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Assessments of genetic diversity of fig (*Ficus carica L.*) in the West Bank based on Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) markers.

By

Khulood Saleh AlKaraki

In Partial Fulfillment of the Requirements for the Degree of
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The undersigned hereby certify that they have read and recommend to the Faculty of Scientific Research and Higher Studies at the Palestine Polytechnic University and the Faculty of Science at Bethlehem University for acceptance a thesis entitled:

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Assessments of genetic diversity of fig (*Ficus carica L.*) in the West Bank based on Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) markers.

khulood Saleh al Karaki

Abstract:

Fig (*Ficus carica L.*, Moraceae) is considered to be one of the most old and ancient type of fruits that grow in the Mediterranean region. Fig recently has attracted much of attention to researchers because it has medical importance and nutritional value. Identification of the polymorphism among Palestinian fig cultivars is essential step in crop improvement. Researches that were conducted in Palestine on genetic diversity of fig germplasm have been limited. There were just two studies were established in Palestine on genetic diversity among fig cultivars. RAPD fingerprinting was firstly used as molecular technique in both studies to characterize the polymorphism among Palestinian fig cultivars. However, this technique has limitation of reproducibility due to random nature of the primers. No study examined molecular polymorphism among Palestinian fig cultivars based on microsatellite marker and inter simple sequence repeat (ISSR). The aim of this study is to analyze the genetic diversity among 20 Palestinian fig cultivars were collected from different regions (northern, middle. Southern) of Palestine by using simple sequence repeat (SSR) and inter simple sequence repeat (ISSR). A set of 6 ISSR primers were used to analyze the diversity among which produced 56 loci of DNA, a mean of 9.3 DNA fragments per primer. We measured the efficiency of ISSR primers by calculating the resolving power (RP) and polymorphic information content (PIC). The total six ISSR primers produced mean of resolving power value (RP) of 10.8, mean of polymorphic information content (PIC) of 0.27 and percent of polymorphic marker of 81%. The primers UCB 812 and UCB 817 revealed high RP values 13.4 and 14.4 respectively. Therefore, these two ISSR primers were the most

useful to characterize the genetic diversity among Palestinian fig cultivars. Additionally, a set of seven SSR primers were used also to analyze the genetic diversity and produced 24 alleles, a mean of 3.3 alleles per primer and 88% percent of polymorphic SSR marker. The diversity parameters of SSR markers was measured to evaluate the efficiency of SSR markers, mean of polymorphic information content value (PIC) of 0.44 and mean of observed Heterozygosity value of 0.49. SSR Primers MFC3 and FCUP 68-1 revealed high PIC values of 0.7198 and 0.6838 respectively. Therefore, these two SSR primers were the most useful to characterize the genetic diversity among Palestinian fig cultivars. Clustering analysis was applied (UPGMA dendogram) for both markers' datasets and for combined ISSR and SSR data to elucidate the genetic relationships among Palestinian varieties. The genetic distance range was 0.000-0.789 for SSR and 0.050-0.644 for ISSR markers. The results of genetic distance suggest a wide range of genetic distance for SSR marker over ISSR marker. Despite the different dendogram trees produced by each marker, four genotype pairs were common between the two marker dataset trees and combined marker data tree. The correlation test between the employed Jaccard coefficients revealed no significant relationship ($r = 0.0246$). Results contribute to better utilization of more than one marker; this is of great importance to estimate the level of diversity among figs.

تحديد الطرز الجينية للتين في الضفة الغربية باستخدام SSR و ISSR

ملخص

ذُكرت شجرة التين في الكتب المقدسه باعتبارها شجره مباركه حيث ان التين حديثا جذب الكثيرمن اهتمام الباحثين لما له من اهميه غذائية وطبيه، فهو يعتبر من احد اكثر الفواكه التي تنمو في منطقه حوض البحر المتوسط منذ زمن قديم. أيجاد التنوع الجيني للأصناف المختلفة لثمره التين من الخطوات المهمة من اجل تحسين محاصيل التين، الا ان الدراسات في فلسطين حول التنوع الجيني لهذه الثمرة وايجاد صلات القرابة بين اصناف التين محدودة وما انجز منها كان مقتصر على تقنيه (RAPD) ولكن هذه التقنية لديها قيود في العمل وهي الطبيعة العشوائية للبادئات Primers. لذللك كان الهدف من هذه الدراسه هو تحديد االطرز الجينيه والعلاقات التطوريه بين اصناف التين المختلفه في فلسطين باستخدام تقنيات تستخدم لأول مره في فلسطين وهي Inter Simple repeat ISSR) Sequence)و(SSR) Simple Sequence Repeat.

حيث تم جمع 20 صنف من اصناف التين المختلفة من ثلاث مناطق في فلسطين (الشمال، الوسط، الجنوب) ومن ثم استخراج المادة الوراثية من كل صنف باستخدام طريقه CTAB و Qiagen DNeasy plant mini kit، ومن ثم مضاعفه المادة الوراثية في تقنيه ISSR باستخدام سته بادئات (Primers) فكان من النتائج ان 81% Percent of polymorphic ISSR marker بين الاصناف المدروسة من التين، وايضا تم مضاعفه المادة الوراثية في تقنيه SRR باستخدام سبعه بادئات (Primers) فكان من النتائج ان % 88% percent of polymorphic SSR marker بين الاصناف المدروسة من التين.

ولقد تم استخدام كلا من نتائج ISSR وSSR في عمل مصفوفات لانشاء شجرتين للتطور من اجل توضيح العلاقات الوراثية بين اصناف التين المختلفة، فتبين من النتائج ان هناك اختلاف جيني على مستوى االمادة الوراثية بين اصناف التين المدروسة الا ان هناك اربعه ازواج من الاصناف وهي (عناقي وخرطماني) (قراوي وسماري) (بياضي وحماري) (تلحمي وحماضي) يوجد هناك تقارب جيني بينهم باستخدام كلا من تقنيه SSR او SSR، وان تقنيه SSRلها فعاليm جيده على تقنيه ISSRفي تحديد االطرز الجينيه الجيني بين اصناف التين.

DECLARATION

I declare that the Master Thesis entitled "Assessments of genetic diversity of fig (*Ficus carica L.*) in the West Bank based on Simple Sequence Repeat (SSR) and Inter-Simple Sequence Repeat (ISSR) markers." is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Name and signature: Khulood Saleh AlKaraki

Date 18.10.2019

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Dedication

My thanks are due to everyone supported and assisted me to do this thesis.

I extend my endless thankfulness to my husband, kids, and my brothers for their continuous support.

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List of abbreviations:

AFLP	Amplified Fragment Length Polymorphism
FAMD	Fingerprinting Analysis with missing Data
ISSR	Inter Simple Sequence Repeat
PCR	Polymerase chain Reaction
RAPD	Random Amplified polymorphic DNA
RELP	Restriction Fragment length Polymorphism
Rp	Resolving power
PIC	Polymorphic information content
He	Expected Heterozygosity
Ho	Observed Heterozygosity
ISSR	Inter simple sequence Repeat
SSR	Simple Sequence Repeat

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CHAPTER ONE

Introduction

1.1 Background:

The fig tree is mentioned in holy books as a blessed tree. The fig tree has been mentioned in the Bible and the Quran. In sura al-Teen of the Quran, the medicinal benefit of fig is discussed as “I swear by the fig and the Olive” (Sura alteen no. 95 verse no. 1, Farooqi 1998). Also, the fig tree is mentioned in folkloric songs, sayings and stories and these fig fruits have a strong relation with healthy food in our heritage (Shtayeh et al. 1991).

Ficus carica L, commonly known as "Fig", it is a well-known type of fruits. Fig is classified as part of ", Moraceae" family. It is one of the eldest and most ancient fruits that grow in the Mediterranean region. Worldwide, this region is the most known for producing Figs. Carbonized figs were discovered since 11,400–11,200 years ago in an early Neolithic site in the Jordan Valley and this discovery means that figs were first domesticated during the early Neolithic Revolution before cereal domestication. Since then, fig cultivation spread to the neighboring western Asia and other Middle-East regions, and also they spread to other regions of the world (Basheer-Salimia et al. 2012).The fig tree is an ancient tree as mentioned before and it has been cultivated since long time and this produced hundreds of cultivars which present worldwide.

The fig was diversified to many cultivars after their cultivation spread and for their delicious taste and unique pollination system, this kind of fruits are grown now in moderate temperature of the world countries (Basheer-Salimia et al. 2012). However there is a reduction of fig cultivated area due to a decrease in the number of fig genotypes selected and maintained since ancient time and replacement of fig with olive trees, so the identification of fig germplasm and its diversity is needed as an important step in an efficient management and preserve of viable fig genetic resources for breeding programs (Giraldo et al. 2008).

Fig recently has attracted much of attention because it has a medical importance and nutritional values (Flaishman et al. 2008, Faostat 2009). *F. carica* has been traditionally used for its

medicinal benefits for treatment different diseases such as metabolic, cardiovascular and respiratory diseases (Duke 2002, Mawa, Husain et al. 2013). In addition to that, the leaves, roots and fruits of *F. carica* that is known as "common fig "are used to treat different disorders like gastrointestinal and inflammatory disorders. The fruits of ficus carica can be eaten fresh or dried and it is a good source of minerals, vitamins, and dietary fibers. The dry fruit of *F. carica* is a supplement food for a diabetic patient. Also, Ficus carica paste is recommended for tumors to relieve pain.

1.2 Taxonomy and morphology:

Ficus carica L, which is locally known as "common fig" has chromosomal number equals 26 Figs are diploid species that belong to the order of Urticales and the family Moraceae. This Moraceae family has over 1400 species that classified into about 40 genera. The genus "Ficus", has about 700 species, which are found mainly in the tropical areas and are divided into six sub-genera and this division of the genus Ficus is based on morphology (Giraldo et al. 2005).

Because ficus genus includes significant nutritional values, it is economically and medically important, and also it plays an important part of the biodiversity in the rainforest ecosystem, for these important points, Fig is considered to be an important genetic resource. In addition to that, it is also a good source of food for fruit-eating animals in tropical areas (Rønsted et al. 2007).

"*F. carica L*" is an important member of the genus Ficus. The fig tree is classified as gynodioecious based on Morphological information, but functionally, the fig is considered dioeciously with two tree morphs: Capri fig and edible fig. The fig tree is a many-branched tree. It is ordinarily deciduous shrub or small tree, its tall ranges from 3 to 9 m (10 to 30 ft.) tall. This genus is referred to as “common fig”. The average life span of fig trees is between 50 and 70 years. The wood of fig tree is light and soft and easily decayed with little or no use. The roots of fig tree are strongly penetrated in the soil; this strong penetration let the root to absorb water from distant wet area. The fig trees containing cells that secrete wax and produce milky exudates that characterized to all fig cultivars (CRANE and Brown 1950).This latex is used as defense against mechanical wounding and\or against insects, microorganisms and fungi as it has been suggested from previous studies (John 1992).

