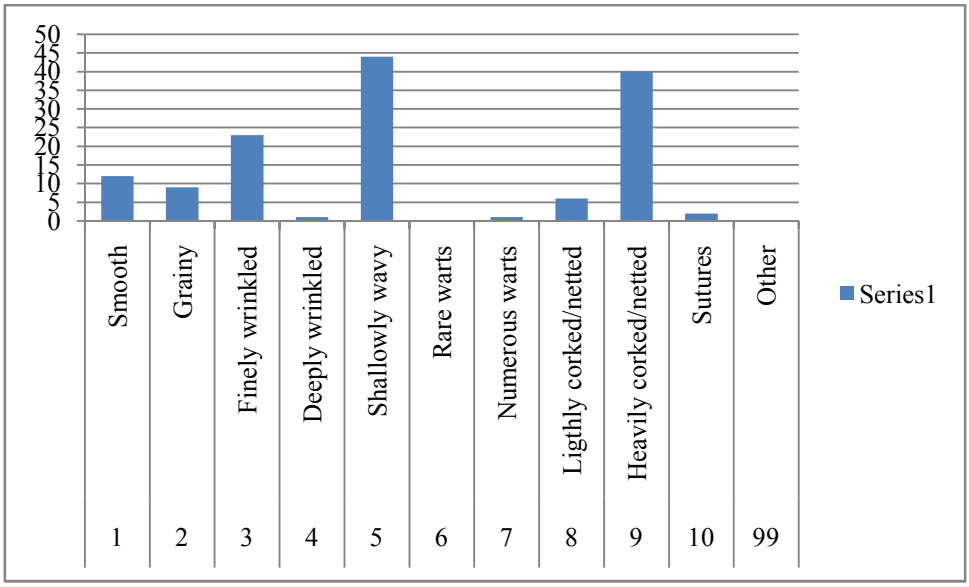

Figure B-9. Fruit Surface

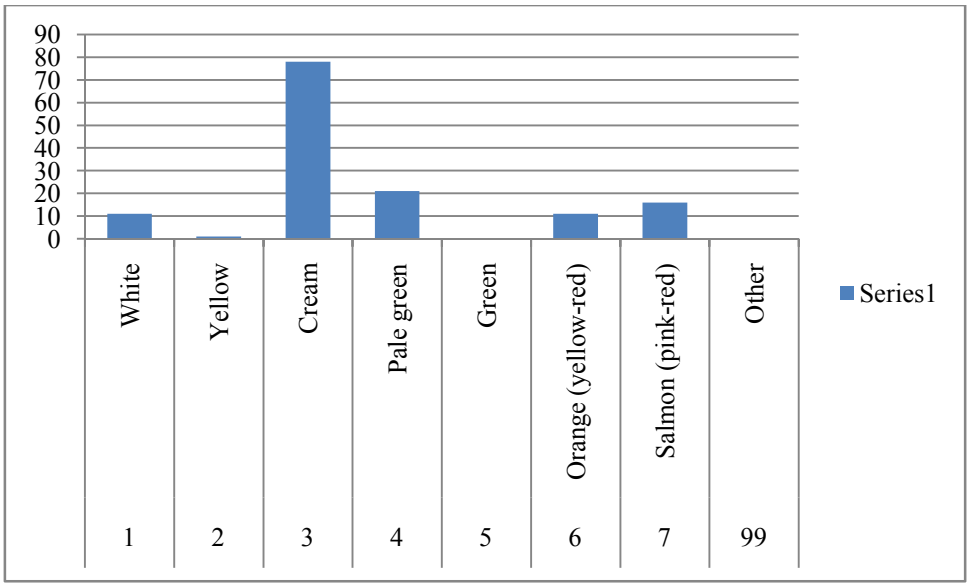

Figure B-10. Main Colour of Flesh

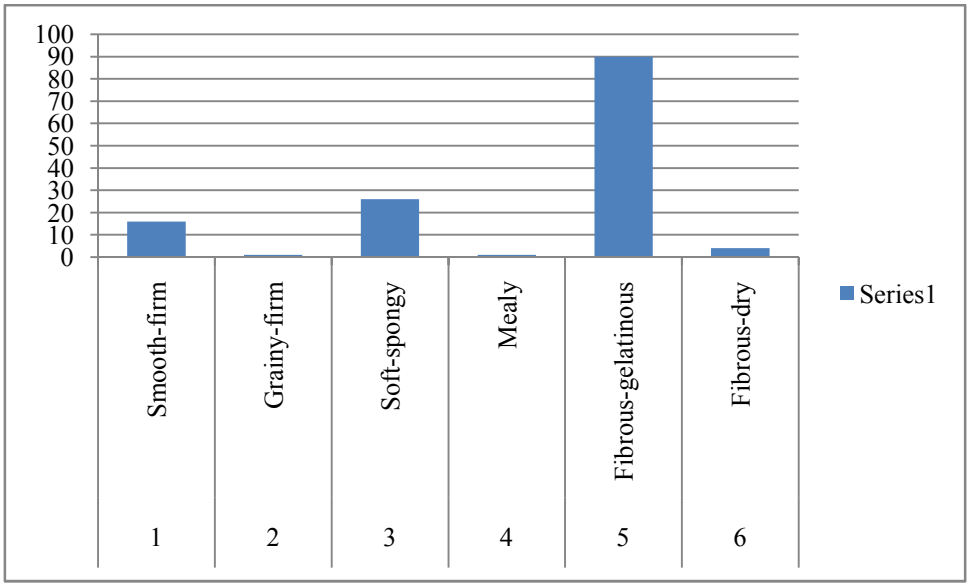

