An-Najah National University

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Assessment of biodiversity among Palestinian landraces of *Cucumis melo* L. groups based on morphological descriptors and molecular markers (RAPD and ISSR)

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Dedication

I dedicate my thesis to Allah.

I dedicate my thesis also to my family. A Special gratitude for my loving parents, Aysha & Bassam Mallah for their encouragement and support. Also I dedicate this work to my brothers, my wife "Alaa", and to my sons Jehad & Batool.

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إقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Assessment of biodiversity among Palestinian landraces of *Cucumis melo* L. groups based on morphological descriptors and molecular markers (RAPD and ISSR)

دراسة التنوع الحيوي للأصناف البلدية الفلسطينية لمجموعات *Cucumis melo* L.
باستخدام واصفات مورفولوجية و الكاشفات الجزيئية (RAPD , ISSR)

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل أي جزء منها لم يقدم من قبل لنيل أية درجة أو لقب علمي أو بحث لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced. Is the researcher's own work and has not been submitted from anywhere else, for any other degree or qualification.

Student's name:	**اسم الطالب:**
Signature:	**التوقيع:**
Date:	**التاريخ :**

Table of Contents

List of Tables

List of Figures

List of abbreviations

IPGRI	International Plant Genetic Resources Institute
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
RE	Restriction Enzymes
DNA	Deoxyribonucleic acid
cDNA	Complementary DNA
AFLP	Amplified Fragment Length Polymorphism
RAPD	Random Amplified Polymorphic DNA
UV	Ultraviolet
Kb	Kilo base pair
ISSR	Inter Simple Sequence Repeats
SSR	Simple sequence Repeat
VNTR	Variable Number Tandem Repeat
SNPs	Single Nucleotide Polymorphisms
UAWC	Union of Agricultural Work Committees
CSB	Community-Based Seed Bank
BERC	Biodiversity and Environmental Research Center
ARIJ	Applied Research Institute- Jerusalem
CTAB	Cetyl Trimethyl Ammonium Bromide
Tris	Trisamine
HCL	Hydrochloric acid
EDTA	Ethylenediamine tetraacetic acid
DTT	DL-Dithiothreitol
CI	Chloroform: isoamyl alcohol
EtOH	Ethyl alcohol
sdH2O	Sterile distilled water
µg	Microgram
µl	Microliter
ml	Milliliter
ng	Nanogram
TAE	Tris base, acetic acid and EDTA
dNTP	Deoxynucleotide
MgCl2	Magnesium chloride
KCl	Potassium chloride
BSA	Bovine Serum Albumin
Rp	Resolving power
Ib	Band informativeness
AvIb	Average band informativeness
Cm	Centimeter
Mm	millimeter
PCBS	Palestinian Central Bureau of Statistics

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Abstract

Background: Economically; melons (snake cucumber and cantaloupes) are important crops cultivated in Palestine. Traditional melons are rain-fed crops. Although melons are differ in morphological traits such as shape, fruit color, taste, and flavor, low genetic variations between these crops is present.

Objectives: The aims of this study are to study the genetic variations between and within melon groups in Palestine using genetic markers (RAPD & ISSR), and to determine the relationships between molecular and morphological characterization, also to evaluate the efficiency of RAPD and ISSR genetic markers in discriminating between and within landraces of melon groups.

Methods: Biodiversity among 44 Palestinian landraces of melon was studied using RAPD and ISSR genetic primers, and morphological descriptors. Similarity matrixes and dendrograms were generated using SPSS (version 16) software. Resolving Power (Rp) was calculated for each primer.

Results: Morphological descriptors separated melons into two ‘groups’, Fakus (*flexuosus*) with two phenotypic subgroups (white and green), and *cantalupensis*.

From 14 RAPD primers used 132 bands were amplified, 75 bands were polymorphic (57%) and 57 were monomorphic (43%). Cluster analysis by RAPD results divided Palestinian melons into two clusters: Cluster I (contain all *flexuosus* accessions) and cluster II (contain all *cantalupensis* accessions). The highest similarity between *flexuosus* and *cantalupensis* accessions by RAPD primers was 0.86.

Nine ISSR primers produced 71 bands; all bands were monomorphic, so that there are no genetic variations revealed between melon accessions by ISSR primers. This indicated the highly genetic similarity between these groups.

Conclusions: RAPD primers proved efficient in discriminating between Palestinian melon groups, and gave an indications or marks about genetic variations within *Flexuosus* accessions. No genetic variations between Palestinian melon groups were observed when ISSR primers were used.
Results strongly indicated the importance of study the origin and diversity of Palestinian landraces of melons.

CHAPTER ONE

INTRODUCTION

1.1 Importance of melon and other cucurbit crops in Palestine.

The *Cucurbitaceae* family includes 118 genera and 825 species. The most economically important crop species are melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus*) and members of the genus *Cucurbita* L., including summer and winter squash, pumpkins, and gourds (Jeffrey, 1980).

Cucurbit crops are widely consumed in large quantities in the traditional diet and grown over a large area of the Middle East. In Palestine, cucurbit crops are the most widely grown vegetables (PCBS, 2010) (Table 1.1).

Table 1.1 Cucurbit crops area (donum) and production (ton/year) in Palestine.

Crop	Total Area (donum*)	Total production (Ton)
Cucumber	32,348	171,065
Squash	28,185	37,372
Muskmelon	1,203	2,020
Snake cucumber	6,171	2,917
Pumpkin	1,494	1,091
Gourd	617	241
Watermelon	3,540	1,028

***One donum = 1000 m^2. PCBS, 2010, Agriculture Statistics, Ramallah.**

1.2 Characterization of *Cucumis melo*.

Cucumis melo is considered the most diverse species of the genus *Cucumis*. Large morphological variations exist in fruit characteristics such as size, shape, color, texture, taste and composition (Bates & Robinson, 1995). The species comprises wild and cultivated varieties; *Cucumis melo* including sweet "dessert" melons, as well as non-sweet forms that are consumed raw, pickled or cooked. *Cucumis melo* (2n = 2x = 24) are dicotyledonous plants that are located in tropical, subtropical and temperate climates (Decker-Walters *et al*., 2002). The common name is melon but also called sweet melon, round melon, muskmelon, casaba, cantaloupe and winter melon (Nayar & Singh, 1998; Decker-Walters *et al*., 2002). *Cucumis melo* L. species includes a non sweet cultivars or groups as snake melon (*Cucumis melo* var. *flexuosus*) (Stepansky *et al*., 1999).

The name of genus *Cucumis* comes by its first descriptor Linné 1753, who described five species of cultivated melons. Which were later united in a single species: *Cucumis melo* by Naudin (1859). The extensive variation found in *C. melo* led scientists to propose intraspecific classification schemes (Stepansky *et al*., 1999; Szamosi *et al*., 2010). Munger & Robinson (1991) proposed a simplified division of *C. melo* into a single wild variety, *C. melo* var. *agrestis*, and six cultivated ones including *flexuosus*.

Melon varieties are classified into seven varieties (Munger & Robinson, 1991). Taking into consideration the descriptions by Naudin (1859), Pangalo (1929), Grebenscikov (1953) and Hammer *et al.* (1986) these seven varieties were listed by (Stepansky *et al*., 1999) as:

1. ***Cucumis melo* var. *agrestis*:** thin-stemmed, monoecious plants growing as weeds in African and Asian countries. Very small (<5 cm), inedible fruits with very thin mesocarp and tiny seeds.

2. ***Cucumis melo* var. *cantalupensis*:** Medium-large size fruits, smooth, scaly or netted rind of variable color. Fruits are aromatic with sweet, juicy flesh, and abscise at maturity. Includes also former var. *reticulatus*. Andromonoecious flowering in most genotypes, hairy ovary including dessert melon types such as Galia, Ananas, Charentais, "American shippers".

3. ***Cucumis melo* var. *inodorus*:** Large-sized winter melons, with non-aromatic, non-climacteric and longstoring fruits, with thick, and smooth or warty rind including sweet dessert melons from Asia and Spain, such as Honeydew and Casaba type-cultivars. Usually andromonoecious, and hairy ovary.

4. ***Cucumis melo* var. *flexuosus*:** Fruits are very elongated, non-sweet, eaten immature as cucumbers are found in the Middle East and Asia, where similar, less elongated types, *adzhur* and *chate*, have also been reported as ancient vegetable crops. Usually is monoecious.

5. ***Cucumis melo* var. *conomon*:** Far-Eastern cultivars, where the smooth, white-fleshed, thin rind fruits are eaten as pickles; includes also sweet, crisp fruits eaten with their rind. Andromonoecious vines bear dark, spiny leaves, sericeous ovaries corresponds to Naudin's var. *acidulus*.

6. ***Cucumis melo* var. *chito* and *dudaim*:** were described by Naudin and grouped together by Munger and Robinson. The former was reported as American wild origin, with small plum-size, aromatic fruits used as pickles, monoecious vines and sericeous ovaries. The second is of Persian origin, andromonoecious, sericeous ovaries, bears small, aromatic, red or brown-striped fruits, grown as ornamentals in oriental gardens.

7. ***Cucumis melo* var. *momordica*:** A group added by Munger & Robinson (1991) it includes Indian accessions with monoecious vines, sericeous ovaries and large, non-sweet fruits with thin rind that splits at maturity.

1.3 "Fakus" melon landraces diversity in Palestine.

Local traditional varieties (landraces) and their wild relatives represent genetic resources, essential for crop breeding (Simmonds, 1993). They harbor precious genetic variation that constitutes a "safety valve" against evolving disease and pests and climatic changes, maintaining long-term food security and sustainability of plant production.

Landraces of cucumber-looking melons of ancient domestication, called Fakus (*C. melo* var. *flexuosus*), are grown in the open field on significant scale in Palestinian villages, where they exhibit good climatic adaptation, and some stress tolerance and disease resistance traits. Fakus is a rain-fed crop, thought to be resistant to soil-borne diseases.

There are two main sub-cultivars of *C. melo* var. *flexuosus* in Palestine; white and green, commonly. Known as "sahori" and "baladi", many synonyms for *flexuosus* such as: "sahori abyad", "sahori akhdar", "baladi abyad", and "baladi akhdar" are also present.

1.4 Morphological and molecular characterization.

To determine the variations between and within species; there are two systems that have been used: morphological descriptors which depend on morphological and agronomic traits as leaf, fruit, seeds, and flowers, while the second system use molecular markers which depend on nucleic acid (DNA) or protein level (Gupta *et al*., 1998; Kumar *et al*., 2009).

Morphological descriptors are highly dependent on environmental factors like temperature, light, and lack of water or chemical structure of soil that may induce change in morphological and agronomic traits. So that morphological descriptors cannot give accurate and clear information about plant accession or species. Whereas, molecular markers are not affected by environmental factors and they are more stable than morphological descriptors. Therefore the combination between both morphological and molecular markers is widely used to study the variations within and between plant species (Kumar *et al*., 2009).

1.4.1 Morphological descriptors.

The most diverse varieties in the genus *Cucumis* is *Cucumis melo*. Morphologically; there are significant variations in fruit traits such as color, size, shape, texture, and taste (Zhang *et al*., 2012).

Morphological characterization has been carried out mainly according to the combined standards of Descriptor Lists of IPGRI (The International Plant Genetic Resources Institute) and others (Stepansky *et al*., 1999; IPGRI, 2003; Soltani *et al*., 2010).

1.4.2 Molecular markers.

Molecular markers can be divided into four main groups: protein-based systems, hybridization-based systems, PCR-based systems, and sequencing-based systems (Gupta, 1994; Monforte *et al*., 2004).

1.4.2.1 Protein-based molecular marker systems (Allozymes).

Allozymes also known as isozymes are defined as multiple forms of enzymes. Enzymes as any protein have a specific sequence of amino acids, this sequence encoded by specific genes. Nucleotides may alter in DNA sequence genes (genes which encode enzymes proteins), so that alteration may occur in amino acid sequence in a particular protein, leading to enzyme polymorphisms between individuals having the same function. The alteration leads to variation in conformation and net charged, so the electrophoretic mobility changed, so can be detect the variation between individuals by staining (Korzun *et al*., 2001).

Allozymes as biochemical analysis have been used to delineate phylogenetic relationships, estimate genetic variation, characterization of plant genetic resource management and plant breeding. The disadvantages of using allozymes analysis are lowering of abundance and relatively have low level of polymorphism (Bretting & Widrlechner, 1995; Staub & Serquen, 1996).

1.4.2.2 Hybridization-based molecular marker systems.

Restriction Fragment Length Polymorphism (RFLP) was first used for genetic mapping in 1975 (Helentjaris *et al.*, 1986), and is considered to be the most widely used as hybridization-based molecular markers in plant genomics. RFLP has been used for genetic diversity and phylogenetic studies within and between populations. RFLP has a high reproducibility, in addition to need to know DNA sequence (Kiss *et al*., 2011).

In general, the principle of RFLP is variations within and between species by patterns derived from cleavage DNA sequence by specific restriction enzymes (Endonucleases). DNA fragmented by these enzymes, each restriction enzyme (RE) has a different recognition sites in a DNA sequence. DNA sequence variations between individuals lead to alter the recognition sites of the same restriction enzyme, so DNA fragmented by RE for some individuals will give a different patterns on gel electrophoresis (Gnavi *et al*., 2010; Vyskot *et al*., 1991).

DNA sequence may differ in a few nucleotides due to point mutation, insertion/deletion, translocation, inversion or duplication in genome, these processes may lead to change the recognition sites between two individuals when using one restriction enzyme. A specific banding pattern revealed by transferring Fragments to a nitrocellulose membrane

(Southern Blotting) labeled with probes which hybridized with these fragments.