The reproduction systems of species in the genus Ficus are unique. For successful pollination and reproduction of species of *F. carica* to happen, the species of ficus can only be pollinated

by their associated agaonid wasps (Hymenoptera; Chalcoidea; Agaonide), and in turn the wasps can only lay eggs within their associated fruit. (Janzen 1979). So, pollination occurs in a closely interaction with only one species of specialized fig wasp, which crawls inside a small hole in the tip of the syncomium. The female wasp lay eggs inside the receptacle then dies, but the offspring generally complete their lifecycles, hatching into larvae, pupating, and becoming as adults, that leave the syncomium when the fruit ripens. The seeds that develop are embedded in a fleshy wall, which turns sweet and juicy when the "fruit" is ripe. (Mawa et al. 2013)

1.2.1 Fig Leaf:

Ficus carica is known for its fragrant leaves that are large and lobed. The leaves are 12- 25 cm long and 10 - 18 cm across. The leaf contains 3-7 deep lobes (Mubaslat 2012).

1.2.2 Fig Fruit:

The edible part of fig is the fruit which is fleshy, hollow, and receptacle (Dueñas et al. 2008). The fig fruit is 3–5 cm long, with a green skin, which has sometimes purple color when ripening.

Fig is not an individual fruit, it is a false fruit (Lisci and Pacini 1994). It is a syconium-a type of specialized hollow, fleshy branch or receptacle with numerous an inflorescence flowers (an arrangement of multiple flowers) which is forming into fruit inside of it, after cross-pollination with a tiny fig wasp *Blastophaga psenes*. This hollow shell of receptacle tissue including hundreds of individual pedicellate drupelets that develops from the individual female flowers lining the receptacle wall.

The mature fruit of the edible fig has a tough skin, a whitish interior rind, and a sweet, gelatinous pulp of the individual ripe drupelets. The seeds are within the drupelets characterized that either non-present to crunchy (Stover et al. 2007). Fruit starts to mature at July and may continue to mature until temperature decrease between October and December. At the end of the growth period, the leaves of fig tree fall and the tree enters the dormancy period. Reproductive buds that do not produce fruit during the growing season stay dormant in all the winter until the spring, when first breba crop give rise (Flaishman et al. 2008).

1.3 Medical Importance:

Today, fig is an important crop worldwide, this importance due to its dry and fresh consumption. According to the Dietary Reference Intakes data (Elçi, et al.), published by the Food and Nutrition Board of the U.S.A, figs are considered to be the superior and excellent source of minerals and vitamins, whereas per 100 g of fig there are the following elements: iron, 30%; calcium, 15.8%; potassium, 14%; thiamin (B1) 7.1%; and riboflavin (B2) 6.2%. Figs are sodium free, fat and cholesterol free (Wang et al. 2003, Vinson et al. 2005). Also, dried figs have relatively high amounts of crude fibers (5.8%, w/w), figs contain higher amount of fibers than all other common fruits (Vinson et al. 2005).Around 28% of the fiber in the fig is soluble type, and this type aid and help in controlling blood sugar, cholesterol level in blood and weight loss (Solomon et al. 2006).

F. carica L has many phenolic compounds which are suitable and appropriate for human health, because they are able to act as an antioxidant by different ways: reducing agents, hydrogen donators, free radical scavengers, singlet oxygen quenchers.(Çalişkan and Polat 2011). Also, latex that is secreted from tree has been showed to suppress the growth of various tumors (Ullman 1952).

Fruit, root, and leaves of fig are used in traditional medicine to treat various diseases such as gastrointestinal (colic, loss of appetite, and diarrhea), respiratory (sore throats, coughs, and bronchial problems), and cardiovascular disorders and inflammatory disorders. The *F. carica* has methanol extract that acts as a strong antibacterial activity against oral bacteria. It has been investigated that gathering methanol extract from fig with ampicillin or gentamicin were powerful against oral bacteria, so that approved and showed that figs could act as a natural antibacterial agent (Duke 2002).

There are many biological activities of *ficus carica* and these activities can be explored in the future for using them in healing methods in the future. The anti-cancer activity of Ficus carica is one of *ficus carica* biological activities, a mixture of 6-O-acyl- d-glucosyl-sitosterols, That is isolated from latex of *ficus carica* that acts as an effective cytotoxic agent that showed in vitro inhibitory effects on proliferation of various cancer cell lines (Yancheva et al. 2005).

1.4 World Market of fig:

F. carica has been cultivated for a long time in different regions of world for its edible fruit. It is supposed that *F. carica* was found in Western Asia and spread from there to the Mediterranean by humans. The major producers of figs are Turkey, Egypt, Morocco, Spain, Greece, California, Italy, Brazil, and other regions that are characterized by mild winters and hot dry summer (Tous and Ferguson 1996).

According to the data base of FAO that is published in 2009, amounts are given in tons of produced fig crops in different countries, Table 1.1.

In addition to that, according to FAO database in 2013, Turkey is known to be the largest world producer with 298,914 tons and this amount represents 26.8% of total world production. In 2013, 70% of fig crop was produced by five countries which are; Turkey, Egypt, Algeria, Morocco and Iran. This information ensures that the majority of fig production is still located in Mediterranean region of the world. However, there are other countries also produce fig crop and they play role in increasing the fig market in world since 2000, such as, the United States, Brazil, India, Japan, and China (http://faostat3.fao.org).

Table 1.1: Amounts in tons of fig crop in major producers in descending order.

Turkey	(280,000 Ton)	**Syria**	(43,000 T)
Egypt	(190,000 T)	**Italy**	(18,000 T)
Greece	(80,000 T)	**Tunisia**	(13,000 T)
Morocco	(67,000 T)	**Lebanon**	(9,000 T)
Spain	(61,000 T)	**Palestine**	(7,500)
Algeria	(60,000 T)	**Jordan**	(3,600 T)
USA	(45,000 T)		

Source: http://apps.fao.org/ "FAO statistics database on the World Wide Web," 2009.

In addition to that, according to FAO database in 2013, Turkey is known to be the largest world producer with 298,914 tons and this amount represents 26.8% of total world production. In 2013, 70% of fig crop was produced by five countries which are; Egypt, Algeria, Morocco and Iran. This information ensures that the majority of fig production is still located in

Mediterranean region of the world. However, there are other countries also produce fig crop and they play role in increasing the fig market in world since 2000, such as, the United States, Brazil, India, Japan, and China. (http://faostat3.fao.org).

In Palestine, which is characterized by a wide range of environmental conditions and rich of natural biodiversity, in Palestine fig trees are grown since long time in all the country and are mostly located on marginal lands, in combination with other fruit trees (mainly olive and grape), or are scattered at the periphery of orchards and in home gardens.(Basheer-Salimia et al. 2012).

According to the ministry center of agriculture in Ramallah that has been monitoring a decline in the production of fig in northern region of west bank. This decline in fig production was attributed to FMD (fig mosaic disease), that is pathogenic agent which is mainly caused by fig mosaic virus in northern region of west bank in Palestine, where 60% of infected samples of fig caused by FMV (fig mosaic virus) infection (Mahmoud 2015).

The fig producing region is characterized by hot dry summer. The winter temperatures limit and may damage the fig tree (Shtaya et al. 2013), (Ferguson et al. 1990).

1.5 Disease of Fig:

Understanding of plant pathogen is an important and necessary to human because they cause damage to plant and plant products. Disease can be caused by pathogen such as bacteria, fungi and virus. *Ficus carica* is exposed to pathogen and disease.

Recently, in different countries many types of viruses were detected infect fig trees (Elçi, Serçe et al. 2012). Recent studies have reported fig mosaic disease (FMD), that was observed in leaf tissues of fig which had symptoms of viral infection (Grbelja and Erić 1983). There have been reported several viruses infecting fig trees. Fig mosaic virus (FMV), fig cryptic virus (FCV), fig badnavirus 1 (FBV1). These viruses which infect plant cause problems such as affect crop production and fruiting plant period.

According to study was established at al Najah university in Palestine aimed to study the sanitary assessment of viral disease infecting fig trees in northern west bank (Mahmoud 2015). The study has indicated and investigated that (FMV) was the main incidents of FMD in Northern region in west bank and this result was in agreement to previous survey study of

Alkowni research (Alkowni, Chiumenti et al. 2015). Nablus province has the most infected rate around 88%, 83% in Jenin and 80% in Ramallah.

Palestine maintained a high incidence around 60% of FMV infection compared with previous studies done in turkey (Elçi et al. 2012) and Egypt (Elbeshehy and Elbeaino 2011). In this research the main symptoms of virus infections are mosaic, yellowing on leaves and ring spots on fruits, mosaic color is characterized by light green to yellow with normal green color of leave or fruit. Their recommendation in this study was to initiate sanitation program for the production and usage of healthy propagating plant material.

1.6 *F. Carica* Cultivars in Palestine:

Types of fig are described according to cropping and pollination characteristics.(Flaishman, Rodov et al. 2008). The fig types that are grown commercially are: the Common type, which develops fruits parthenocarpically, either brebas (first crop) or main crop (second crop); the Smyrna type, non-parthenocarpic, which requires pollination with pollen from profichi of the caprifig to develop the main crop; and the San Pedro type, which produces brebas parthenocarpically and the main crop after caprification. So, every fig variety should be one of these types: caprifig, Smyrna, San Pedro or Common. The fig germplasm consists of numerous landraces and cultivars that are selected in the past by farmers for their fruit qualities and kept in orchards and farms.

In Palestine, more than 50 varieties of fig were identified(Shtayeh et al. 1991). Fig cultivars grow with their wild forms and they adapt together, and they can be found growing all over the country with high level of phenotypic difference in fruit color, size, shape, and flavor. Local fig cultivars are available with different local names which these names were mainly given based on skin ground color, internal color, and maturity date as follows:

Green or yellow varieties, e.g.: Khdari, Biadi, Mowazi,

Greenish violet varieties, e.g., Khortmani, Enaqi, Hmadi,

Blakish violet to black colored varieties, e.g, Kharobi, Swadi,

The most famous local names for cultivars are khdari, Hmadi, Biadi, Khurtmani, Inaqi, Swadi and Mwazi. The discrimination and the difference between these cultivars are important for purposes of crop improvement and plant genetic resources conservation and protection. The

first and an initial classification of the Ficus carica cultivars was performed morphologically that based on tree leaf, and fruit characteristics, However, these morphological characters are influenced by plant age, phenological stage, cultivation conditions, and environmental conditions, so these morphological characters are exposed to phenotypic modifications. (Ali-Shtayeh et al. 2014).These phenotypic modifications can lead to a large number of homonymous and synonymous names and the occurrence of misnamed genotypes presence (Basheer-Salimia et al. 2012).