Figure B-11. Flesh Texture

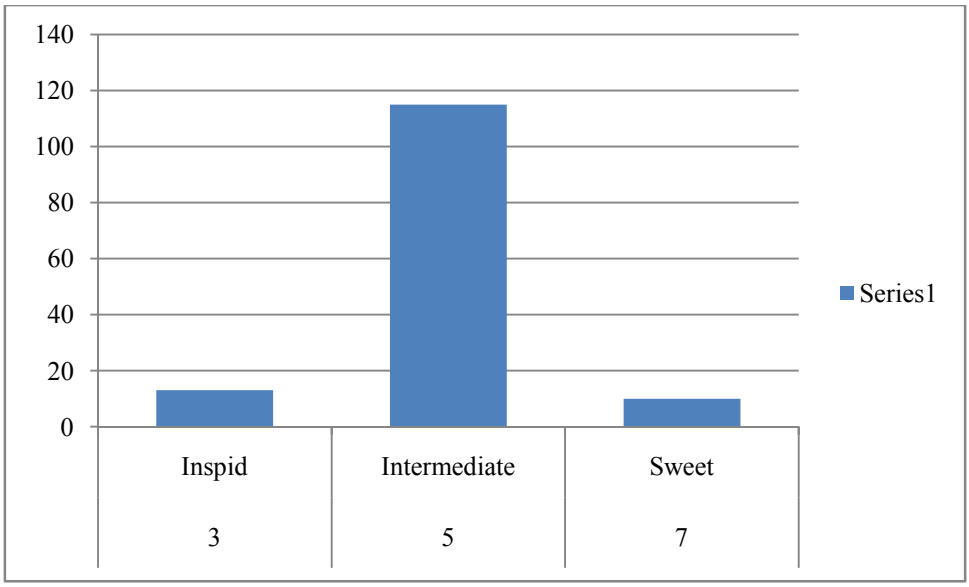

Figure B-12. Flesh Flavour

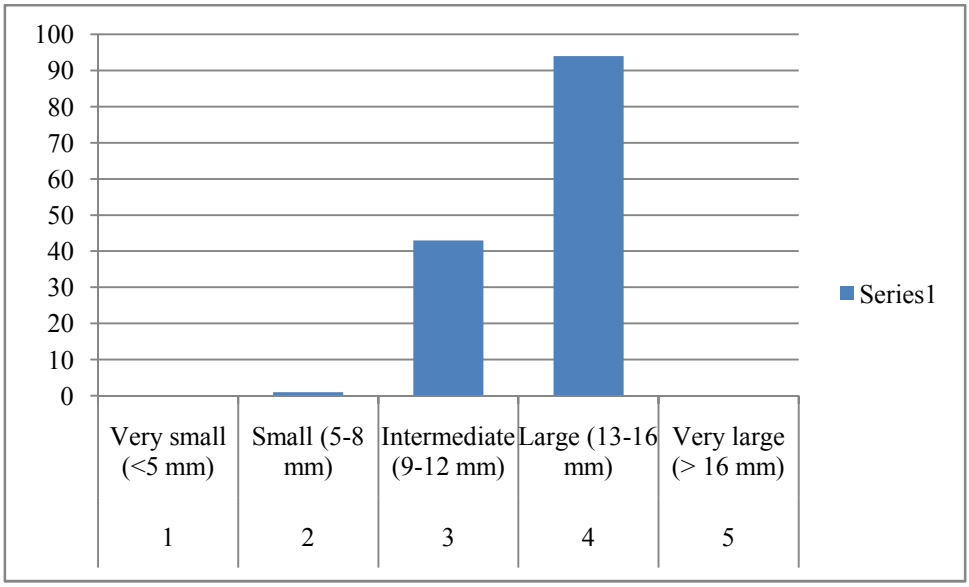

Figure B-13. Seed Size