Labeling of the probe may be performed with a radioactive isotope or with alternative non-radioactive stains, such as digoxigenin or fluorescein. These probes are mostly species-specific single locus probes of about 0.5–3.0 kb in size, obtained from a cDNA library or a genomic library (Miller & Tanksley, 1990; Landry & Michelmore, 1987; Neale & Williams, 1991).

1.4.2.3 PCR-based molecular markers.

1.4.2.3.1 Amplified Fragment Length Polymorphism (AFLP).

AFLP technology (Vos *et al*., 1995) is a combination between power of RFLP and flexibility of PCR-based technology. AFLP is amplification of DNA fragments produced from cutting with restriction enzymes. Selective amplification by primers designed with corresponding adaptor and restriction site specific sequences.

Polymorphism detected by banding pattern on gel electrophoresis. AFLP is high reproducible and can produce 50-100 informative bands and no sequence data for primer construction are required. AFLP used for gene mapping and linkage, in addition to discrimination between individuals (Alonso *et al*., 1998; Matthes *et al*., 1998).

1.4.2.3.2 Random Amplified Polymorphic DNA (RAPD).

RAPD is a PCR-based technology by using short (~10bp) and single primers to amplify genomic DNA (Welsh & McClelland, 1990; Williams *et al.,* 1990). These primers (arbitrary nucleotide sequence) can anneal randomly on many loci on genomic DNA strands in PCR reactions, so low annealing temperature (~35 ºC) is used. These oligonucleotides serve as both forward and reverse primer (Russell *et al*., 1997).

PCR products separated on agarose gel electrophoresis and visualized under UV transilluminator after staining with ethidium bromide. Visualized bands scored by presence "1" or absence "0". These polymorphisms are considered to be primarily due to variation in the primer annealing sites, and each primer gives separate bands, so many primers used to study genetic variations. These primers can amplify fragments 0.5-5 Kb.

RAPD-PCR can detect polymorphisms between and within species, and widely used because it's informative, easy to use, cheap, quick, and no sequence information are needed. There are hundreds of primers used and commercially available (Arif *et al*., 2010).

The main limitation of RAPDs is their low reproducibility, and highly standardized experimental procedures are needed because of their sensitivity to the reaction conditions. RAPDs primers are able to amplify genomic DNA fragments from contamination, so precautions are needed.

RAPDs primers are dominant markers (Bardakci, 2001; Srivatsava & Nidhi, 2009).

1.4.2.3.3 Inter Simple Sequence Repeats (ISSR).

ISSR is a PCR-based technology reported by Zietkiewicz *et al*. (1994). Primers used in this PCR are simple sequence repeat primers (e.g. [AC]n) to amplify regions between identical microsatellite repeat regions oriented in opposite directions (Zietkiewicz *et al*., 1994; Gupta *et al*., 1994; Gupta *et al*., 2000).

Single primer (15-30bp) used to target multiple genomic loci on DNA to amplify mainly inter simple sequence repeats of different sizes. It is recommended to use high annealing temperature to maintain high stringency. PCR products separated by gel electrophoresis after staining with ethidium bromide. Bands scored as RAPD by presence "1" or absence "0". Although the specificity of microsatellite primers, but sequence information not required. ISSRs are also easy to use, quick, and cheap, and used for taxonomic studies of closely related species and genetic mapping (Godwin *et al*., 1997; Kojima *et al*., 1998).

1.4.2.3.4 Simple sequence Repeat (SSR).

In genomic DNA there are non coding sequences and repeated many times along DNA called Variable Number Tandem Repeat (VNTR) (Powell *et al*., 1996). There are variations in number of nucleotide repeat between individuals. VNTRs contain two families: minisatellites and microsatellites. Microsatellites or Simple Sequence Repeat (SSR) are repetitive sequence consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species, while minisatellites consisting of large repeats more than penta-nucleotide (Cardle *et al*., 2000).

Importance of repetitive sequence comes from variation of number of repeats in different alleles between individuals. Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, so it used to study genetic diversity. SSR markers used also for studies of gene duplication or deletion, marker assisted selection, and fingerprinting (Foster *et al*., 2010).

1.4.2.4 Sequencing-based molecular markers systems.

Sequencing systems are more accurate and give more information than other types of molecular markers systems. But cost and time are the most limitations to use it. Single nucleotide polymorphisms (SNPs) are the most usable example of these systems. When a single nucleotide (A, T, G or C) is altered (point mutation) it leads to DNA sequence variations called Single Nucleotide Polymorphisms (SNPs), also pronounced "snips". SNP used as molecular marker due to SNPs have a high level of polymorphism due to their high frequency of occurrence in the genome, so it used as powerful molecular marker. SNPs used for various applications as genetic maps, and for discrimination between homozygous and heterozygous alleles (Ching *et al*., 2002; Alves *et al*., 2008).

1.5 Literature review.

In the last two decades, DNA fingerprinting has been used to resolve taxonomic relationships, providing a quantitative measure for genetic diversity between genera and species (Silberstein *et al*., 1999). The sensitivity of these methods also allows genotyping varieties or cultivars within a species, and these have been utilized to explore melon diversity in different collections and germplasm sections.

Molecular characterization of melon by genetic markers has been carried out by many researchers; RAPD markers (Stepansky *et al*., 1999; Mliki *et al*., 2001; López-Sesé *et al.*, 2003; Staub *et al*., 2004; Nakata *et al*., 2005; Dhillon *et al*., 2007; Tanaka *et al*., 2007; Yi *et al*., 2009; Soltani *et al*., 2010; Yildiz *et al*., 2011; Ismail *et al*., 2012; Zhang *et al*., 2012).

Soltani et al. (2010) study the diversity among Iranian landraces of melons groups by RAPD primers and morphological descriptors, their results shown variations between and within accession, cluster analysis did not separate between groups of melon.

Zhang *et al*. (2012) study the diversity of South Asian landraces of *Cucumis melo* and *Cucumis sativus* by RAPD and SSR markers, their results had shown a higher diversity of *Cucumis melo* accessions than *Cucumis sativus* accessions.

Erdinc *et al*. (2013) study the genetic diversity of Turkish landraces of melon by RAPD and ISSR primers, their results also shown variation between accessions.

ISSR markers were also used by many researchers (Stepansky *et al*., 1999; Sestili *et al*., 2008; Yildiz *et al*., 2011); AFLP (Yashiro *et al*., 2005); SSR markers (Monforte *et al*., 2003; Sestili *et al*., 2008; Emmanouil *et al*., 2009). A linkage map presented for *Cucumis melo* by (Silberstein, *et al*., 2003) using RFLP, AFLP, ISSR, and RAPD marker.

RAPD and ISSR markers selected in this study to characterize the traditional landraces of *Cucumis melo* groups in Palestine, because it is informative, easy to use, cheap, quick, and no sequence information required.

1.6 Aims of this study.

1. For characterizing and detecting polymorphisms among local Fakus and sweet melon varieties in the West Bank, and in addition, investigating the genetic relationships among these genotypes accessions.

2. To determine the relationships between molecular markers and morphological descriptors results.

3. To evaluate RAPD and ISSR genetic markers discrimination efficiency.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Plant Material

In this study 44 accessions of local landraces seeds of sweet melon and "Fakus" melon were systematically collected from farmers in the West Bank areas included seeds (16 accessions) were provided by National Seed Bank of Union of Agricultural Work Committees (UAWC) were used.

Distribution of the collection sites, accession name, variety, and common name are listed in Table 2.1. Collection sites are shown in the West Bank map in Figure 2.1. Seeds have been deposited at Community-Based Seed Bank (CSB) in Biodiversity and Environmental Research Center (BERC), Til, Nablus.

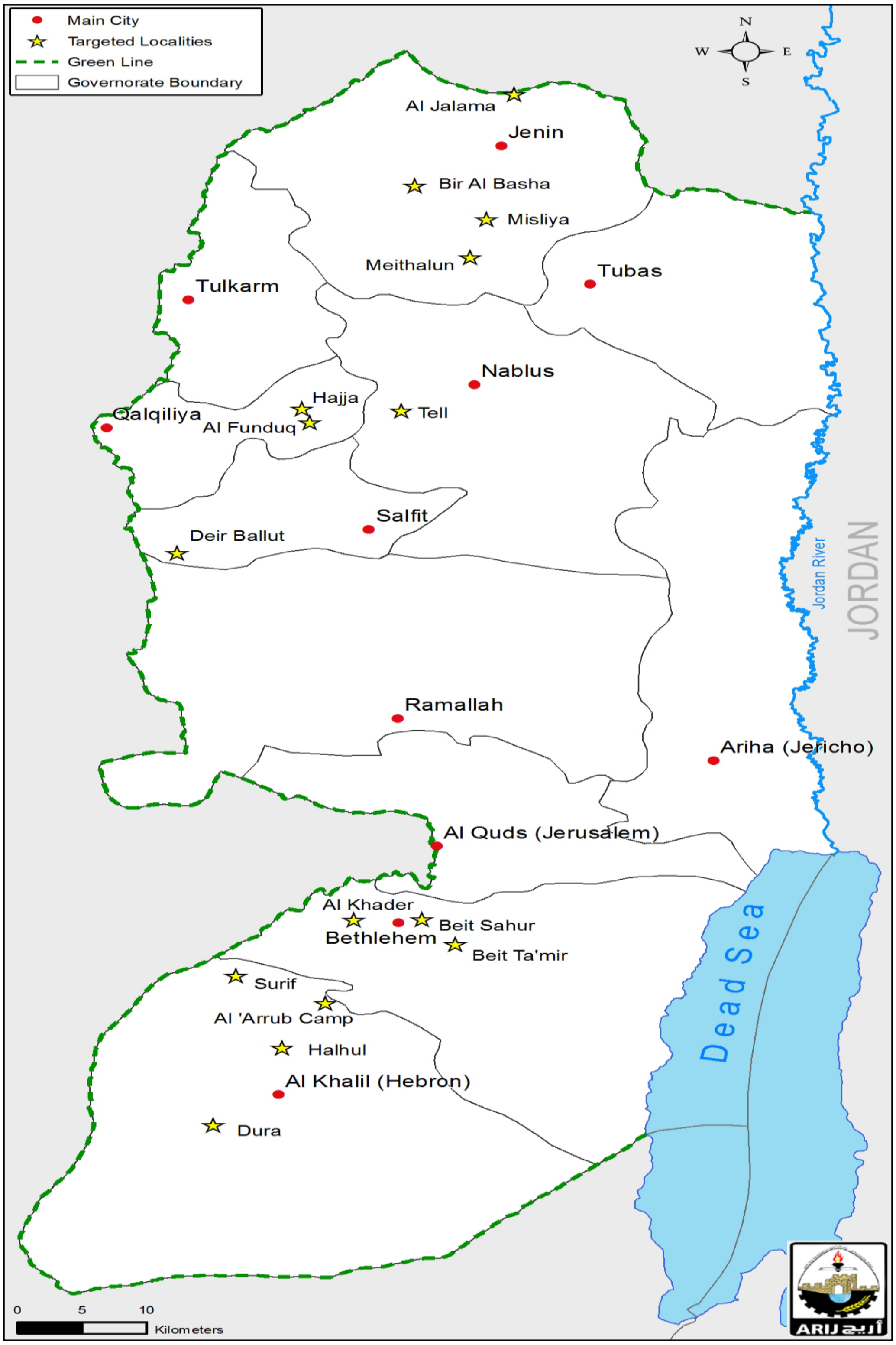

Figure 2.1: Map of West Bank locations. "*" indicates fourteen collection sites where melon landraces were collected. (Source: the Applied Research Institute - Jerusalem (ARIJ)).

Table 2.1 Table of melon accessions with their number, accession name, variety, common name, and the location they were collected from. (BERC: Biodiversity and Environmental Research Center; UAWC: Union of Agricultural Work Committees).