Researches that were established in Palestine on genetic diversity of fig germplasm have been limited. There are just two studies have been conducted. One of them in northern region of west bank in Palestine (Ali-Shtayeh et al. 2014) and second one in southern region of Palestine (Basheer-Salimia et al. 2012).All of these researches have just used RAPD fingerprinting as dominant molecular marker to study the polymorphism and none of these researches were representative of all fig cultivars in all regions of Palestine. Therefore, the aim of the study is to establish phylogenetic tree of fig cultivars in Palestine by using co-dominant markers which are microsatellite markers.

1.7 Diversity and Genetic DNA Marker:

Fig cultivars displays a large genetic variability because of the old selection of fig individuals that carried out over many centuries by farmers, and are maintained and kept by cutting as a way of vegetative propagation (Mars 2001).

The cultivar variability exists in fig shape and fruit color. There are over 650 varieties of fig arising from diverse countries that have a warm and dry climate. However, varietal identification of fig cultivars depends on morphological characters which are influenced by modifications of environmental conditions(Mamouni et al. 2001). For example, in Tunisia, as in other Mediterranean countries, fig has been traditionally cultivated since long times in diverse conditions. The identification of the cultivars in Tunisia are usually based on the color, size and time of fruit ripening or geographical origin, as what done in Palestine, and this result in and lead to confusion in nomenclature of fig cultivars there. (Essid et al. 2015)

Also, in northern Morocco, fig cultivars are much diversified, and they have many genotypes. However, because of numerous cases of synonymy (several denominations for the same genotype) and homonymy (several genotypes under the same denomination), pomological

characterization is considered to be insufficient to build reference genotypes for fig breeding programs and crop improvement in the region (Khadari et al. 2005).

Also, in Palestine, it has been approved in a research was established by Salimia in 2012 that morphological characters are insufficient to differentiate between the fig cultivars. The research revealed that genetic pattern for some cultivars was in contradictory to morphological descriptors and this result ensures that morphological characters can be influenced by modification of environment that lead to confusion in nomenclature of fig cultivars (Basheer-Salimia et al. 2012)

In Palestine, according to the researcher's knowledge, two researches were conducted to establish the polymorphism among the fig cultivars. Both employed RAPD as genetic, molecular marker and morphology characterization to characterize the diversity among the fig cultivars. None of these researches include and represent growing fig cultivars from all the regions in Palestine (northern, southern, and middle), one of these researches includes fig cultivars from northern region and other study includes fig cultivars from southern region. Both of these researches used molecular marker(RAPD fingerprinting and morphology markers) as methods to assess the similarities and polymorphism between fig cultivars, one of these researches that is done by Basheer-Salimia revealed and approved that dendrogram which was established by molecular markers differ from that dendrogram which was established by morphological descriptor according to certain kind of cultivars, so this result support the need to use molecular and genetic marker to differentiate between fig cultivars and show that RAPD fingerprinting is an efficient tool to characterize Palestinian fig genotypes rather than usage morphological characterizations alone where morphological characterizations was insufficient to be used without complementary of molecular markers(Basheer-Salimia et al. 2012).

However, the second research that was conducted by Shtayeh who revealed that the diversity among fig cultivars could be obtained by usage of both molecular markers and morphology descriptors together rather than usage of molecular markers or morphology descriptors alone. and the limitations of modification in morphology descriptions can be eliminated by application of morphology descriptions on fig trees that grew under the same environmental conditions (Ali-Shtayeh et al. 2014).

There are reasons behind the imitations and shortages of morphological and phenotypic characters in characterization fruits, which are; the limited number of phenotypic characters,

low heritability of them and it is difficult to differentiate between different cultivars before plant has attained the adult phase of life. In addition to that, morphological descriptors is limited due to phenotypic variability that is affected by environmental conditions (Guasmi et al. 2006).

Another marker was used in genotype fingerprinting and has been applied in several fruit species including fig which are Isozymes. The differences in the amino acid composition of isozymes were used to investigate the genetic variation within species and phylogenetic relationships between species. However, their usage has been limited because of the small number of isozyme systems available, the low level of polymorphism obtained(few alleles per locus), and the influence of environmental factors (Khadari et al. 2005).In addition to that, isozymes are not genetic materials, they are products of gene expression, so they could be affected by environmental factors.

Therefore, the attention of researchers is going to the molecular markers. The molecular markers are based on the nucleotide sequence mutations within the individual's genome, they arise from different classes of DNA mutations such as substitution mutations (point mutations), or errors in Rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA, they are usually located in non-coding regions of the DNA. They are the most reliable markers available which proved to be powerful tools to estimate genetic diversity of species, and genotype identity (Al-Samarai and Al-Kazaz 2015).

In fact, molecular markers have many advantages on conventional morphological based methods; these markers are selectively neutral because they are usually located in non-coding regions of DNA. So, they are stable and detectable in all tissues regardless of growth, differentiation, development and defense status of the cell (Kordrostami and Rahimi 2015). In addition, DNA markers are not affected by the environmental factor. (Basheer-Salimia, Awad et al. 2012).Also, DNA markers are widely used as a type of marker for distinction between individuals because of their abundance and they are unlimited in number. Also, DNA markers have been used in construction of linkage maps and they have numerous applications in plant breeding such as assessing the level of genetic diversity within cultivars and fingerprinting the germplasms (Kordrostami and Rahimi 2015).

So, the emergence of new polymerase chain reaction (PCR)-based molecular markers, such as randomly amplified polymorphic DNA (RAPD), amplified fragment-length polymorphisms (AFLPs), and simple sequence repeats (SSR), has created the opportunity for genetic

characterization of germplasms because they are highly polymorphic and are not readily influenced by environmental conditions (Khadari et al. 2005). Needless to say, DNA markers have also disadvantages as well, most of these markers protocols are time consuming and expensive and the application is complicated (Soliman et al. 2003).

1.8 Dominant and Co-dominant Markers:

DNA markers are useful in detecting differences between cultivars of the same or different species. These markers are called polymorphic; markers that do not distinguish between genotypes are called monomorphic. Polymorphic markers may also describe as co- dominant or dominant based on whether or not the marker can detect difference between homozygotes and heterozygotes. Co- dominant markers indicate differences in size while Dominant markers are either present or absent (Collard et al. 2005).

1.8.1 Different Types of DNA Markers:

There are different molecular tools like RAPDs, ISSRs, AFLPs, RFLPs or SSRs which have been used for fig germplasm characterization and diversity analyses (Essid et al 2015). However, Microsatellites are reliable tool for characterization and genetic diversity in fig (Khadari et al. 2005; Do Val et al. 2013).

1.8.2 **RFLP and RAPD**:

The first use of RFLP markers were mainly in 1980s and 1990s in plant genetic studies, for that they considered, as (first generation molecular markers),(Jones et al. 2009). The polymorphisms detected by RFLPs are as a result of changes in nucleotide sequences in recognition sites of restriction enzymes, or due to mutation events (insertions or deletions) of several nucleotides leading to obvious shift in fragment size (Tanksley et al. 1989).

The main advantages of RFLP markers are co-dominance, high reproducibility, no need of prior sequence information, and high locus-specificity. By using RFLP markers, genetic maps have been established in several crop species including rice maize, wheat (Cho et al. 1998). However, RFLP were few used since the last decade in genetic research and plant breeding. Because RFLP markers have disadvantages which are, it is too time-consuming procedure and it requires relatively large amounts of pure DNA, tedious experimental procedure. Additionally, each point mutation has to be analyzed individually (Edwards and Batley 2010; Wong 2013).

RAPD markers have been used in different plant species for assessment of genetic variation in populations and species, fingerprinting and study of phylogenetic relationships among species and subspecies (Gupta et al. 1999). RAPD has become a powerful and accurate tool for analyzing the genetic relatedness and diversity in figs. RAPD fingerprinting firstly was used to characterize and detect similarities and polymorphism among some fig cultivars in Palestine because it is simple, efficient, fast, simple method and does not need prior knowledge of sequence produce abundant polymorphic fragments (Basheer-Salimia et al. 2012).

RAPDs are based on PCR amplification of random DNA segments with primers of random nucleotide sequences of about 10 bp in length that were inexpensive and easy to use. The primers bind to complementary DNA sequences and where two primers bind to the DNA sample in close enough for successful PCR reaction (Williams et al. 1990).

However, RAPD markers have disadvantages which are, they are dominant markers, and Polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity. In addition to that, these markers could not detect allelic differences in heterozygotes. Also, because of their random nature of amplification, short primer length, experimental reproducibility can vary with reaction conditions. (Yang et al. 2015).

1.8.3 Microsatellites:

Microsatellite markers have been demonstrated in several fields, including forensic science, paternity test (Achtak et al. 2009)**.** Microsatellites are tandemly repeated arrays of sequences consisting of di-, tri-, or tetra-nucleotide core units and are abundant, and highly polymorphic (Rahman et al. 2000; Hayden and Sharp 2001) and flanked by highly conserved sequences(Chambers and MacAvoy 2000).

Closely related species like inbred populations, or geographically close populations can easily be distinguished by microsatellite-based markers while other molecular tools do not prove useful. Microsatellite primers derived from one species can be used for studying closely related species (Ganal 1995).

SSR markers have been widely used for cultivar characterization and for genetic diversity studies of fig (Achtak et al.2009). However, in Palestine SSR markers have not been used for

genetic diversity studies of fig cultivars, this research is the first one in Palestine that studies genetic diversity of fig cultivars by SSR markers.

1.8.3 a. SSR:

During 1990s, Simple sequence repeats (SSRs) which is also known as microsatellites were established and provided as a choice for many genetic researches. They are randomly tandem repeats of short nucleotide motifs (2 - 6 bp) (Dunn et al. 2005). And they are classified as mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats. The sequences of di-, tri- and tetra nucleotide repeats are the most common choices for molecular genetic studies. They are tandemly repeated (usually 5-20 times) in the genome with a minimum repeat length of one. They present both in coding and noncoding regions. (AT) n and (GT) n are the most common repeats found in plants with a high frequency of occurrence in the UTRs of coding regions (Al-Samarai and Al-Kazaz 2015).