No.	Accession name	Variety	Common name	Location
1	BERC-NTF01	*flexuosus*	Akhdar	Tell/nablus
2	BERC-NTF02	*flexuosus*	Akhdar	Tell/nablus
3	BERC-HHF03	*flexuosus*	Sahori abyad	Halhul/Hebron
4	BERC-HHF04	*flexuosus*	Sahori abyad	Halhul/Hebron
5	BERC-HHF05	*flexuosus*	Sahori abyad	Halhul/Hebron
6	BERC-HHF06	*flexuosus*	Sahori akhdar	Halhul/Hebron
7	BERC-HHF07	*flexuosus*	Sahori abyad	Halhul/Hebron
8	BERC-BAF08	*flexuosus*	Sahori abyad	Al khader/Bethlehem
9	BERC-BTF09	*flexuosus*	Sahori akhdar	Beit Ta'mir/Bethlehem
10	BERC-BBF10	*flexuosus*	Sahori akhdar	Beit Sahur/Bethlehem
11	BERC-HSF11	*flexuosus*	Sahori abyad	Surif/Hebron
12	BERC-BAF12	*flexuosus*	Sahori abyad	Beit Sahur/Bethlehem
13	BERC-SDF13	*flexuosus*	Akhdar	Deir Ballut/Salfit
14	BERC-JJF14	*flexuosus*	Abyad	Al Jalama/Jenin
15	BERC-JBF15	*flexuosus*	Abyad	Bir Al Basha/jenin
16	BERC-JMF16	*flexuosus*	Abyad	Meithalun/jenin
17	BERC-JMF17	*flexuosus*	Abyad	Meithalun/jenin
18	BERC-JMC18	*Cantalupensis*	Baladi	Meithalun/jenin
19	BERC-JMC19	*Cantalupensis*	Baladi	Meithalun/jenin
20	BERC-JMF20	*flexuosus*	Akhdar mkhatat	Meithalun/jenin
21	BERC-QMF21	*flexuosus*	Baladi abyad	Meithalun/jenin
22	BERC-QFF22	*flexuosus*	Baladi akhdar	Al Funduq/Qalqiliya
23	BERC-QHF23	*flexuosus*	Baladi akhdar qaser	Hajja/Qalqiliya
24	BERC-JSF24	*flexuosus*	Abyad taweel	Misliya/Jenin
25	BERC-JMF25	*flexuosus*	Abyad	Meithalun/jenin
26	BERC-JMF26	*flexuosus*	Abyad	Meithalun/jenin
27	BERC-QHF27	*flexuosus*	Akhdar	Hajja/Qalqiliya
28	BERC-NTF28	*flexuosus*	Akhdar	Tell/nablus
29	BERC-HSF29	*flexuosus*	Sahori abyad taweel	Soref/Hebron
31	UB-14-08	*flexuosus*	Sahori abyad	Halhul/Hebron (UAWC)
32	UB-147-10	*flexuosus*	Sahori abyad-tawel	Deir Ballut/Salfit (UAWC)
33	UB-177-10	*flexuosus*	???????	Dura/Hebron (UAWC)
34	UB-193-10	*flexuosus*	Sahori abyad qaser	Dura/Hebron (UAWC)
35	UB-196-11	*flexuosus*	Akhdar tawel	Dura/Hebron (UAWC)
36	UB-201-11	*flexuosus*	Sahori abyad-tawel	Dura/Hebron (UAWC)

37	UB-203-11	*flexuosus*	?????	Dura/Hebron (UAWC)
38	UB-220-12	*flexuosus*	?????	Dura/Hebron (UAWC)
39	UB-234-12	*flexuosus*	Sahori abyad-tawel	Dura/Hebron (UAWC)
40	UB-243-12	*flexuosus*	Sahori abyad	Dura/Hebron (UAWC)
41	UB-246-12	*flexuosus*	Akhdar tawel	Halhul/Hebron (UAWC)
43	UB-59-09	*flexuosus*	Sahori abyad qaser	Dura/Hebron (UAWC)
45	UB-84-10	*flexuosus*	??????	Dura/Hebron (UAWC)
49	UB-97-10	*Cantalupensis*	Baladi	Meithalun/jenin (UAWC)
50	UB-229-12	*Cantalupensis*	Baladi	Al 'Arrub Camp/Hebron (UAWC)

2.2 Morphological characterization.

In April 2012, 10 seeds of each accession were grown in the greenhouse in plastic pots for two weeks, and then the seedlings plants were transplanted in the field.

For morphological characterization, thirty eight accessions, (6 accessions of *flexuosus* were excluded), were used. Morphological descriptors were recorded using ten plants per accession according to combined standards as described by IPGRI (The International Plant Genetic Resources Institute) and from studies about morphological characterization of *Cucumis melo* (Stepansky *et al*., 1999; IPGRI, 2003; Soltani *et al*., 2010).

In total, 17 traits were recorded (Table 2.2). Weights are measured by balance, and diameters measured by caliper.

Table 2.2 Morphological descriptors were used in this study. For fruit characters: n= 10 fruits for *flexuosus* accessions, and n= 3 fruits for *cantalupensis* accessions. For descriptors (Stem thickness and Flower size) n= 3 plants. For Seeds weight character, n= 100 seeds.

No.	Traits	Standard	Notes	Reference
1	Fruit shape	1: Oblate, 2: Elongate	Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	IPGRI, 2003
2	Fruit size	1: (300-375g), 2: (376-450g)	Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	IPGRI, 2003
3	Fruit length/width ratio [L/W]	1: (<4), 2: (4-4.7), 3: (4.8-5.5)	The length from stem end to blossom end of the fruit divided by the width at the broadest point. Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	IPGRI, 2003
4	Predominant fruit skin color	1: White, 2: Green, 3: Orange	Predominant color is the color, which covers the largest surface area of the fruit. In case the two colors have the same surface area the lighter color will be considered the predominant one. Scored 10 days after fruit set for *flexuosus* accessions, and 30 days for *cantalupensis* accessions	IPGRI, 2003
5	Secondary fruit skin color	1: White,2: Pale green, 3: Green, 4: Orange	Secondary color is the color that covers the second largest area of the fruit. In case two colors have the same surface area the lighter color will be considered the predominant one. Scored 10 days after fruit	IPGRI, 2003

			set for *flexuosus* accessions, and 30 days for *cantalupensis* accessions	
6	Secondary skin color pattern (skin design)	1: No secondary skin color, 2: Speckled (spots <0.5cm), 3: Striped (bands that run from peduncle to blossom scar), 4: Short streaked (elongated marks that are continuous from one end the other.	Design produced by secondary skin color. Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	IPGRI, 2003
7	Skin texture	1: wrinkled, 2: ribbed	Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	Stepansky, 1999
8	Flesh color	1: white, 2: green, 3: Pale orange	Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	Stepansky, 1999
9	Taste	1: insipid (non-sweet), 2: sweet	Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	Stepansky 1999
10	fruit hair	1: presence, 2: absence	Scored 10 days after fruit set for *flexuosus* accessions, 30 days for *cantalupensis* accessions	Soltani, 2010
11	Sex type	1: monoecious, plant bears staminate and pistillate flowers, 2: andromonoecious, with staminate and perfect flower		Stepansky, 1999

12	Ovary shape	1: Flat, 2: Round, 3: Long, 4: Very long		IPGRI, 2003
13	Ovary pubescence length	1: Short (<1cm), 2: Intermediate (1-5cm), 3: Long (>5cm)		IPGRI, 2003
14	Stem thickness	1: (7-8 mm), 2: (8.1-9mm)	measured on fifth node of main stem, n= 3 plants	Stepansky, 1999
15	Flower size	1: (19.5-21mm), 2: (21.1-22.5mm), 3: (22.6-24mm)	diameter of flowers, n= 3 plants	Stepansky, 1999
16	Hair density	1: sparse, 2: medium, 3: dense	evaluated on fifth node of main stem	Stepansky, 1999
17	Seeds weight	1: (3-3.5g), 2: (3.6-4g), 3: (4.1-4.5g)	average of 100 seeds from original gene bank sample	Stepansky, 1999

2.3 Molecular characterization.

2.3.1 DNA extraction.

For molecular characterization, one leaf per accession was collected from the field and directly stored in liquid nitrogen. Leaf samples ground by using mortar and pestle in liquid nitrogen to fine powder and genomic DNA was extracted by using the CTAB method (Permingeat *et al*., 1998).

DNA Extraction protocol by CTAB method: In 1.5ml tube; ~50mg of each sample taken, 500µl H-buffer (100mM Tris-HCl, pH 8.0, 20mM EDTA, 1.4M NaCl, 2% CTAB, 0.5M glucose, and 100mM DTT) added for each sample and shaken for 1 hour at 60ºC, 500µl CI (Chloroform:isoamyl alcohol (24:1)) added for each sample and shaken for 5 minutes at room temperature, tubes were spun down by centrifuge at maximum speed for 5 minutes at 4ºC, upper phase was collected, and transferred to new tubes

(Figure 2.2), isopropanol was added for each sample; about 0.8 Volume, tubes inverted twice, a precipitate became visible, pellet was spun down at maximum speed for 10 minutes at 4ºC, the pellet was washed by 70% EtOH, spun down at maximum speed for 5 minutes at room temperature, EtOH was evaporated (10 minutes) at room temperature; the pellet was dissolved in 100µl sdH2O, and Stored at -20ºC (Appendix A).

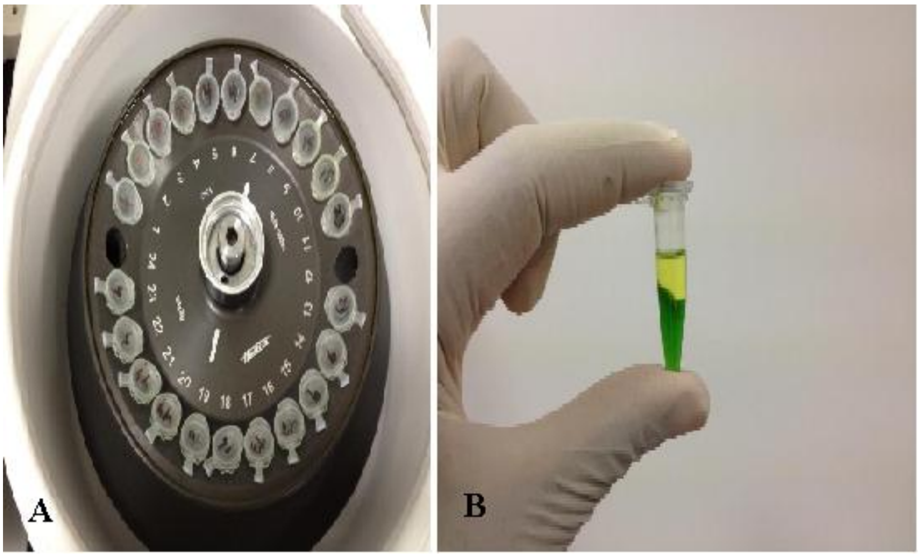

Figure 2.2 Samples sorting in the centrifuge (A) and one tube after centrifugation (B).

DNA was purified by adding 1µl of a 10 µg/ml of RNase A to DNA samples and incubated at 37ºC for 30 min. DNA recovered by adding 1/10 volume of 3M sodium acetate (pH 6.8) and 2 volumes of isopropanol to the DNA containing solution. Incubated on ice for 10 min, and centrifuged at maximum speed for 5 min at room temperature. Supernatant was discarded carefully. Then washed with 70% ethanol and samples were left to dry and then were dissolved in 100 sdH2O.

DNA concentrations were measured by using multiscan plate (biotech) and all samples were diluted to 30ng/μl for polymerase chain reaction (PCR) amplification. DNA integrity was checked by agarose gel electrophoresis (1% agarose, 1X TAE buffer, and 0.5 μg/ml ethidium bromide). DNA samples were loaded as follows: 2μl from DNA sample, 3μl from 6X sample loading buffer. The gel was run at voltage 120V for 30 min in 1X TAE buffer. DNA bands were visualized under UV transilluminator and photographed.

2.3.2 Random Amplified Polymorphic DNA (RAPD) assay.

Fourteen RAPD primers were used. RAPD-PCRs were performed using random decamer sets (Operon Technologies, Alameda, Calif., sets OPA, B, D, L, and R), according to Williams *et al.* (1993).

The reactions were performed twice for each primer by thermocycler in 25μL reaction volumes containing the following: 30 ng genomic DNA, 0.2 μmol/L primer, 0.5 U *Taq* DNA polymerase (hy labs), 0.1 mmol/L of each dNTP (GeneDirex), 1.5 mmol/L MgCl2, and reaction buffer (1.5 mmol/L MgCl2, 10 mmol/L Tris-HCl (pH = 9), 50 mmol/L KCl, 0.1% volume fraction of Triton X-100, and 0.2 mg/mL bovine serum albumin (BSA)). Amplification included 40 cycles of 1 min at 94 °C, 90 s at 36 °C, 2 min at 72 °C, with 2 min initial denaturation, and 5 min final extension.

RAPD-PCR products were separated by gel electrophoresis (1.5 % agarose, 1X TAE buffer, and 0.5 μg/ml ethidium bromide). PCR products were loaded in the gel as follows: 8µl of PCR product and 3µl of 6X sample loading buffer. Gel was run at voltage 120V for 1 hour in 1X TAE buffer, bands were visualized under a UV transilluminator and photographed.

2.3.3 Inter Simple Sequence Repeats (ISSR) assay.

Inter SSR analysis was performed according to Gupta *et al.* (1994) and Stepansky *et al.* (1999), using the ISSR primer set (9 primers) of the University of British Columbia, Vancouver. ISSR-PCRs performed by thermocycler in 25 µL reaction volume included 30 ng genomic DNA, 1 µmol/L primer, 0.5 U *Taq* DNA polymerase, 0.2 mmol/L of each dNTP (GeneDirex), 1.5 mmol/L MgCl2, and reaction buffer (10 mmol/L Tris–HCl (pH = 8.4), 50 mmol/L KCl, 0.1% Triton X-100, and 0.2 mg BSA/mL).

Amplification included 35 cycles of 1 min at 95 °C, 30 s at the annealing temperature (5°C below the approximate melting point temperature (Tm) of each primer), and 5 min at 72 °C, with 2 min initial denaturation, and 5 min final extension. ISSR-PCR products visualized as RAPD procedure.

One kb DNA ladder marker (GeneDirex) was used as marker and loaded in the first lane of all gels.

2.4 Data scoring and analysis.

For Morphological Characterization; all variables were converted to binary variables. The commercial software package SPSS 16 was used to develop similarity matrices based on the Jaccard coefficient. These data were then used to construct dendrogram for cluster analysis based on the Jaccard coefficient.

For Molecular Characterization; RAPD and ISSR markers resulting bands were scored for each primer based on the molecular size.

Reproducible amplified DNA fragments were transformed into binary character matrices (1 for presence, 0 for absence). The commercial software package SPSS (version 16) was used to develop similarity matrices based on the Jaccard coefficient. These data were then used to construct dendrogram for cluster analysis based on the Jaccard coefficient. Two separate dendrograms for ISSR and RAPD data were generated.

Percentage of polymorphism and Resolving power (Rp) were calculated for each primer.

Average band informativeness (AvIb) is a measure of closeness of a band to be present in 50% of the genotypes under study, and resolving power (Rp) is the sum of Ib values of all the bands amplified by a primer. Band informativeness (Ib) and resolving power (Rp) were calculated as given by Prevost & Wilkinson (1999). The formulas used for the above-mentioned parameters are: (i) Band informativeness of a given band: Ib = 1

$-$ (2 $\times|0.5 - p|$), where p is the proportion of the total genotypes containing the band; (ii) resolving power of a primer is the sum of band informativeness: $Rp = \Sigma Ib$.