SSRs are frequently highly polymorphic sequences normally present in animal and plant species (Kalia et al. 2011), and can be used to study the relationship between inherited traits within a species. Microsatellite markers are often obtained from non- coding/anonymous genomic regions, such as genomic survey sequences (GSSs).Because of that, development of SSR markers used to be expensive and laborious (Mir et al. 2013). This assay of SSR PCR is easily detectable by gel electrophoresis for few to hundreds of samples, which could be inexpensive by researchers with limited resources. Polymorphism is based on the variation in the number of repeats in different genotypes. In recent years, SSR markers can easily be developed in silico due to the availability of sequence information for many plant species that is available in online databases and can be scanned for identification of SSRs (Varshney et al. 2005).

(SSRs) have many advantages over RFLP, RAPDs. They are more variable and informative, they are co- dominant which can distinct between individual heterozygotes from homozygotes, this method requires low amount of template DNA. (Al-Samarai and Al-Kazaz 2015). In addition to that, microsatellite markers or simple sequence repeats permit the identification of multiple alleles present in populations, and the production of easily interpretable results with high reproducibility due to all of these characteristics of SSR markers are considered to be a useful and a reliable tools for the genetic identification of individuals. (Do Val et al. 2013).

On the other hand, these markers have several disadvantages: expensive, laborious and time consuming. The low frequency of SSRs in plants also blocks the isolation of SSRs(Al-Samarai and Al-Kazaz 2015) And another possible problem, which is associated with the use of simple sequence repeat markers is the occurrence of null alleles (Callen et al. 1993) In addition that microsatellites have a major drawback which is the need to isolate them de novo when a new species is investigated (Zane et al. 2002; Squirrell et al. 2003).

1.8.3 b. ISSR:

The ISSR technique is used widely in areas of genetic diversity, phylogenetic studies, and evolutionary biology of crop species. Inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellites repeat regions oriented in opposite direction. The microsatellite repeats used as primers can be di-nucleotide, tri-nucleotide, tetra nucleotide or penta-nucleotide. The primers used can be either unanchored (Gupta and Varshney 2000) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz et al. 1994).

ISSR techniques are very much like (RAPD) technique, except that ISSR primers are designed from microsatellite regions. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter- SSR sequences of different sizes. These regions are 100–3,000-bp long. The annealing temperatures used in the PCR reaction for ISSR are higher in comparison to RAPDs. ISSRs are much more informative than RAPDs (Korbin et al. 2002) and ISSR have more polymorphic information content (PIC) than amplified fragment length polymorphism (AFLP) (Blair et al. 1999). ISSRs are economical, fast, and easy to use.

The major advantages of ISSR technique are no prior requirement of the genomic sequence; the PCR products obtained are specific to microsatellite sequences, so ISSRs are more reliable than arbitrary sequence-based primer techniques and effective for distinguishing closely related species. The main disadvantages of ISSR technique are unclear fingerprints can be resulted sometimes if ISSR primers are less specific to genome and poor reproducibility of ISSR results if DNA quality is poor (Sarwat 2012; Chatti et al. 2010).

1.9 Research Objectives:

Plant identification and estimation of their relationships and diversity are established on the basis of morphological and characteristics. Here in Palestine, fig identification is based on morphological characteristics.

It is difficult to differentiate between fig genotypes only based on their external structure (phenotype), especially for leaf and fruit characters, because these may vary according to development and environmental conditions. This could lead to misidentification, so it is necessary to use stable markers like molecular DNA markers, for safely assessing genetic relationships.

RAPD fingerprinting was firstly used as dominant marker to characterize and detect similarities and polymorphism among some fig in Palestine, because it is simple, efficient, fast, simple method and does not need prior knowledge of sequence and produce abundant polymorphic fragments (Basheer-Salimia et al. 2012). However, it has been approved from a comparative perspective that RAPD and microsatellite markers for fig characterization that ISSR and SSR markers are more informative than RAPD markers (Achtak et al. 2009).

Therefore, the specific aims of this study are:

- To assess the molecular polymorphism in a set of Palestinian fig cultivars from southern, middle, Northern areas of the West Bank in the Palestine, by using both dominant and co-dominant markers, ISSR and SSR.
- To establish a phylogenetic tree and to study the genetic relatedness and diversity among Palestinian fig (*ficus. carica L*) cultivars using molecular markers; SSR and ISSR.
- To examine the efficiency of the seven SSR Primers and six ISSR Primers for determining genetic diversity among studied cultivars.
- To compare between both ISSR and SSR techniques.

CHAPTER TWO

Methodology

2.1 Sample Collection

Twenty local cultivars from different regions in the West Bank were included in this study as shown in Table 2.1 below. Cultivars Shehami and Qrawi were collected from two locations to investigate the possibility of misnaming by farmers of different cultivars in different regions. From each cultivar, 5 young leaves were collected in April-May 2018. Cultivars were identified at the time of collection with the help of an agronomist from the Ministry of Agriculture. Sample were then labeled and stored in clean plastic bags at -20°C till DNA extraction.

Table 2.1: The name and collection place of the 20 local studied cultivars.

No	Cultivar	Location	No	Cultivar	Location
1	Mwazi موازي	Hebron	**11**	Qrawi B قراوي	Bethlehem
2	Dafor دافور	Hebron	**12**	Talhami تلحمي	Bethlehem
3	Shehami H شحامي	Hebron	**13**	Besatee بساطي	Bethlehem
4	Khdari خضاري	Hebron	**14**	Swadi سوادي	Bethlehem
5	Biadi بياضي	Hebron	**15**	Neami نعيمي	Bethlehem
6	Ruzzi رزي	Hebron	**16**	Shehami B شحامي	Bethlehem
7	Smari سماري	Hebron	**17**	Khortmani خرطماني	Ramallah
8	Qrawi H قراوي	Hebron	**18**	I'naqi عناقي	Nablus
9	Sebaei سباعي	Bethlehem	**19**	Hmari حماري	Nablus
10	Barqawi برقاوي	Hebron	**20**	Hmadi حماضي	Ramallah

2.2 DNA extraction:

About 100 mg of young fresh leaves from each cultivar were cut into small pieces by a sterile blade and were ground to a fine powder using a mortar and pistil in liquid nitrogen. For the ISSR-PCR, genomic DNA was extracted and purified using the Qiagen DNeasy plant mini kit according the manufacturer instructions. For the SSR-PCR the DNA was extracted using the CTAB (cetyl trimethylammonium bromide) method. This is because fig leaves are rich with polysaccharides and polyphenols were the Qiagen kit could not produce sufficient amounts of DNA for both methods. Therefore, an optimized CTAB protocol was used to achieve higher yields of genomic DNA which were then purified from RNA using RNase A and from excess proteins and polysaccharides by using chloroform:isoamyl alcohol (24:1). DNA was precipitated by adding 0.7 volume cold isopropanol. Pellet was washed with ice cold 70% ethanol, air-dried and then resuspended in 200 µl of ultra-pure water. The quantity and quality of the DNA was determined using a Nanodrop spectrophotometer and also as visualized in 1% agarose gel stained by Ethidium Bromide under UV light. Finally, the DNA samples were stored at -20°C till use.

2.3 Primers and PCR Assays

A set of 7 SSR primers and 6 ISSR primers were used in the PCR amplifications (Tables 3.2 and 3.3).These primers were selected based on previous studies (Essid, Aljane et al. 2015; Ikegami, Nogata et al. 2009; Do Val, Souza et al. 2013; Achtak, Oukabli et al. 2009; and Bandelj, Javornik et al. 2007).

Table 2.2: List of ISSR Primers.

Primer	Sequence 5`→3`	Tm
UBC 807	AGAGAGAGAGAGAGAGT	50°C
UBC 808	AGAGAGAGAGAGAGAGC	52°C
UBC810	GAGAGAGAGAGAGAGAT	50°C
UBC 812	GAGAGAGAGAGAGAGAA	50°C
UBC 817	CACACACACACACACAA	50°C
UBC 818	CACACACACACACACAG	52°C

Table 2.3: List of SSR Primers.

Primer	Sequence 5`→3`	Allelic size range bp	Tm
LMFC30	F:TTGTCCGTTTCTTATACAAT R:TCTTTTTAGGCAGATGTTAG	231 -258	52◦C
MFC3	F: ATATTTTCATGTTTAGTTTG R: AGGATAGACCAACAACAAC	132-134	54◦C
MFC9	F:GGAGGCAAAACGACAAACGACT R: CAAGGAACCAAGCGGGAGGG	190 -211	58◦C
FCUP068-1	F: GGAATTACCGTCCATGGCTA R:CGCCACTCTCTCTCTCCACT	176-206	58◦C
MFC4	F: CAAACTTTTAGATACAACTT R: TTCTCAACATATTAACAGG	198 -221	54◦C
FCUP008-2	F: CATACACTTTCATGGAGCACAAA R: CCCAGATGTTTGGTGAAGG	158-184	58◦C
LMFC12	F:TTAAACCCTACTTTCAACAAT R:GTAATCCCCCGAGATATAGT	376	52◦C

2.3.1 ISSR- PCR

The ISSR-PCR reaction mixture was prepared using the AccuPower Hot Start PCR premix (Bioneer Corporation Hylabs). The following were added to each reaction; 20 - 30 ng of genomic DNA, 30pg of primer and an additional 0.5mM $MgCl_2$. The total volume of each reaction was adjusted to 20 µl with DNase-free ddH_2O. Amplifications were performed in 9600 Perkin Elmer Thermal Cycler as follows: one step of 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing depending on primer (Table 2.2) for 40 seconds, and an extension at 72°C for 90 seconds. A final extension step for 5 minutes at 72°C was added.

2.3.2 Agarose Gel Electrophoresis

The amplification products were separated by electrophoresis on 1.5% agarose gels stained with Ethidium Bromide in 1x TEB at 120 volts for 1-1.5 hr. Gels were photographed using a BioDoc -it imaging system, UVP. Only clear bands were scored and their sizes were estimated using a 100 bp DNA ladder.

2.3.3 SSR-PCR amplification

The SSR-PCR reaction mixture was prepared using the AccuPower Hot Start PCR premix (Bioneer corporation Hylabs). The following were added to each reaction; 20 - 30 ng of genomic DNA, 0.25 of each primer and an additional 0.5-1mM $MgCl_2$. The total volume of each reaction was adjusted to 20 µl with DNase-free ddH_2O. Amplifications were performed in 9600 Perkin Elmer Thermal Cycler as follows: one step of 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing depending on primers (Table 3) for 35 seconds, and an extension at 72°C for 40 seconds. A final extension step for 5 minutes at 72°C was added.

2.3.4 Metaphore Gel Electrophoresis

DNA fragments within the size ragen of 20-800bp that differ in size by 2% can be resolved in metaphore gel. This provides twice the resolution capabilities of the finest normal agarose. To prepare 4% gel, Metaphore agarose was soaked in 1x TEB (Tris/Borate/EDTA) buffer for 1 hr and melted at 75°C for few minutes. The SSR-PCR products were resolved in the prepared metaphore gels at 120 volt for 2hr. Gels were stained with Ethidum Bromide and photographed using a BioDoc -it imaging system, UVP.

2.4 Bands Scoring and Statistical Analysis

Amplification products (bands) were scored as either present (1) or absent (0) for each locus using the GelAnalyzer software and corrected manually (Khakabimamaghani, Najafi et al. 2013). ISSR bands were transformed into a binary matrix. Matrix statistics, band counts, and genotypic identities were revealed using the FAMD (Schlüter 2013) and the Excel softwares. Using the NTSYSpc software (Rohlf, 2008), a genetic distance matrix was estimated based on Jaccard's similarity coefficient, which is defined as: similarity index = (number of

electrophoretic bands in common)/(number of bands not in common + number of bands in common)] . Another similarity matrix was estimated based on the Nei 1972 coefficient. A comparison plot and an MxComp correlation following Mantel test were conducted for the Jaccard and Nei matrices. The similarity matrices were then used to construct a dendrogram and phylogenetic trees following the unweighted pair group method with arithmetic mean (UPGMA) and Neibour Joining (NJ) method for Cluster analysis, respectively. Dendrograms and phylogenetic trees were displayed by the MEGA software (Tamura, Dudley et al. 2007). The principal coordinate analysis was also performed using the NTSYSpc software to validate the tree. The bootstrap values were generating using 1000 replicates with the FAMD software

To compare the efficiency of primers in identifying different fig genotypes, for each primer, the total number of bands, the polymorphic bands, band informativness (Ib) and the primer resolving power (Rp) were calculated. Band informtivness is a measure of closeness of a band to be present in 50% of the genotypes, Band informtivness of a given band (Ib) = 1-[2X(0.5-P)] where P is the proportion of the total genotypes containing the band. Resolving power (Rp) is the sum of Ib values of all the bands amplified by a primer Rp = ΣIb. Ib and Rp were calculated as reported by Prevost and Wilkinson, 1999.

The polymorphic information content (PIC) for the ISSR markers was calculated following the formula PIC = 2*fi*(1-fi) where fi is the frequency of the amplified allele (band present) and (1-fi) is the frequency of the null allele (band absent) (Soengas, Velasco et al. 2006). For SSR markers, PIC values were calculated following the formula $PIC= 1-\Sigma Pi^2 \Sigma\Sigma\ 2\ Pi^2Pj^2$ (Botstein, White et al. 1980), where pi and pj are the frequencies of the i^{th} and j^{th} alleles in the population, respectively. High value means very good primer for genetic diversity, for co-dominant marker as in the case of SSR, the PIC value ranges from 0 to 1 where those higher than 0.5 are considered highly informative. PIC value for co dominant marker was calculated by power maker version 3.25 (Liu and Muse 2005).

The expected heterozygosity (He), which is the probability of an individual's heterozygosity for the relevant locus was calculated according to the equation He = 1-Σpi2 (Nei 1973), where Pi is the frequency of ith allele among the total number of alleles, Pi depicts the proportion of samples carrying the ith allele. The observed heterozygosity (Ho) is the part of heterozygous genes in the population. It is calculated for each locus as the total number of heterozygotes

divided by the sample size. He and Ho were calculated by GenAlex version 6.51 (Peakall and Smouse 2006).

The ability of informative SSR primers to differentiate between verities was assessed by the probability of Identity (PI) i.e. the probability of two cultivars sharing the same genetic profile by chance. PI was calculated by GenAlex version 6.51 (Peakall and Smouse 2006) according to the formula $PI = \Sigma\ Pi^2 +_i \Sigma\Sigma\ 2(PiPj)^2$, where pi and pj are the frequencies of the ith and jth alleles in the population.(Paetkau, Calvert et al. 1995). PI expresses the likelihood of finding two Individuals with the same genotype for a certain Loci in the population. As a rule, the more discriminative an SSR marker is, the lower would be its PI value (Korkovelos, Mavromatis et al. 2008).

The fixation index (F) is an inbreeding coefficient that reflects the deviation of the observed heterozygosity of an individual relative to the expected heterozygosity under random mating according to Hardy-Weinberg equilibrium. F was calculated by GenAlex version 6.51 (Peakall and Smouse 2006) following the formula F= 1- Ho/He.

CHAPTER THREE

Results

3.1 ISSR-Binary matrix statistics

Six primers were screened for their ability to generate ISSR polymorphic DNA bands. A total of 56 DNA fragments (loci) ranging in size from 300 -1500 bp were scored (Table 3.1 and Fig. 3.1). About 82% of these bands (46) are polymorphic while the rest of amplified loci (10 bands) are monomorphic, that is present in all the cultivars. The results show that the average number of amplified DNA loci using the six ISSR primers is 9.3. The number of amplicons produced by the ISSR primers ranged from 6 to 11.

The mean of polymorphism percent of the used ISSR markers is 81.8%. Moreover, the number of polymorphic bands produced by each primer ranged from 5 for UCB 808 to 10 for UCB 818 with an average of 7.6 per primer (Table 3.1). These numbers reflect a high level of polymorphism among the Palestinian fig cultivars

Table 3.1: Characteristics of ISSR banding profiles produced in Palestinian figs. T: total number of loci, P: no of polymorphic bands, M: no of monomorphic bands, Rp: resolving power, PIC: polymorphism information content, %P: percentage of polymorphic bands.

Primer	T	P	M	Rp	PIC	%P
UCB 810	8	6	2	11	0.262	75
UCB 807	10	8	2	8.6	0.196	80
UCB 812	11	9	2	13.4	0.313	82
UCB 817	10	8	2	14.4	0.305	80
UCB 818	11	10	1	11.3	0.259	91
UCB 808	6	5	1	6.2	0.298	83
Mean	9.3	7.6		10.8		81.8
Total	56	46	10	64.9		

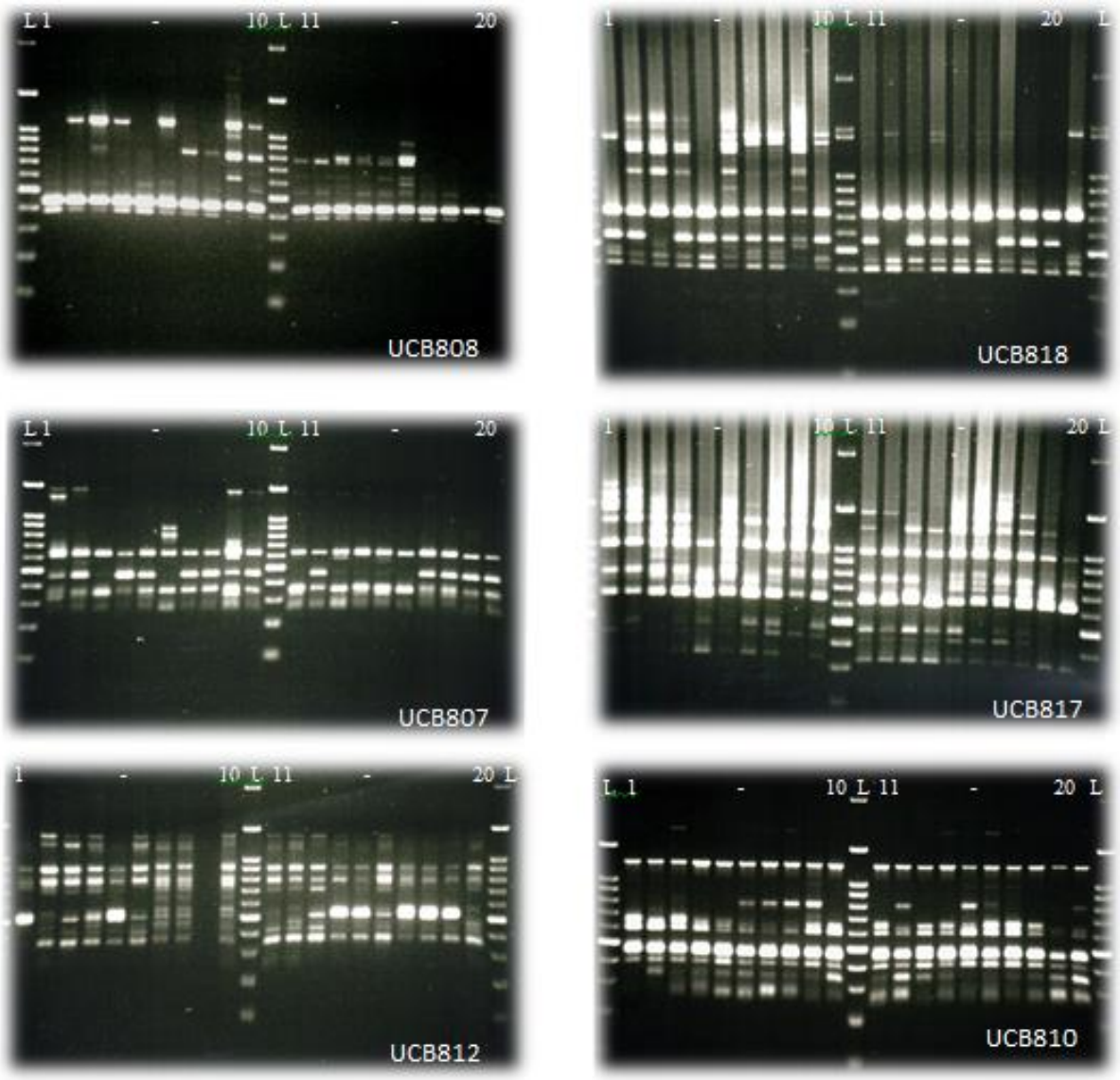

Figure 3.1: ISSR-PCR of 20 fig cultivars. Six primers were used. Lanes 1-20 correspond to the cultivars as shown in Table 3.1. L: 100bp DNA ladder.

3.2 ISSR-allelic frequency per cultivar

The frequency of ISSR bands for each cultivar was calculated as the number of band presences (1) for that cultivar divided by the total number of loci produced by all primers. It is a measurement of the capacity of the marker to produce DNA bands. The higher the frequency, the larger is the number of detected bands. In this study, the frequencies for ISSR markers ranged from 0.39 to 0.69 (Table 3.2). For example, in Sebaei and Smari cultivars 38 bands out of a total of 56 loci were obtained. In the Hmari cultivar only 22 bands were detected.

Table 3.2: Frequency of band presences per cultivar. The frequency was caluclated as the number of band presences (1) for a cultivar divided by the total number of loci.