CHAPTER THREE

RESULTS

3.1 Morphological Characterization.

The data of morphological descriptors for 38 melon accessions were listed in Table 3.1 and Appendix B. Four descriptors (sex type, ovary shape, ovary pubescence length, and hair density) gave similar results for all melon accessions.

For all melon accessions, sex type was monoecious, ovary shape was round, ovary pubescence length was short (<1cm), and hair density was medium.

Fruit shape was elongated for all *flexuosus* accessions and oblate for all *cantalupensis* accessions. Predominant fruit skin color was white or green for *flexuosus* accessions and orange for *cantalupensis* accessions.

Secondary fruit skin color and pattern among *flexuosus* accessions were varied; *flexuosus* accession which gave white in predominant color gave white or pale green in Secondary fruit skin color, and gave white or striped secondary fruit skin color pattern (Figure 3.1).

Fruit length/width ratio [L/W] ranged from 4-5.5 for *flexuosus* accessions, and <4 for *cantalupensis* accessions.

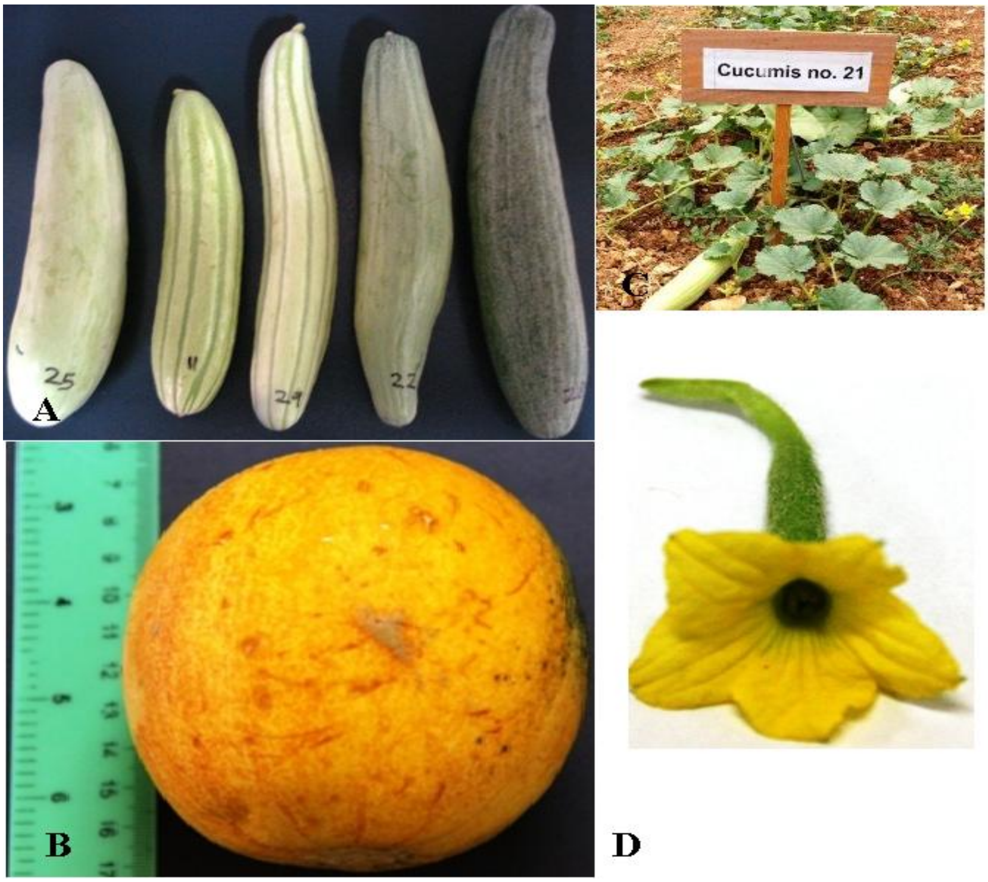

Figure 3.1 Morphological variations within and between Palestinian melons. A: *flexuosus* fruits, B: *cantalupensis* fruit, C: whole plants in the field, D: flowers.

Accessions *of cantalupensis* had short streaks on fruits, but these were lacking on *flexuosus* accessions. Taste of *flexuosus* accessions was insipid (non-sweet) while *cantalupensis* accessions had sweet taste.

Fruit sizes, Stem thickness, male flower size, are varied between all Palestinian *Cucumis melo* accessions; 300-450g, 7-9 mm, and 19.5-22.5mm respectively (Table 3.1).

Similarity matrix and Dendrogram of 13 morphological descriptors generated for 38 accessions shown in Table 3.2 and Figure 3.2 respectively.

Table 3.1 Morphological descriptors scored on melon accessions.

No.	Fruit shape[1]	Predominant fruit skin color[2]	Secondary fruit skin color[3]	Secondary skin color pattern[4]	Skin texture[5]	Flesh color[6]	Taste[7]	Fruit hair[8]	Stem thickness[9]	Male Flower size[10]	Fruit size[11]	Fruit length/width ratio[12]	Seeds weight[13]
1	2	2	3	2,3	2	2	1	1	1	2	1	2	1
2	2	2	3	2,3	2	2	1	1	1	1	2	2	1
3	2	1	2	3	2	1	1	1	1	3	2	2	1
4	2	1	2	3	2	1	1	1	1	1	1	2	1
5	2	1	2	3	2	1	1	1	1	1	2	2	1
7	2	1	2	3	2	1	1	1	1	2	1	2	1
8	2	1	2	3	2	1	1	1	1	2	2	2	1
9	2	2	2	2,3	2	2	1	1	1	3	1	2	1
11	2	1	2	3	2	1	1	1	2	2	1	2	1
13	2	2	2	2,3	2	2	1	1	2	2	2	3	1
14	2	1	1	1	2	1	1	1	1	3	2	2	2
15	2	1	1	1	2	1	1	1	2	3	1	2	1
16	2	1	1	1	2	1	1	1	1	2	2	2	1
17	2	1	1	1	2	1	1	1	2	2	2	3	1
18	1	3	4	4	1	3	2	2	2	3	2	1	2
19	1	3	4	4	1	3	2	2	2	1	2	1	3
49	1	3	4	4	1	3	2	2	2	1	2	1	2
50	1	3	4	4	1	3	2	2	2	1	2	1	1
20	2	2	3	2,3	2	2	1	1	2	2	2	2	3
21	2	1	1	1	2	1	1	1	2	3	2	3	3
22	2	2	3	2,3	2	2	1	1	2	3	2	2	1
23	2	2	3	2,3	2	2	1	1	2	2	1	2	1
24	2	1	1	1	2	1	1	1	2	2	2	2	3
25	2	1	1	1	2	1	1	1	2	2	2	3	3
27	2	2	3	2,3	2	2	1	1	2	2	2	2	1
28	2	2	3	2,3	2	2	1	1	2	3	2	3	1
29	2	1	2	3	2	1	1	1	2	3	2	3	2
31	2	1	2	3	2	1	1	1	2	2	2	2	1
32	2	1	2	1	2	1	1	1	2	3	1	3	2
34	2	1	2	1	2	1	1	1	2	2	2	2	1
35	2	2	2	2,3	2	2	1	1	2	3	2	2	1
36	2	1	2	3	2	1	1	1	2	2	2	3	2
37	2	1	2	3	2	1	1	1	2	1	1	3	3
39	2	1	2	3	2	1	1	1	2	2	1	2	2
40	2	1	2	3	2	1	1	1	2	3	2	2	3
41	2	2	2	2,3	2	2	1	1	2	1	2	2	1
43	2	1	2	3	2	1	1	1	2	3	2	3	2
45	2	1	2	3	2	1	1	1	2	3	2	3	2

1- Fruit shape: 1: Oblate, 2: Elongate, 2- Predominant fruit skin color: 1: White, 2: Green, 3: Orange, 3- Secondary fruit skin color: 1: White, 2: Pale green, 3: Green, 4: Orange, 4- Secondary skin color pattern: 1: No secondary skin color, 2: Speckled, 3: Striped, 4: Short streaked, 5- Skin texture: 1: wrinkled, 2: ribbed, 6- Flesh color: 1: white, 2: green, 3: Pale orange, 7- Taste: 1: insipid (non-sweet), 2: sweet, 8- Fruit hair: 1: presence, 2: absence, 9- Stem thickness : 1: (7-8 mm), 2: (8.1-9mm), 10- Male Flower size: 1: (19.5-21mm), 2: (21.1-22.5mm), 3: (22.6-24mm), 11- Fruit size: 1: (300-375g), 2: (376-450g), 12- Fruit length/width ratio [L/W]: 1: (<4), 2: (4-4.7), 3: (4.8-5.5), 13- Seeds weight: 1: (3-3.5g), 2: (3.6-4g), 3: (4.1-4.5g). For fruit descriptors: n= 10 fruits for *flexuosus* accessions, and n= 3 fruits for *cantalupensis* accessions. For characters (Stem thickness and Male Flower size) n= 3 plants. For Seeds weight character, n= 100 seeds. Numbers of accessions are as listed in Table 2.1.

From dendrogram; three clusters revealed: cluster I: white subcultivar of *flexuosus* (Fakus), cluster II: green subcultivar of *flexuosus* (Fakus), cluster III: *cantalupensis* (sweet melon).

Cluster I (white *flexuosus*) subdivide into two sub clusters: Ia and Ib. Ia contained 15 accessions (14 accessions from Hebron area, and one accession form Bethlehem area), and these accessions represents all accessions of "white" *flexuosus* collected from southern areas of the West Bank.

Sub cluster Ib contained 8 accessions (7 accessions from Jenin area, and one accession from Salfit area), and these accessions represent all accessions of "white" *flexuosus* collected from northern areas of the West Bank.

Table 3.2 Similarity matrix by Jaccard Coefficient for Morphological descriptors of 38 Palestinian melon accessions.