Cultivar	Freq.	Cultivar	Freq.	Cultivar	Freq.	Cultivar	Freq.
Mwazi	0.50	**Ruzzi**	0.68	**Qrawi B**	0.57	**Shehami B**	0.60
Dafor	0.57	**Smari**	0.69	**Talhami**	0.61	**Khortmani**	0.55
Shehami H	0.55	**Qrawi H**	0.69	**Besatee**	0.55	**I'naqi**	0.52
Khdari	0.66	**Sebaei**	0.69	**Swadi**	0.53	**Hmari**	0.39
Biadi	0.48	**Barqawi**	0.64	**Neami**	0.55	**Hmadi**	0.52

3.3 Number of ISSR bands scored for each primer:

A Total of 1120 data entries were analyzed for the ISSR markers; 649 data are present bands (1) and 471 are absent bands (0). The total data entries and the number of data presences (1) per each primer are shown in Table 3.3.

Table 3.3: Total data entries TDE and the number of data presences (1) per each primer.

Primer	TDE	Present (1)	Primer	TDE	Present (1)
UCB 810	160	110	**UCB 817**	200	144
UCB 807	200	86	**UCB 818**	220	113
UCB 812	220	134	**UCB 808**	120	62

3.4 ISSR Genotypic identities

In three of the cultivars, namely Mowazi, Ruzi and Sebaei, one or more genotypic identities were detected by two of the tested ISSR primers (UCB807 and 818, Table 3.4). A genotypic identity means the presence of a band at that locus only for the specified cultivar. It is worth mentioning that cultivars Ruzi and Sebaei share a genotypic identity at locus 5 of the UCB 818 primer. Such identities would facilitate future identification of these cultivars.

Table 3.4: ISSR genotypic identities of three Palestinian fig cultivars as detected in two primers. The identities were detected for three cultivars in two primers. The loci number is the descending order of the unique band as appeared in the agarose gel.

Cultivar	Loci number	
	UCB807	UCB818
Mowazi	2	-
Ruzi	3, 4	5*
Sebaei	5	8, 5*

* This identity is shared by Ruzi and Sebaei cultivars only.

3.5 Evaluation the efficiency of ISSR primers for diversity identification:

3. 5.1 Resolving power:

As shown in Table 3.1 above, the powerfulness of the employed ISSR primers to detect variation among the Palestinian fig cultivars (Resolving power) was high. The values of Rp range from 6.2-14.4 for primers UCB808 and UCB817, respectively with an average of 10.8 for all primers.

3.5.2 Polymorphic information content (PIC):

PIC was calculated for each ISSR marker to evaluate the informativeness of each primer in revealing genetic diversity among its loci. In other words, how polymorphic is a specific locus among the cultivars. It was calculated as the average of PIC values of all loci produced by an ISSR primer. A locus is considered with intermediate diversity, when PIC values are between 0.25 and 0.5. As shown in Table 3.1, the average PIC values of the tested 6 ISSR primers ranged from low (0.96) to intermediate (0.305). However, higher values of PIC were detected for several single loci and reached up to 0.495. The average PIC values were lowered due to the presence of monomorphic bands at some loci.

3.5.3 Correlation among the two genetic matrices:

As shown in Table 3.5 and Figure 3.2, a high fit between the Nei72 and the Jaccard matrices was revealed by Mantel test where a high correlation Cophenetic coefficient (r=0.934) was achieved. Note that a value of 1 means identical matrices.

Table 3.5: MxComp Matrix correlation for fig cultivars ISSR clustering analysis generated by NTSYS.PC v 2.10e.

2-way Mantel test - Mantel (1967) method	Tests for associations: Matrix correlation: **r = 0.93408**
N = 190 points	(= normalized Mantel statistic Z)
Mean X = 0.3743, SSx = 4.1972	Approximate Mantel t-test: t = 7.5621
Mean Y = 0.2855, SSy = 1.9651	Prob. random Z < obs. Z: p = 1.0000

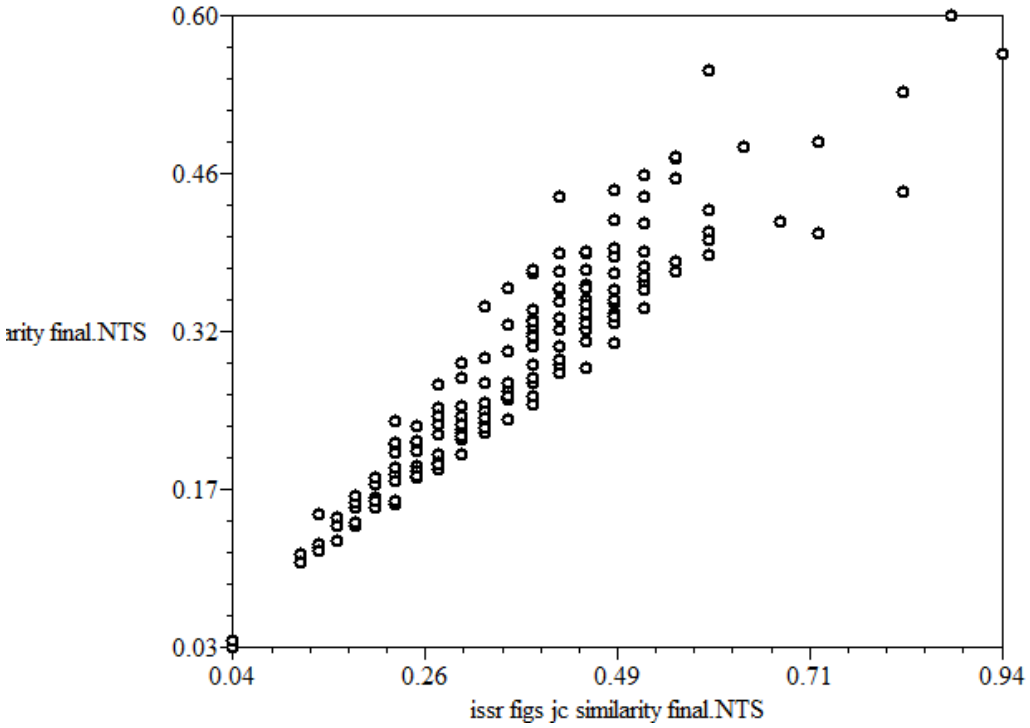

Figure 3.2: Nei and JC matrix comparison plot following Mantel test (see Table 3.5).

3.6 Cluster Analysis following ISSR results:

The genetic relationships among the fig genotypes are illustrated by two trees; the first is a dendrogram based on UPGMA analysis following a similarity matrix generated by Jaccard's coefficient with bootstrap value of 1000 replicates, and the second is a phylogram based on Neighbor Joining analysis following a similarity matrix generated by Nei 1972. There are slight differences in the clustering patterns of in each tree. There is however, an overall agreement between both trees and the clustering pattern produced by the principal coordinates analysis (Figure 3.5). It worth mentioning that similar clustering patterns were also produced by a dendrogram produced using the Nei72 coefficient and a phylogram generated using the Jaccard's coefficient (data not shown). This is in accord with high correlation value (Table 3.5) between the two similarity matrices.

The 20 fig cultivars clustered into 4 groups in the consensus dendrogram; I, II, III and IV (Figure 3.3). It is worth mentioning that in all of the generated trees using various similarity methods and coefficients, the following pairing patterns (sub-clusters) were always the same; Inaki with Khurtumani, Besati with QurawiB, Hemari with Biadi, Smari with QurawiH, Ruzi with Sebaei, and Hemadi with Talhami. A cluster encompassing Dafor, Khdari, ShehamiH and Barqawi was also present in all of the produced dendrograms with slight changes in the subclustering patterns. From this study, it was apparent that the least similar or most distant cultivars to the rest of the Palestinian cultivars are ShehamiB and Swadi followed by Mowazi

Interestingly, the genetic dissimilarity between ShehamiB and ShehamiH as well as between QurawiB and QurawiH may suggest a case of homonymy, different genotypes under the same denominations. Such results would help agronomists and farmers reevaluate their nomenclature strategies.

It is worth mentioning that in all of the generated trees using various similarity methods and coefficients, the following pairing patterns (sister groups) were always the same; Inaki with Khurtumani, Besati with QurawiB, Hemari with Biadi, Smari with QurawiH, Ruzi with Sebaei, and Hemadi with Talhami. A cluster encompassing Dafor, Khdari, ShehamiH and Barqawi was also present in all of the produced dendrograms with slight changes in the sub clustering patterns.

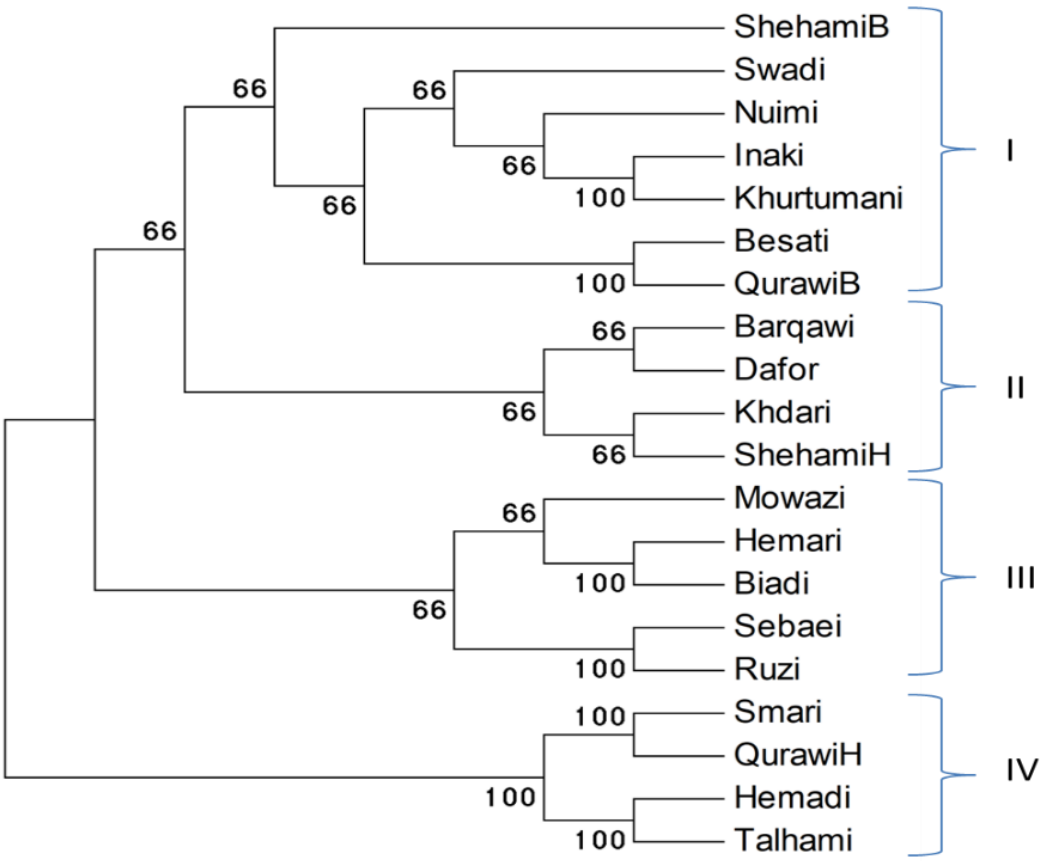

Figure 3.3: Dendrogram of 20 Palestinian fig cultivars based on UPGMA analysis using the maximum similarity (Jaccard's coefficient) method generated by FAMD. Bootstrap values are shown on the nodes, 1000 replicates. The tree is displayed by MEGA4.

The phylogram (Figure 3.4) encompasses 3 clusters. The pairing patterns between the cultivars within the clusters are similar to those of the dendrogram (Figure 3.3). However, the place of some pairs in the phylogram is different from their places in the dendrogram. For example, Ruzi and Sebai sub-cluster belong to the Dafor cluster (II) in the phylogram but to the Biadi cluster (III) in the dendrogram. Mowazi, ShehamiB, Swadi and Nuimi clustered distantly from the other cultivars. A small new cluster containing Besati and QurawiB only has been evident in the phylogram.

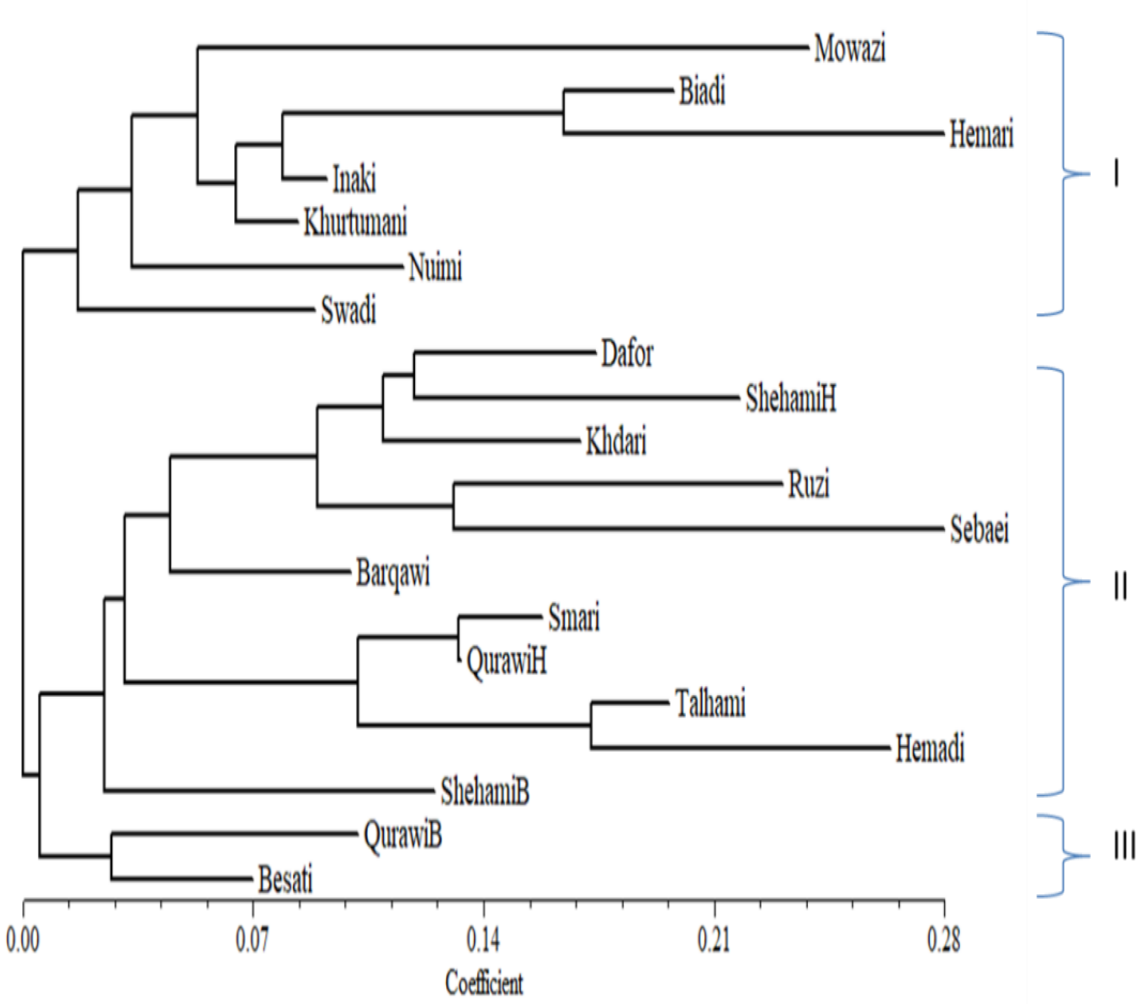

Figure 3.4: Phylogram of 20 Palestinian fig cultivars based on weighted Neighbor NJ analysis using the similarity matrix generated by Nei 1972.

In addition, the analysis of data by principle coordinate of analysis (PCA) was used to confirm the rigidity of the phylogenetic trees. As shown in Figure 3.5, the 2-dimensional plot is in support with clustering patterns seen in both trees.

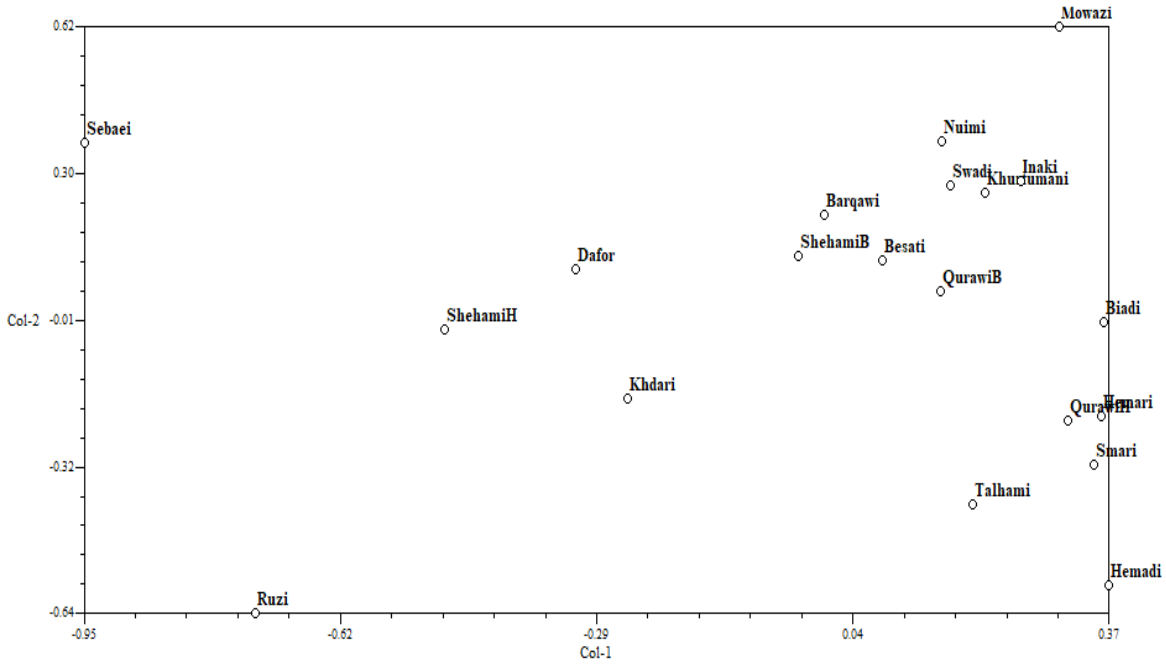

Figure 3.5: 2-D Plot of the principal coordinate analysis of the 20 Palestinian fig cultivars.

3.7 SSR analysis of fig cultivars:

3.7.1 Genetic polymorphism and SSR PCR analysis of Fig cultivars:

Seven SSR markers were used in this study with the aim to detect genetic diversity in fig cultivars. A total of 24 loci DNA fragments (loci) were detected by electrophoresis on 3- 4% Metaphor gels as shown in Figure 3.6 and Table 3.6 ranging in size from 130 – 360 bp. Twenty one bands (88 %) of these loci are polymorphic and only 3 bands (12%) are monomorphic as shown in (Table 3.6) The genetic diversity was measured and assessed by percent of polymorphic bands. The polymorphic percent of SSR marker was 88.0 %, with mean of 3 polymorphic bands per each primer. These results reflect a high level of polymorphism among the tested Palestinian Fig cultivars. This indicates the availability of diverse genetic resources that could be incorporated into the future breeding programs.

Table 3.6 Percentage of polymorphic SSR marker, total No of loci scored and No of polymorphic bands

Marker	No of loci scored	No of polymorphic bands	Percentage of polymorphic marker
MFC9	4	4	**100%**
MFC3	5	5	**100%**
MFC4	3	3	**100%**
LMFC12	3	2	**67%**
FCUP00 82	2	1	**50%**
FCUP 68-1	5	5	**100%**
LMFC30	2	1	**50%**
Total	**24**	**21**	
Mean	**3.4**	**3**	**88%**