	1	2	3	4	5	7	8	9	11	13	14	15	16	17	18	19	49	50	20	21	22	23	24	25	27	28	29	31	32	34	35	36	37	39	40	41	43	45
1	1.00																																					
2	0.75	1.00																																				
3	0.42	0.50	1.00																																			
4	0.50	0.50	0.73	1.00																																		
5	0.42	0.59	0.86	0.86	1.00																																	
7	0.59	0.42	0.73	0.86	0.73	1.00																																
8	0.42	0.42	0.73	0.63	0.73	0.73	1.00																															
9	0.65	0.56	0.50	0.50	0.42	0.50	0.50	1.00																														
11	0.50	0.35	0.63	0.73	0.63	0.86	0.63	0.42	1.00																													
13	0.56	0.56	0.42	0.35	0.42	0.42	0.59	0.65	0.50	1.00																												
14	0.35	0.42	0.73	0.53	0.63	0.53	0.53	0.35	0.44	0.29	1.00																											
15	0.29	0.23	0.44	0.44	0.37	0.44	0.44	0.42	0.53	0.35	0.63	1.00																										
16	0.42	0.42	0.63	0.53	0.63	0.63	0.63	0.29	0.53	0.35	0.86	0.53	1.00																									
17	0.29	0.29	0.44	0.37	0.44	0.44	0.63	0.29	0.53	0.50	0.63	0.73	0.73	1.00																								
18	0.00	0.04	0.09	0.00	0.04	0.00	0.04	0.04	0.04	0.08	0.09	0.09	0.04	0.09	1.00																							
19	0.00	0.08	0.04	0.04	0.09	0.00	0.04	0.00	0.04	0.08	0.04	0.04	0.04	0.09	0.71	1.00																						
49	0.00	0.09	0.05	0.05	0.10	0.00	0.05	0.00	0.05	0.09	0.05	0.05	0.05	0.10	0.69	0.69	1.00																					
50	0.04	0.14	0.10	0.10	0.15	0.05	0.10	0.04	0.10	0.14	0.10	0.10	0.10	0.15	0.57	0.69	0.82	1.00																				
20	0.65	0.65	0.35	0.29	0.35	0.35	0.35	0.40	0.42	0.65	0.29	0.23	0.35	0.35	0.08	0.13	0.09	0.09	1.00																			
21	0.17	0.23	0.44	0.30	0.37	0.30	0.44	0.29	0.37	0.35	0.63	0.73	0.53	0.73	0.14	0.14	0.10	0.10	0.35	1.00																		
22	0.65	0.75	0.50	0.35	0.42	0.35	0.35	0.56	0.42	0.65	0.42	0.35	0.35	0.35	0.13	0.08	0.09	0.14	0.75	0.35	1.00																	
23	0.87	0.65	0.35	0.42	0.35	0.50	0.35	0.56	0.59	0.65	0.29	0.35	0.35	0.35	0.04	0.04	0.04	0.09	0.75	0.23	0.75	1.00																
24	0.29	0.29	0.44	0.37	0.44	0.44	0.44	0.17	0.53	0.35	0.63	0.53	0.73	0.73	0.09	0.14	0.10	0.10	0.50	0.73	0.35	0.35	1.00															
25	0.23	0.23	0.37	0.30	0.37	0.37	0.53	0.23	0.44	0.42	0.53	0.63	0.63	0.86	0.09	0.14	0.10	0.10	0.42	0.86	0.29	0.29	0.86	1.00														
27	0.75	0.75	0.42	0.35	0.42	0.42	0.42	0.47	0.50	0.75	0.35	0.29	0.42	0.42	0.08	0.08	0.09	0.14	0.87	0.29	0.87	0.87	0.42	0.35	1.00													
28	0.56	0.65	0.42	0.29	0.35	0.29	0.42	0.65	0.35	0.75	0.35	0.42	0.29	0.42	0.13	0.08	0.09	0.14	0.65	0.42	0.87	0.65	0.29	0.35	0.75	1.00												
29	0.23	0.29	0.63	0.44	0.53	0.44	0.53	0.35	0.53	0.42	0.44	0.44	0.37	0.44	0.25	0.14	0.21	0.15	0.35	0.53	0.42	0.29	0.44	0.44	0.35	0.42	1.00											
31	0.42	0.42	0.73	0.63	0.73	0.73	0.73	0.35	0.86	0.59	0.53	0.44	0.63	0.63	0.09	0.09	0.10	0.15	0.50	0.44	0.50	0.50	0.63	0.53	0.59	0.42	0.63	1.00										
32	0.23	0.17	0.44	0.44	0.37	0.44	0.44	0.42	0.53	0.35	0.44	0.73	0.37	0.53	0.14	0.04	0.10	0.05	0.23	0.63	0.29	0.29	0.44	0.53	0.23	0.35	0.63	0.44	1.00									
34	0.35	0.35	0.63	0.53	0.63	0.63	0.63	0.29	0.73	0.50	0.63	0.53	0.73	0.73	0.09	0.09	0.10	0.15	0.42	0.53	0.42	0.42	0.73	0.63	0.50	0.35	0.53	0.86	0.53	1.00								
35	0.56	0.65	0.59	0.42	0.50	0.42	0.42	0.65	0.50	0.75	0.42	0.35	0.35	0.35	0.13	0.08	0.09	0.14	0.65	0.35	0.87	0.65	0.35	0.29	0.75	0.75	0.50	0.59	0.35	0.50	1.00							
36	0.35	0.35	0.63	0.53	0.63	0.63	0.63	0.29	0.73	0.50	0.44	0.37	0.53	0.53	0.14	0.09	0.15	0.10	0.50	0.44	0.42	0.42	0.63	0.53	0.50	0.35	0.73	0.86	0.53	0.73	0.50	1.00						
37	0.35	0.35	0.53	0.73	0.63	0.63	0.44	0.35	0.73	0.35	0.37	0.44	0.37	0.37	0.04	0.14	0.10	0.10	0.42	0.44	0.35	0.42	0.53	0.44	0.35	0.29	0.53	0.63	0.53	0.53	0.42	0.63	1.00					
39	0.42	0.29	0.53	0.63	0.53	0.73	0.53	0.35	0.86	0.42	0.37	0.44	0.44	0.44	0.09	0.04	0.10	0.05	0.42	0.37	0.35	0.50	0.53	0.44	0.42	0.29	0.63	0.73	0.63	0.63	0.42	0.86	0.73	1.00				
40	0.29	0.35	0.73	0.53	0.63	0.53	0.53	0.35	0.63	0.42	0.53	0.44	0.44	0.44	0.14	0.14	0.10	0.10	0.50	0.63	0.50	0.35	0.63	0.53	0.42	0.42	0.73	0.73	0.53	0.63	0.59	0.73	0.73	0.63	1.00			
41	0.56	0.75	0.50	0.50	0.59	0.42	0.42	0.56	0.50	0.75	0.35	0.29	0.35	0.35	0.08	0.13	0.14	0.20	0.65	0.29	0.75	0.65	0.35	0.29	0.75	0.65	0.42	0.59	0.29	0.50	0.87	0.50	0.50	0.42	0.50	1.00		
43	0.29	0.35	0.73	0.53	0.63	0.53	0.53	0.35	0.63	0.42	0.53	0.44	0.44	0.44	0.14	0.14	0.10	0.10	0.50	0.63	0.50	0.35	0.63	0.53	0.42	0.42	0.73	0.73	0.53	0.63	0.59	0.73	0.73	0.63	1.00	0.50	1.00	
45	0.23	0.29	0.63	0.44	0.53	0.44	0.63	0.42	0.53	0.50	0.44	0.53	0.37	0.53	0.14	0.14	0.10	0.10	0.42	0.73	0.42	0.29	0.53	0.63	0.35	0.50	0.73	0.63	0.63	0.53	0.50	0.63	0.63	0.53	0.86	0.42	0.86	1.00

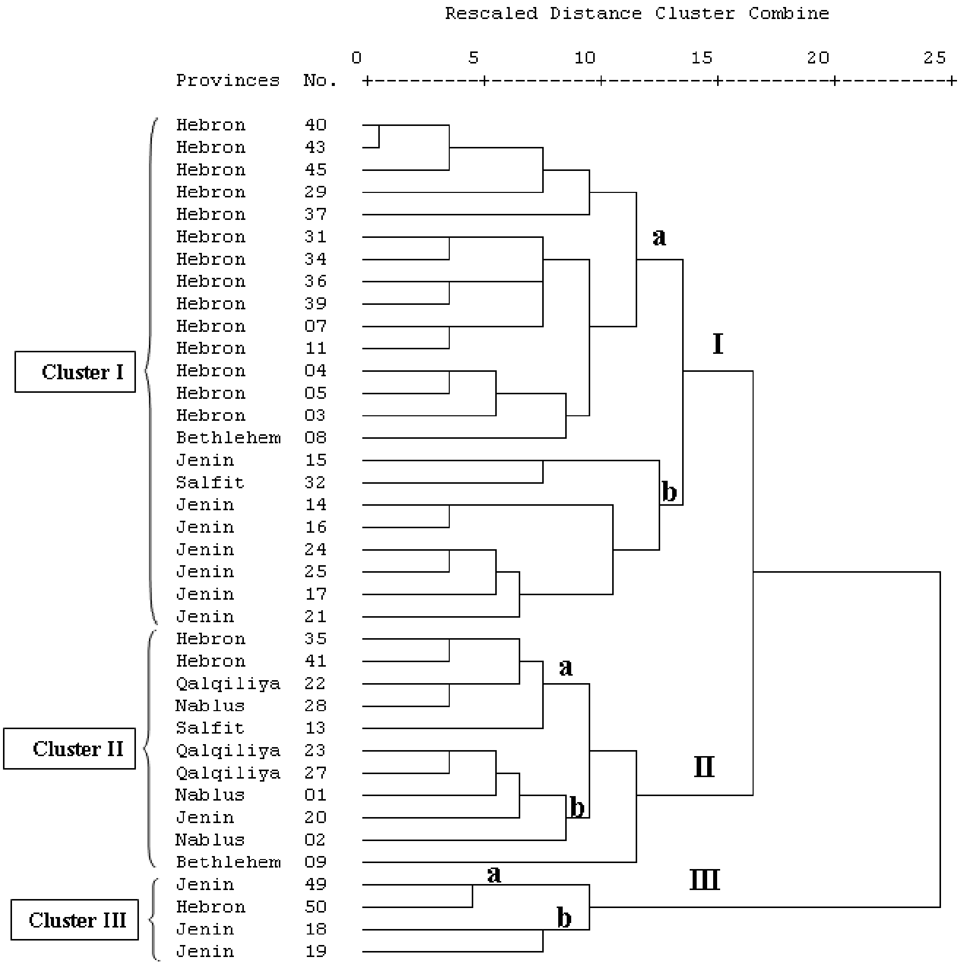

Figure 3.2 Clusters analysis of morphological descriptors of 38 Palestinian melon accessions. Numbers of accessions are as listed in Table 2.1.

Morphological descriptors differentiate between "white" *flexuosus* ecotypes, especially secondary skin color pattern descriptor. Sub cluster Ia accessions showed striped pattern, while no secondary skin color pattern was shown in sub cluster Ib.

In cluster I, the highest similarity (1.0) was found between accessions UB-243-12 & UB-59-09, these two accessions were collected from the same area (Dura/Hebron). The lowest similarity (0.30) was between accessions BERC-HHF07 & BERC-QMF21. BERC-HHF07 accession was collected from Hebron area, while BERC-QMF21 accession was collected from Jenin area.

Cluster II (green *flexuosus*) subdivided into two sub clusters: IIa and IIb. IIa contained 5 accessions (2 from Hebron area and 3 form Northern areas of the West Bank). IIb contained 5 accessions (2 from Hebron area and 3 from Northern areas of the West Bank). Cluster II also contained one accession from Bethlehem area in a separate branch.

In cluster II, the highest similarity (0.87) was found between several accessions including BERC-NTF01 & BERC-QHF23, BERC-JMF20 & BERC-QHF27, BERC-QFF22 & BERC-QHF27, BERC-QFF22 & BERC-NTF28, BERC-QFF22 & UB-196-11, and between BERC-QHF23 & BERC-NTF28.

The lowest similarity (0.56) was found between several accessions including BERC-NTF1 & BERC-SDF13, BERC-NTF1 & BERC-QHF23, BERC-NTF1 & BERC-NTF28, BERC-NTF1 & UB-196-11, BERC-NTF1 & UB-246-12, BERC-NTF2 & BERC-BTF09, BERC-NTF02 & BERC-SDF13, BERC-BTF9 & BERC-QFF22, BERC-BTF9 & BERC-QHF23, and between BERC-BTF09 & UB-246-12.

Cluster III (*cantalupensis*) was subdivided into two sub clusters: IIIa and IIIb. IIIa contained 2 accessions (one accession from Hebron area & one accession from Jenin area), IIIb contained 2 accessions from Jenin area.

In cluster III, the highest similarity (0.82) was found between accessions (UB-97-10 & UB-229-12). While the lowest similarity (0.57) was found between two accessions (BERC-JMC18 & UB-229-12).

The highest similarity between *flexuosus* and *cantalupensis* accessions by morphological descriptors (0.25) was found between BERC-HSF29 & BERC-JMC18.

Accessions included morphological characterization (UB-203-11 & UB-84-10) defined as white *flexuosus*, and located in sub cluster Ia (these two accessions wasn't have common name).

3.2 Molecular Characterization.

3.2.1 DNA quality and quantity.

DNA check on agarose gel electrophoresis for all accessions (44) had tight bands, no streaky bands, no degraded bands, no shearing bands, and no RNA appears (Figure 3.3).

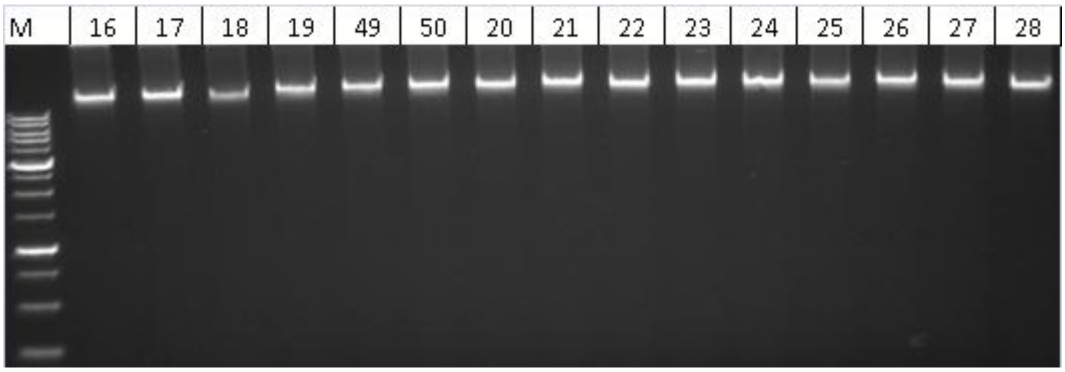

Figure 3.3: DNA check for 15 DNA samples extracted from melon accessions. Lane 1: (M: Marker) 1Kb ladder (5µl), the other lanes: DNA samples for 15 melon accession (3µl DNA and 3µl loading dye).

DNA concentrations (ng/µl) and Abs 260/280 (nm) for all DNA samples are shown in Table 3.3. Abs 260/280 ratio indicates the quality of DNA samples, and best ratio for DNA is around 1.8, all DNA melon samples is around 1.8.

Table 3.3 DNA concentration and Abs 260/280 ratio for all DNA melon samples.

No.	260/280	ng/µL	No.	260/280	ng/µL
1	1.71	1415.91	21	1.73	2055.71
2	1.83	356.63	22	1.88	652.55
3	1.86	466.67	23	1.91	617.19
4	1.76	2064.16	24	1.83	238.04
5	1.90	797.24	25	1.85	2306.12
6	1.85	2189.30	26	1.92	517.80
7	1.80	106.54	27	1.86	1883.83
8	1.90	1623.53	28	1.91	2897.76
9	1.84	127.00	29	1.89	1460.71
10	1.89	3471.84	31	1.74	2272.38
11	1.74	3361.84	32	1.90	518.85
12	1.82	250.43	33	1.83	93.26
13	1.96	543.37	34	1.78	2843.35
14	1.76	2860.37	35	1.91	1978.43
15	1.93	211.15	36	1.84	2176.85
16	1.91	328.47	37	1.87	2064.18
17	1.82	2390.47	38	1.77	3256.14
18	1.86	2954.54	39	1.80	946.11
19	1.80	746.57	40	1.89	2492.69
49	1.75	1722.15	41	1.85	889.98
50	1.88	618.73	43	1.82	1492.40
20	1.89	2822.54	45	1.79	1065.70

3.2.2 RAPD analysis.

A total of 14 RAPD primers were used for the determination of genetic variation in 44 traditional landraces of melon gave 132 bands, 75 of them were polymorphic bands (57%), and 57 were monomorphic bands (43%). The sizes of amplified bands by RAPD primers ranged from 250 bp to 3000 bp.

In total, the average number of amplified bands was 9.43 bands, the average number of monomorphic bands was 4.07 bands, and the average number of polymorphic bands was 5.36 bands. Figure 3.4 & Figure 3.5 show examples of check RAPD-PCR products on agarose gel electrophoresis.

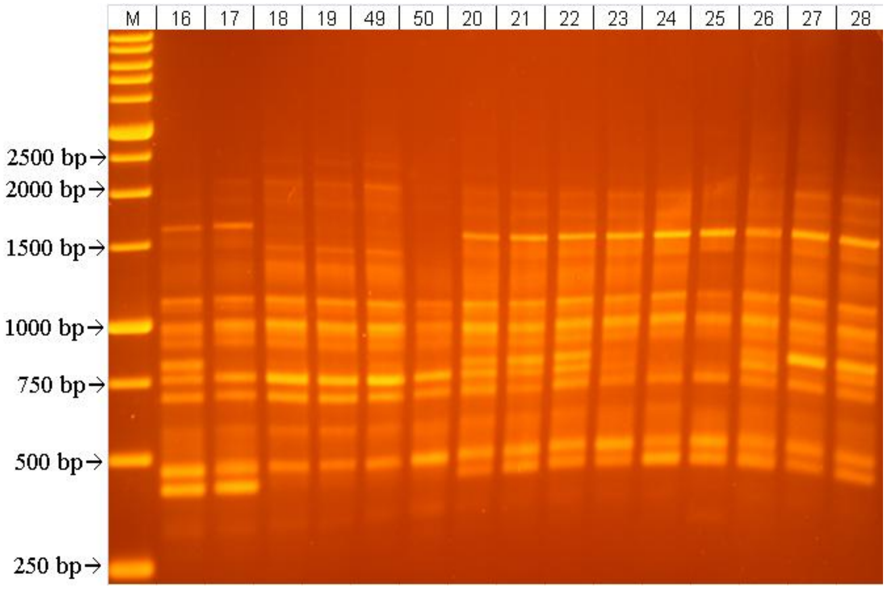

Figure 3.4 RAPD-PCR products by OPD08 primer checked on 1.5% agarose gel electrophoresis. Lane1: (M: Marker) 1Kb ladder, tphe other lanes for melon accessions number as listed in Table 2.1.

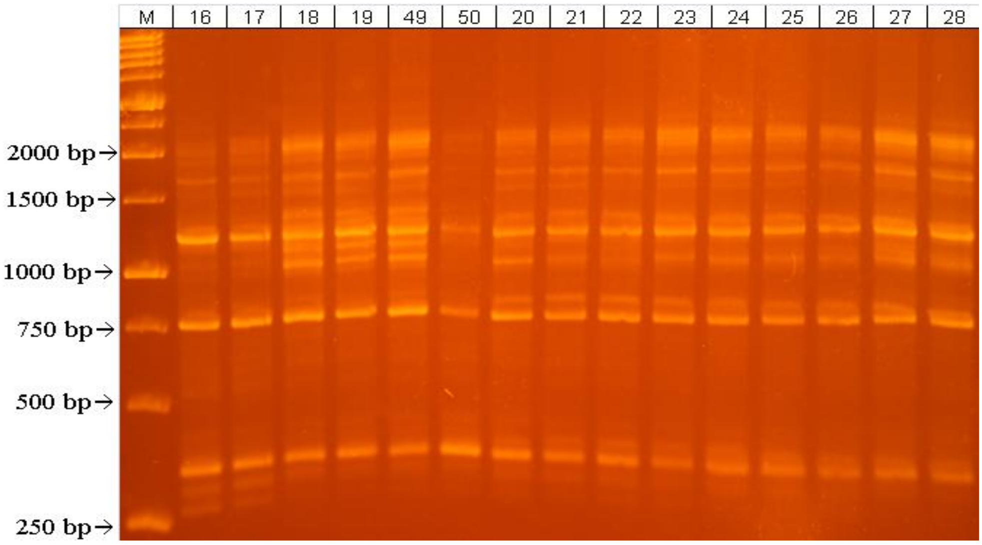

Figure 3.5 RAPD-PCR products by OPD07 primer checked on 1.5% agarose gel electrophoresis. Lane1: (M: Marker) 1Kb ladder, the other lanes for melon accessions number as listed in Table 2.1.

The highest resolving power (Rp) value was 8.23 and it was for OPD07 RAPD primer, but the lowest Rp value was 2.64 for OPA16 RAPD primer. Table 3.4 shown the number of bands amplified for each primer, number of polymorphic and monomorphic bands, Percentage of polymorphic bands, Rp values, and sequence (5'-3') for each primer.

Table 3.4 Fourteen RAPD primers used in this study, with total No. of bands, No. of monomorphic and polymorphic bands, Percentage of polymorphic bands, Rp values, and sequence (5'-3') for each primer.

Primer name	Total No. of bands	No. of monomorphic bands	No. of ploymorphic bands	Percentage of polymorphic bands (%)	RP value	Sequence (5'-3')
OPA07	6	3	3	50.00	4.50	GAAACGGGTG
OPA10	9	2	7	77.78	5.14	GTGATCGCAG
OPA16	5	1	4	80.00	2.64	AGCCAGCGAA
OPA18	8	1	7	87.50	5.43	AGGTGACCGT
OPB06	11	1	10	90.91	6.20	TGCTCTGCCC
OPC08	6	2	4	66.67	4.39	TGGACCGGTG
OPD07	11	2	9	81.82	8.23	TTGGCACGGG
OPD08	10	3	7	70.00	6.00	GTGTGCCCCA
OPD11	10	7	3	30.00	5.41	AGCGCCATTG
OPD13	11	10	1	9.09	5.73	GGGGTGACGA
OPD20	12	3	9	75.00	7.34	ACCCGGTCAC
OPL07	15	12	3	20.00	7.77	AGGCGGGAAC
OPR02	10	5	5	50.00	6.02	CACAGCTGCC
OPR10	8	5	3	37.50	5.27	CCATTCCCCA
Sum	132	57	75			
Average	9.43	4.07	5.36	57.00		

Similarity matrix and Dendrogram of 14 RAPD primers generated between 44 melon accessions shown in Table 3.5 and Figure 3.6 respectively.

From RAPD primers dendrogram; two clusters were revealed: cluster I: contained all *flexuosus* accessions, cluster II: contained all *cantalupensis* accessions.

Cluster I (*flexuosus*) subdivided into two sub clusters: Ia and Ib. Ia contained 17 accessions, while Ib contained 23 accessions. Cluster II (*cantalupensis*) subdivided into two sub clusters: IIa and IIb. IIa contained 3 accessions (accessions collected from Jenin), while IIb contained one accession (collected from Hebron).

Table 3.5: Similarity matrix by Jaccard coefficient for 14 RAPD primers of 44 Palestinian melon accessions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	49	50	20	21	22	23	24	25	26	27	28	29	31	32	33	34	35	36	37	38	39	40	41	43	45
1	1.00																																											
2	0.85	1.00																																										
3	0.90	0.85	1.00																																									
4	0.90	0.89	0.93	1.00																																								
5	0.89	0.87	0.93	0.92	1.00																																							
6	0.89	0.87	0.93	0.92	0.89	1.00																																						
7	0.84	0.78	0.79	0.81	0.78	0.81	1.00																																					
8	0.85	0.82	0.87	0.85	0.86	0.86	0.78	1.00																																				
9	0.83	0.77	0.78	0.76	0.76	0.77	0.85	0.79	1.00																																			
10	0.88	0.86	0.90	0.88	0.86	0.87	0.79	0.89	0.78	1.00																																		
11	0.81	0.84	0.83	0.84	0.82	0.84	0.80	0.81	0.75	0.84	1.00																																	
12	0.84	0.82	0.89	0.85	0.87	0.85	0.81	0.85	0.81	0.90	0.82	1.00																																
13	0.83	0.82	0.80	0.80	0.79	0.81	0.81	0.81	0.84	0.81	0.82	0.83	1.00																															
14	0.87	0.87	0.89	0.90	0.88	0.88	0.79	0.83	0.77	0.88	0.80	0.86	0.82	1.00																														
15	0.86	0.85	0.82	0.83	0.81	0.82	0.81	0.80	0.81	0.79	0.85	0.79	0.88	0.85	1.00																													
16	0.87	0.82	0.84	0.85	0.85	0.82	0.83	0.82	0.80	0.83	0.81	0.89	0.81	0.86	0.81	1.00																												
17	0.83	0.84	0.82	0.84	0.86	0.83	0.76	0.83	0.75	0.84	0.79	0.86	0.78	0.88	0.78	0.88	1.00																											
18	0.78	0.79	0.83	0.82	0.80	0.82	0.70	0.78	0.71	0.82	0.73	0.80	0.74	0.83	0.72	0.74	0.83	1.00																										
19	0.78	0.79	0.83	0.82	0.80	0.82	0.70	0.78	0.71	0.82	0.73	0.80	0.74	0.81	0.72	0.74	0.83	0.98	1.00																									
49	0.79	0.80	0.84	0.84	0.82	0.83	0.71	0.79	0.72	0.83	0.74	0.81	0.75	0.83	0.74	0.75	0.85	0.98	0.98	1.00																								
50	0.68	0.66	0.64	0.66	0.65	0.66	0.64	0.62	0.64	0.64	0.60	0.63	0.64	0.68	0.65	0.67	0.69	0.75	0.75	0.74	1.00																							
20	0.83	0.82	0.89	0.87	0.88	0.88	0.80	0.83	0.77	0.85	0.83	0.89	0.80	0.86	0.80	0.89	0.87	0.78	0.78	0.80	0.64	1.00																						
21	0.87	0.87	0.89	0.88	0.88	0.91	0.82	0.86	0.81	0.88	0.83	0.91	0.85	0.89	0.83	0.87	0.90	0.83	0.83	0.84	0.67	0.94	1.00																					
22	0.84	0.88	0.87	0.88	0.89	0.88	0.78	0.82	0.80	0.85	0.80	0.87	0.83	0.87	0.80	0.85	0.89	0.82	0.84	0.84	0.69	0.91	0.93	1.00																				
23	0.89	0.85	0.92	0.90	0.90	0.91	0.82	0.87	0.81	0.90	0.84	0.92	0.85	0.88	0.82	0.89	0.88	0.82	0.82	0.83	0.66	0.94	0.97	0.95	1.00																			
24	0.85	0.86	0.90	0.91	0.89	0.89	0.80	0.82	0.80	0.85	0.82	0.88	0.81	0.89	0.80	0.85	0.89	0.84	0.84	0.85	0.68	0.91	0.93	0.95	0.95	1.00																		
25	0.85	0.86	0.90	0.91	0.89	0.89	0.80	0.82	0.80	0.85	0.82	0.88	0.81	0.89	0.80	0.85	0.89	0.84	0.84	0.85	0.68	0.91	0.93	0.95	0.95	1.00	1.00																	
26	0.87	0.85	0.91	0.88	0.89	0.91	0.81	0.87	0.82	0.88	0.81	0.91	0.84	0.88	0.81	0.88	0.89	0.82	0.82	0.84	0.66	0.94	0.96	0.95	0.98	0.95	0.95	1.00																
27	0.86	0.87	0.91	0.92	0.90	0.90	0.81	0.85	0.80	0.87	0.82	0.89	0.84	0.91	0.82	0.88	0.88	0.83	0.83	0.85	0.67	0.94	0.95	0.96	0.97	0.98	0.98	0.96	1.00															
28	0.87	0.85	0.92	0.89	0.88	0.91	0.81	0.85	0.82	0.88	0.83	0.90	0.84	0.89	0.83	0.88	0.88	0.82	0.82	0.84	0.66	0.94	0.96	0.95	0.98	0.97	0.97	0.98	0.98	1.00														
29	0.87	0.88	0.90	0.91	0.91	0.91	0.80	0.85	0.79	0.89	0.83	0.88	0.83	0.93	0.83	0.84	0.88	0.84	0.84	0.85	0.66	0.89	0.95	0.92	0.93	0.92	0.92	0.92	0.95	0.92	1.00													
31	0.86	0.84	0.88	0.92	0.89	0.89	0.79	0.85	0.76	0.86	0.84	0.85	0.81	0.88	0.82	0.82	0.84	0.80	0.80	0.82	0.65	0.85	0.88	0.86	0.88	0.86	0.86	0.86	0.89	0.86	0.94	1.00												
32	0.88	0.84	0.93	0.88	0.90	0.91	0.80	0.85	0.80	0.89	0.81	0.90	0.83	0.91	0.81	0.87	0.87	0.82	0.82	0.84	0.66	0.91	0.91	0.92	0.93	0.91	0.91	0.95	0.92	0.94	0.92	0.88	1.00											
33	0.89	0.88	0.92	0.93	0.94	0.88	0.77	0.84	0.76	0.87	0.83	0.83	0.80	0.88	0.82	0.83	0.83	0.80	0.80	0.81	0.65	0.88	0.88	0.90	0.89	0.90	0.90	0.87	0.91	0.88	0.91	0.91	0.88	1.00										
34	0.88	0.87	0.90	0.92	0.92	0.89	0.81	0.82	0.77	0.89	0.84	0.88	0.80	0.90	0.78	0.85	0.87	0.81	0.81	0.82	0.65	0.88	0.90	0.91	0.91	0.91	0.91	0.89	0.92	0.89	0.92	0.90	0.92	0.93	1.00									
35	0.89	0.86	0.89	0.87	0.87	0.90	0.80	0.82	0.78	0.84	0.83	0.84	0.84	0.86	0.89	0.84	0.80	0.77	0.77	0.78	0.67	0.87	0.88	0.87	0.89	0.87	0.87	0.88	0.89	0.90	0.87	0.85	0.88	0.89	0.85	1.00								
36	0.88	0.89	0.90	0.89	0.87	0.94	0.82	0.83	0.81	0.87	0.85	0.87	0.82	0.90	0.82	0.86	0.87	0.80	0.80	0.82	0.67	0.88	0.91	0.91	0.91	0.91	0.91	0.92	0.90	0.92	0.91	0.87	0.95	0.87	0.92	0.88	1.00							
37	0.88	0.90	0.91	0.89	0.88	0.94	0.82	0.83	0.82	0.89	0.86	0.88	0.83	0.90	0.83	0.87	0.86	0.81	0.81	0.82	0.66	0.88	0.91	0.89	0.91	0.89	0.89	0.91	0.90	0.91	0.91	0.88	0.94	0.87	0.92	0.88	0.98	1.00						
38	0.86	0.88	0.91	0.87	0.87	0.90	0.80	0.84	0.82	0.88	0.83	0.89	0.85	0.89	0.85	0.86	0.85	0.81	0.81	0.83	0.67	0.89	0.92	0.91	0.92	0.89	0.89	0.91	0.91	0.92	0.93	0.90	0.94	0.88	0.90	0.88	0.94	0.96	1.00					
39	0.84	0.90	0.88	0.88	0.87	0.88	0.79	0.84	0.82	0.85	0.86	0.86	0.85	0.86	0.85	0.86	0.85	0.78	0.78	0.80	0.65	0.89	0.92	0.91	0.91	0.91	0.91	0.91	0.91	0.93	0.90	0.85	0.90	0.89	0.88	0.87	0.93	0.91	0.92	1.00				
40	0.88	0.89	0.91	0.89	0.86	0.94	0.84	0.85	0.83	0.89	0.86	0.90	0.84	0.90	0.84	0.87	0.86	0.81	0.81	0.82	0.66	0.88	0.93	0.89	0.93	0.91	0.91	0.92	0.92	0.92	0.92	0.89	0.92	0.85	0.91	0.88	0.97	0.98	0.96	0.91	1.00			
41	0.89	0.88	0.92	0.91	0.88	0.96	0.82	0.87	0.79	0.88	0.85	0.86	0.83	0.89	0.85	0.84	0.85	0.84	0.84	0.86	0.69	0.88	0.92	0.90	0.92	0.90	0.90	0.93	0.91	0.93	0.91	0.90	0.93	0.88	0.88	0.90	0.96	0.94	0.92	0.90	0.94	1.00		
43	0.88	0.88	0.91	0.89	0.91	0.92	0.81	0.87	0.80	0.91	0.86	0.90	0.84	0.91	0.84	0.87	0.87	0.82	0.82	0.84	0.65	0.91	0.96	0.92	0.95	0.91	0.91	0.94	0.93	0.94	0.97	0.91	0.95	0.90	0.92	0.87	0.94	0.94	0.96	0.93	0.94	0.93	1.00	
45	0.87	0.88	0.89	0.91	0.91	0.88	0.80	0.86	0.78	0.85	0.83	0.86	0.85	0.89	0.85	0.84	0.85	0.81	0.81	0.83	0.66	0.86	0.89	0.90	0.89	0.89	0.89	0.88	0.91	0.89	0.94	0.94	0.91	0.92	0.91	0.89	0.90	0.90	0.92	0.89	0.90	0.91	0.93	1.00

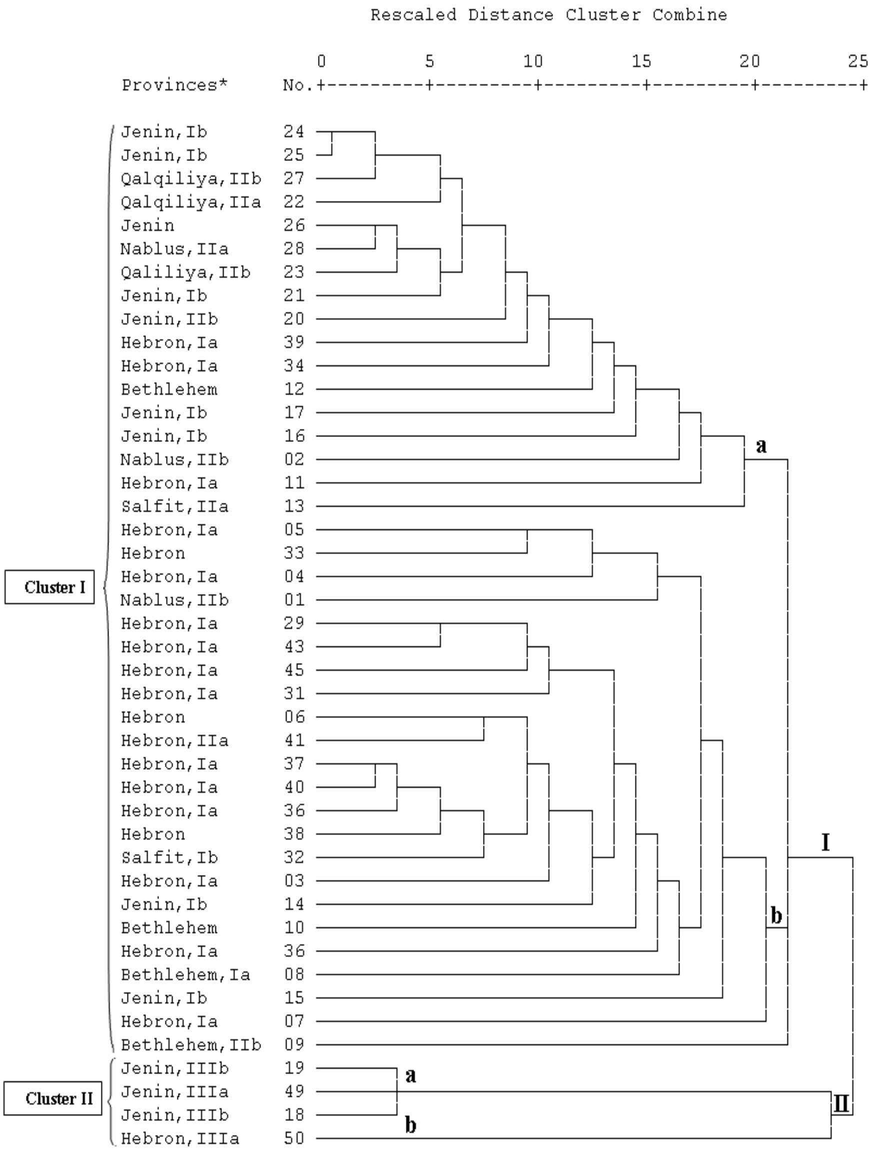

Figure 3.6 Clusters analysis of 14 RAPD primers of 44 Palestinian melon accessions. "*": Provinces listed with cluster result by morphological characterization for each accession. Numbers of accessions are as listed in Table 2.1.

In cluster I, the highest similarity between accessions was 1.0 between (BERC-JSF24 & BERC-JMF25), these two accessions collected from Jenin area (same cluster in morphological analysis).

The lowest similarity was 0.78 between (BERC-SDF13 & BERC-JMF17) accessions; these two accessions were collected from Salfit and Jenin area respectively, and located in separate clusters in morphological analysis.

Cluster I contained many closely related when compared to morphological clusters. Such as (BERC-JSF24 & BERC-JMF25) accessions, these two accessions were collected from Jenin area, the similarity between them by RAPD primers analysis was 1.0 (highest similarity) and located in the same sub cluster in morphological characterization (Ib), similarity between these two accessions by morphological analysis was 0.86.

There are 12 accessions located in sub cluster Ib by RAPD analysis also located in the same sub cluster in morphological analysis (Ia).

In Cluster II, the highest similarity was 0.98 between (BERC-JMC18 & BERC-JMC19 & UB-97-10), and all these accessions were collected from Jenin area. The lowest similarity was 0.74 and it was between (UB-97-10 & UB-229-12) accessions, which were collected from Jenin and Hebron area respectively.

The highest similarity between *flexuosus* and *cantalupensis* accessions by RAPD primers was 0.86 (between UB-246-12 & UB-97-10).

RAPD Primers succeeded to differentiate between *cantalupensis* and *flexuosus* Palestinian traditional landraces, and also between *cantalupensis* accessions according to ecotypes.

Although there are some combinations between morphological clusters and RAPD primers results, RAPD primers failed to reveal real differentiation between *flexuosus* ecotypes. No unknown accessions were defined by RAPD analysis.

3.2.3 ISSR analysis.

A total of 9 ISSR primers were used to determine genetic variations in 44 traditional landraces of melon gave 71 bands; all of them showed monomorphic bands as demonstrated in Figure 3.7 and Table 3.6. The sizes of amplified bands by ISSR primers ranged from 300 bp to 3500 bp.

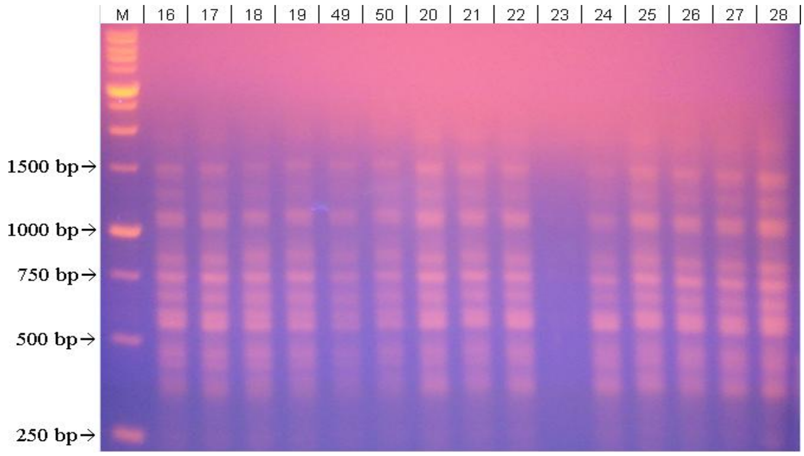

Figure 3.7: ISSR-PCR products by (AC)8YC primer checked on 1.5% agarose gel electrophoresis. Lane1: (M: Marker) 1Kb ladder, the other lanes for melon accessions number as listed in Table 2.1.

Table 3.6 Nine ISSR primers used in this study, with annealing temperature for each primer, Total No. of bands, and No. of monomorphic and polymorphic bands.

Primer (5'-3')	Annealing temperature (°C)	Total No. of bands	No. of monomorphic bands	No. of polymorphic bands
(AC)8T	45	6	6	0
(AG)8T	45	10	10	0
(TC)8C	47	7	7	0
(TG)8G	47	6	6	0
(AC)8G	47	8	8	0
(GGGTG)3	52	9	9	0
(ATG)6	42	6	6	0
(AC)8YC	50	12	12	0
(GA)8YG	50	7	7	0
Sum		71	71	0

CHAPTER FOUR

DISCUSSION

4.1 Morphological characterization.

In this study; all tested Palestinian *Flexuosus* accessions have: elongate shape, white or green skin color, speckled or striped secondary color pattern, ribbed skin texture, white or green flesh color, presence of hair, insipid taste (non sweet) fruits. All of these fruit traits similar to the description of *Cucumis melo* var. *flexuosus* reported by Stepansky *et al*. (1999); and Staub *et al*. (2004).

Green Palestinian *Flexuosus* accessions which clustered in cluster II (Figure 3.2) agreed with *Cucumis melo* var. *flexuosus* description by Pitrat *et al.* (2000), Pitrat defined *Cucumis melo* var. *flexuosus* as: monoecious, very long fruit, light green or striped light green or dark green skin, ribbed or wrinkled, mature fruit not sweet, white flesh, young fruits eaten raw or pickled (like cucumber), climacteric, and medium-size white seeds. It is also similar to description of some accessions reported in Iran by Soltani *et al*. (2010) for Iranian *Flexuosus*.

White Palestinian *Flexuosus* accessions which are clustered in cluster I (Figure 3.2) similar to *Flexuosus* accessions were reported by Staub *et al.* (2000). *Flexuosus* accessions were studied by Nakata *et al*. (2005) were also white *Flexuosus,* but the fruit skin was netted and corrugated.

Sex type of all studied Palestinian *Flexuosus* accessions were monoecious. These results for are in agreement with the results reported in

Iran (Soltani *et al*., 2010), Israel (Stepansky *et al*., 1999), and Greece (Staub *et al*., 2004). In contrast; *Flexuosus* accessions studied by Nakata *et al*. (2005) in Japan reported to be andromonoecious.

Morphological traits are varied among tested Palestinian *Flexuosus* and also between *Flexuosus* group in many countries, this agreed to consideration that *Cucumis melo* is the most diverse species.

For white *Flexuosus*; a distinctive sub cultivar of *Flexuosus* is present in each region of West Bank; sub cluster Ia (Figure 3.2) collected from the southern areas of West Bank, and sub cluster Ib (Figure 3.2) collected from the northern areas of West Bank. Accessions in each sub cluster have distinctive traits.

Similarity matrix of 13 morphological descriptors for Palestinian melons (Figure 3.2) indicated they are closely related with each other within sub clusters, such as between UB-243-12 & UB-59-09, these accessions collected from the same area (Dura/Hebron). The lowest similarity was 0.30 between BERC-HHF7 & BERC-QMF21. BERC-HHF7 accession was collected from Hebron area, while BERC-QMF21 accession was collected from Jenin area.

Morphological results in this study compared with other studies showed that there were variations results for most traits between studies (Stepansky *et al*., 1999; Staub *et al*., 2000; Staub *et al*., 2004; Nakata *et al*., 2005; Soltani *et al*., 2010; Zhang et al., 2012**)**.

Palestinian *cantalupensis* accessions (cluster III, Figure 3.2) which have fruit traits: oblate fruit shape, orange skin color, short streaking pattern, wrinkled, pale orange flesh color, absence of hair, sweet taste, and monoecious sex type. These results are similar to the description of *cantalupensis* by Stepansky *et al*. (1999), Staub *et al*. (2004), Sari *et al*. (2008), and Escribano *et al*. (2011).

Only Seeds weight was varied among *cantalupensis* accessions, 3 of the 4 *cantalupensis* accessions (collected from Jenin area) were close to each other in seeds weight, while the other accession (collected from Hebron area) was not. This indicated that *cantalupensis* accessions separated by geographical regions according to seeds weight.

People in southern West Bank call "fakus sahori" white and green sub cultivar of *Flexuosus*, while in the northern West Bank people call "fakus abyad" and "fakus akhdar" for white and green sub cultivar of *Flexuosus* respectively.

According to this study, these nomenclatures confirmed with white *Flexuosus* which clustered in sub cluster Ia (Figure 3.2), this sub cluster could be called "sahori abyad". Also for white *Flexuosus* which clustered in sub cluster Ib, this sub cluster could be called "fakus abyad".

Morphological characterization succeeded to define the unknown common name accessions (UB-203-11 & UB-84-10) as White *flexuosus*, and located in sub cluster Ia (Figure 3.2).

4.2 Molecular characterization.

4.2.1 RAPD analysis.

RAPD analysis succeeded to discriminate between tested Palestinian landraces of *Flexuosus* and *cantalupensis*. The highest similarity between all accessions (0.86) was between UB-246-12 & UB-97-10 accessions of *Flexuosus* and *cantalupensis* respectively. This indicates the high similarity between Palestinian landraces of melon groups.

The high similarity between Palestinian *Flexuosus* and *cantalupensis* was in agreement with the results obtained by Stepansky *et al*. (1999) where *Flexuosus* accessions were dispersed among the branches, closer to the *inodorus* and *cantalupensis* types. Also agreed with Staub *et al*. (2000); Staub *et al*. (2004); Nakata *et al*. (2005); and Sensoy *et al*. (2007) results.

In contrast, low similarity was showed between Spanish *flexuosus* and *cantalupensis* accessions (López-Sesé *et al*., 2003).

Within studied Palestinian *Flexuosus* accessions (cluster I, Figure 3.6) there were no clear discrimination between accessions when compared with morphological traits, except some accessions that are closely related to each other in morphological and molecular characterization (RAPD). This revealed relative highly genetic diversity between Palestinian *Flexuosus* melons. These results are in agreement with results reported by Soltani *et al*. (2010).

In cluster I (Figure 3.6) the highest similarity between accessions was 1.0 and it was between (BERC-JSF24 & BERC-JMF25), these two *Flexuosus* accessions collected from Jenin area (same cluster in morphological analysis, similarity was 0.86).

Most of *Flexuosus* accessions localized in sub-cluster Ib (Figure 3.6) are localized in one sub-cluster in morphological characterization (Ia, Figure 3.2).

4.2.2 ISSR analysis.

All amplified bands (71) by nine ISSR primers were monomorphic, so that no genetic variations revealed by ISSR primers.

Danin-Poleg *et al*. (1998) used 42 ISSR primers, eight primers (19%) showed no differences between melon genotypes, giving a monomorphic pattern, and eight (19%) primers failed to amplify a clear product. These results consolidated the results of this study. Therefore it is recommended that further analysis would require using more ISSR primers to study the genetic variations between Palestinian melons.

Sestili *et al*. (2008) also used ISSR primers, Out of 90 ISSR primers used, and 39 showed polymorphism among 13 Italian melon accessions. So that 57% of ISSR primers used were monomorphic.

Unknown common name accessions among white or green *Flexuosus* were not defined by molecular markers.

4.3 Conclusions

Phenotypic (morphological and pomological) results have shown to be useful in characterizing melon landraces. Despite considerable phenotypic variability between melon landraces, Palestinian melon groups (*flexuosus* and *cantalupensis*) have shown high genotypic similarity between their different accessions.

RAPD has proved to be a more useful technique in characterizing Palestinian melon genotypes. RAPD primers have succeeded to discriminate between Palestinian melon groups (*flexuosus* and *cantalupensis*).

Here we utilized morphological and genetic characters to further refine this Palestinian melon database for use by both researchers and farmers.

Our results strongly indicate the importance of Palestinian landraces for study of the origin and diversity of melon groups. This study was the first study on genetic characterization of *Cucumis melo* groups, and paves the way for more in-depth research.

4.4 Recommendations

It is recommended to use more RAPD and ISSR primers, and more specific types of molecular markers to reveal the genetic variations between Palestinian melon groups including reference accession.

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Appendixes

Appendix A

Solutions preparations

❖ **H-buffer, 100 ml:** Dissolve 1.21 g of Tris base with 80 ml sdH2O, add 74.4 mg of EDTA, add 8.18 g of NaCl, add 0.9g of glucose, and 4 ml HCL, adjust PH to 8.0, and autoclave. After autoclaving add 2g of CTAB, 1.55g of DTT, and sdH2O to 100 ml total volume. CTAB and DTT were added before use.
❖ **10µg/ml RNase, 10 ml:** Dissolve 0.1 mg of RNase powder in 100 ml sdH2O. Store at -20°C.
❖ **3M sodium acetate (pH 6.8):** In 200 ml sdH2O 40.83 g of Sodium acetate were dissolved, 18 ml of Glacial Acetic Acid were added, and then adjust PH to 6.8, the solution was topped up with sdH2O to 100 ml total volume and autoclaved.
❖ **1X TAE buffer, 500 ml:** TAE buffer was prepared as 50X stock solution. A 50X stock solution (1L) was prepared by dissolving 242g Tris-HCl base in 500 ml of sdH2O, add 57.1ml of glacial acetic acid, and add 100 ml of 500 mM EDTA (pH 8.0) solution, and top up with sdH2O to 1 liter total volume. This stock solution diluted 50:1 with sdH2O to make a 1X TAE working solution.
❖ **0.5µg/ml ethidium bromide** A stock solution of ethidium bromide (10 mg/ml) was prepared as follows 10 mg of ethidium bromide were dissolved in 1ml sdH2O. Stock solution stored in dark bottle in refrigerator. Stock solution was used in agarose gels preparation to final concentration 0.5µg/ml ethidium bromide.

Appendix B

Quantitative morphological descriptors scored on melon accessions

No	Stem thickness (mm)± SD[1]	Flower size (mm) ±SD[2]	Fruit size (g) ±SD[3]	Fruit length/width ratio [L/W] ±SD[4]	Seeds weight (g)[5]
1	7.4±1.26	11.19±1.01	376.93±11.34	4.19±0.27	3.3
2	7.87±1.45	10.10±0.56	391.68±56.96	4.27±0.52	3.4
3	7.70±1.20	11.54±1.04	420.00±34.94	4.66±0.50	3.1
4	7.57±1.25	10.59±0.42	365.33±31.97	4.51±0.37	3.3
5	8.00±1.36	10.83±0.90	428.15±21.14	4.68±0.60	3.2
7	7.37±1.51	11.27±0.71	360.30±43.09	4.12±0.64	3.4
8	7.37±1.40	11.03±0.80	390.90±22.31	4.93±0.32	3.2
9	7.53±1.15	11.68±0.75	347.00±26.86	4.94±0.84	3.4
11	8.80±1.46	11.81±1.21	333.93±63.55	4.13±0.14	3.3
13	7.53±1.47	11.21±0.82	404.08±83.39	5.26±0.36	3.4
14	7.37±1.75	11.50±0.30	382.00±22.16	4.37±0.29	3.6
15	8.37±1.25	12.11±1.59	323.65±36.68	4.80±0.52	3.2
16	7.10±1.20	11.37±0.91	377.93±18.18	4.69±0.29	3.5
17	8.97±1.55	11.17±0.68	412.33±40.03	5.02±0.34	3.5
18	8.93±1.32	11.95±1.16	437.53±67.09	1.78±0.23	3.6
19	8.97±2.17	10.76±0.75	425.07±56.42	1.75±0.13	4.7
49	8.73±1.59	11.36±1.80	435.10±46.17	1.82±0.12	3.8
50	8.87±1.67	10.67±0.97	437.03±50.87	1.94±0.04	3.4
20	8.97±1.40	11.63±0.66	417.35±40.74	4.01±0.52	4.5
21	8.39±1.80	12.14±0.31	397.18±39.66	4.80±0.38	4.1
22	8.77±1.49	11.71±0.82	402.95±66.18	4.47±0.91	3.3
23	8.57±1.83	11.99±1.57	323.63±89.81	4.12±0.76	3.3
24	8.10±1.30	11.59±1.51	385.83±16.99	4.67±0.48	4.5
25	8.83±1.49	11.32±0.40	403.00±63.42	5.26±0.30	4.7
27	8.43±1.86	11.46±0.68	363.05±51.53	4.18±0.70	3.5
28	8.97±1.59	12.10±1.33	359.30±72.68	4.81±1.01	3.4
29	8.67±2.10	11.82±0.97	345.45±72.00	5.35±0.42	3.8
31	8.43±1.60	11.15±0.93	387.05±27.51	4.57±0.46	3.2
32	8.26±1.89	11.74±0.32	355.78±42.20	4.99±0.71	3.8
34	8.8±2.14	11.70±1.66	407.25±70.93	4.40±0.33	3.5
35	8.43±1.42	12.04±0.45	387.43±53.82	4.40±0.53	3
36	8.50±1.20	11.35±1.30	396.75±76.99	4.76±0.48	3.7
37	8.70±1.53	10.71±0.62	334.18±77.30	4.71±0.38	4.4
39	8.90±1.70	11.61±0.78	374.93±64.16	4.36±1.00	3.9
40	8.63±1.78	12.31±1.55	433.10±19.32	4.33±0.67	4.4
41	8.83±1.21	10.35±0.90	424.58±43.91	4.44±0.47	3.2
43	8.93±1.51	12.03±0.75	401.25±25.30	4.77±0.36	3.9
45	8.33±1.20	12.18±1.15	440.33±22.86	4.94±0.27	3.8

1- Stem thickness (mm): measured for 3 plants from each accession with standard deviation , 2- Flower size (mm):measured for 3 plants from each accession with standard deviation , 3- Fruit size (g): measured for 10 plants from each accession of *flexuosus* and 3 plants from each accession of *cantalupensis* with standard deviation, 4- Fruit length/width ratio [L/W]: measured for 10 plants from each accession of *flexuosus* and 3 plants from each accession of *cantalupensis* with standard deviation, 5- Seeds weight (g): measured for 100 seeds from each accession.

جامعة النجاح الوطنية

كلية الدراسات العليا

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية نابلس – فلسطين

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الملخص

المقدمة: اقتصاديا يعتبر الفقوس والشمام من أهم المحاصيل الزراعية في فلسطين, الأصناف المحلية من هذه المحاصيل هي محاصيل بعلية مقاومة للجفاف وللأمراض التي تنتقل عن طريق التربة. على الرغم من اختلاف الصفات المورفولوجية بين الفقوس والشمام مثل الشكل، لون الفاكهة، الطعم، والنكهة، إلا أن الاختلافات الجينية بينهم منخفضة.

الأهداف: تهدف هذه الدراسة إلى دراسة الاختلافات الجينية بين وداخل المجموعات المحلية من الفقوس والشمام في فلسطين باستخدام الكاشفات الجينية (آليات التضخم العشوائي متعدد الأشكال (RAPD) وجملة تكرار التسلسل البسيط (ISSR)، وتحديد العلاقات بين التوصيف الجزيئي والمورفولوجي. وأيضا لتقييم كفاءة الكاشفات في التمييز بين وداخل السلالات المحلية في فلسطين.

طرق البحث: تم دراسة التنوع الوراثي بين 44 سلالة من السلالات المحلية الفلسطينية من مجموعات الفقوس والشمام باستخدام كاشفات RAPD وISSR، بالإضافة إلى دراسة الصفات المورفولوجية.

تم إنشاء مصفوفات التشابه وdendrograms بين السلالات باستخدام البرنامج الإحصائي SPSS (الإصدار 16). و تم حساب قيمة Rp لكل كاشفة.

النتائج: التوصيف المورفولوجي فصل السلالات إلى مجموعتين، المجموعة الأولى تحتوي جميع سلالات الفقوس وفصلت إلى مجموعتين فرعيتين (الأبيض والأخضر)، والمجموعة الثانية تحتوي جميع سلالات الشمام.

باستخدام 14 كاشفة من كاشفات RAPD أنتج 132 حزمة، 75 حزمة كانت حزم متعددة الأشكال (57%) و 57 حزمة كانت أحادية الشكل. تحليل المجموعات لنتائج بادئات RAPD أظهر أن السلالات فصلت الى مجموعتين، المجموعة الأولى وتحتوي جميع سلالات الفقوس والمجموعة الثانية وتحتوي جميع سلالات الشمام، كان أعلى تشابه بين السلالات (0.86).

باستخدام 9 كاشفة من كاشفات ISSR أنتج 71 حزمة, جميعها كان أحادي الشكل, لذلك لم ينتج أي اختلاف جيني بين السلالات باستخدام كاشفات ISSR. وهذا يكشف التشابه الجيني المرتفع بين مجموعات الفقوس والشمام, والسبب يعود إلى أن بادئات ISSR أكثر تحديداً من كاشفات RAPD.

الاستنتاجات: أثبتت الدراسة أن كاشفات RAPD لها كفاءة عالية في التمييز الجيني بين مجوعات الفقوس والشمام في فلسطين، وأعطت مؤشرات أو علامات حول الاختلافات الجينية داخل مجموعة الفقوس. لم يلاحظ أي اختلافات جينية باستخدام كاشفات ISSR.

أشارت النتائج بشدة إلى أهمية دراسة منشأ وتنوع سلالات الفقوس والشمام في فلسطين.