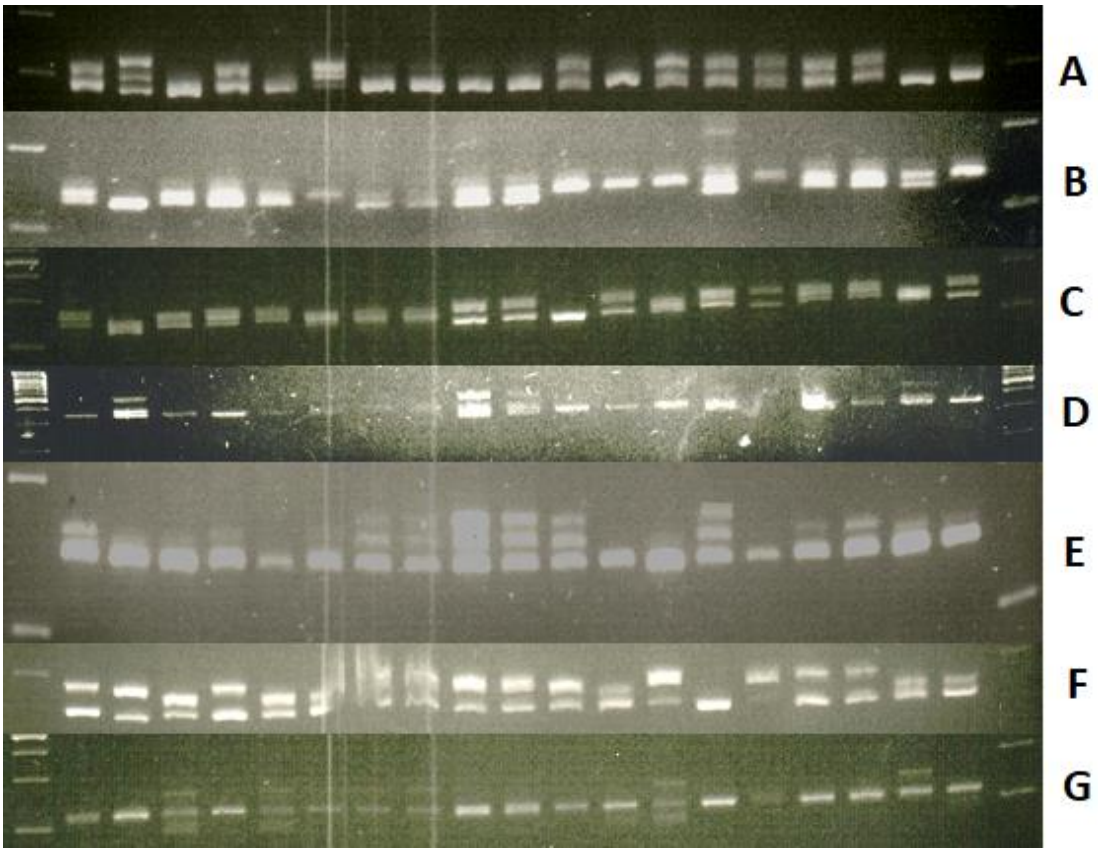

Figure 3.6: SSR-PCR banding profiles. A: MFC9, B: MFC3, C: MFC4, D: LMFC12, E: FCUP0082, F: FCUP68-1, G: LMFC30.

3.7.2 Allelic frequency per cultivar:

In general, different banding patterns were reported in most fig cultivars. The frequency of SSR bands for each cultivar was calculated as the number of band presences for that cultivar divided by the total number of loci produced by all primers. In this study, the highest frequency of SSR bands was in Sebaei cultivar, Barqawi cultivar and Mwazi cultivar (Table 3.7). The common cultivar that has high frequency of both ISSR and SSR bands was Sebaei cultivar where the number of bands in this cultivar was 12 bands. The lowest frequency was for Ruzi cultivar where the number of bands was 9 bands.

Table 3.7 Frequency of band presences per cultivar. The frequency was calculated as the number of band presences (1) for a cultivar divided by the total number of loci.

Cultivar	Freq.	Cultivar	Freq.	Cultivar	Freq.	Cultivar	Freq.
Mwazi	0.50	Ruzzi	0.37	Qrawi B	0.42	Khortmani	0.50
Dafor	0.46	Smari	0.42	Talhami	0.38	I'naqi	0.50
Shehami H	0.46	Qrawi H	0.42	Besatee	0.42	Hmari	0.42
Khdari	0.42	Sebaei	0.50	Swadi	0.46	Hmadi	0.38
Biadi	0.42	Barqawi	0.50	Neami	0.38		

3.7. 3 Number of bands scored for each SSR primer

A Total of 456 data entries were analyzed for the SSR markers; 199 data are present bands and 257 are absent bands (0). The total data entries and the number of data presences per each primer are shown in (Table 3.8)

Table 3.8: Total data entries TDE and the number of data presences (1) per each SSR primer.

Primer	TDE	Present (1)	Primer	TDE	Present (1)
MFC9	76	29	**LMFC12**	57	22
MFC3	95	27	**FCUP0082**	38	28
MFC4	57	32	**FCUP68-1**	95	36
LMFC30	38	25			

3.7.4 Genotypic frequencies and counts of the SSR markers

The number of genotypes ranged from 2 in (Fcup0082, LMFC30) to 9 in MFC3 with an average of 4.6 and a total number of 32 genotypes (Table 3.9). The number of individual cultivars with a certain genotype as well as the frequency of that genotype is shown in Figure 3.7.

Table 3.9 Genotype counts per primer

Marker	Genotype count
MFC9	5
MFC3	9
MFC4	5
LMFC12	3
FCUP0082	2
FCUP68-1	6
LMFC30	2
Mean / Total	4.6/32

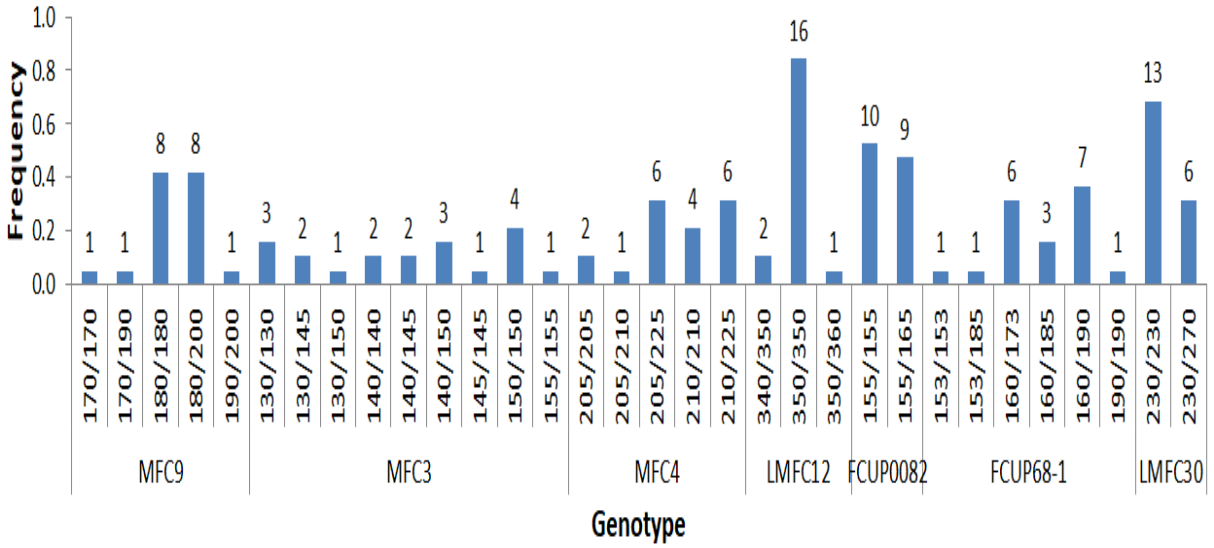

Figure 3.7: Genotypic frequencies of SSR markers. Numbers above columns represent genotype count.

3.7.5 Distribution of the SSR Allelic frequencies

A total of 24 alleles were detected (table 3.6) with an average value of 3.4 alleles per locus. The number of alleles ranged from 2 at loci FCUP0082 and LMFC30 to 5 at locus MFC3 and

FCUP68-1 (Figure 3.8). No alleles were homogenous. High number of observed alleles per locus indicates high genetic variation and the population is under mutation. Allele size varied from 130 bp at locus MFC3 to 360 bp at locus LMFC12, as shown in (figure 3.8). Allele frequency ranged from 0.05 in locus MFC3 to 0.92 in locus LMFC12 with a mean of 0.28.

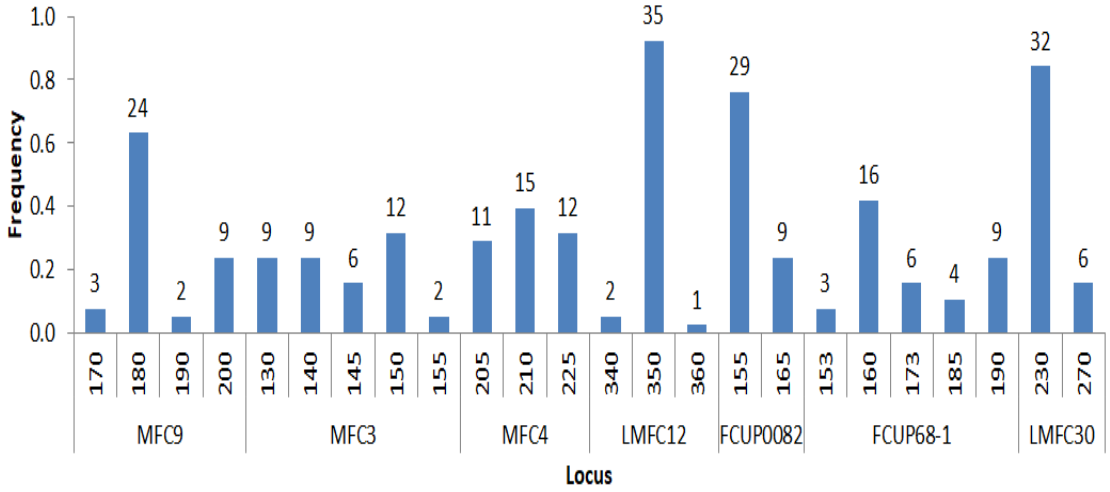

Figure 3.8: Shows Allelic frequencies of SSR markers. Number above columns represents alleles count.

3.7.6 SSR genotypic identities

In two of the cultivars, namely Hemadi and Dafor, genotypic identities were detected by MFC3 and LMFC12, respectively (Table 3.10). It is worth mentioning that in four cases two cultivars shared a genotypic identity. For example cultivars Ruzi and ShehamiH share a genotypic identity at locus 2 of the MFC9 primer. Other examples are shown in Table 3.10.

Table 3.10: SSR genotypic identities of seven Palestinian fig cultivars as detected in four primers. The loci number is the descending order of the unique band as appeared in the agarose gel.

Cultivar	Loci number			
	MFC3	LMFC12	MFC9	FCUP68-1
Hemadi	1	-	-	-
Dafor	-	1	4*	5*
Ruzi	-	-	2*	-
ShehamiH	-	-	2*, 4*	-
Sebaei	-	3*	-	-
Barqawi	-	3*	-	-
Swadi	-	-	-	5*

* These identities are shared by two cultivars.

3.8 Genetic diversity parameters of the SSR markers

3.8.1 Heterozygosity:

Most common parameter of variability in population is an estimate of both observed Heterozygosity (Ho) and expected Heterozygosity (He). The expected Heterozygosity (He) is calculated for each locus and population by utilizing the Hardy–Weinberg formula, He =1-Σp2i, where pi is the frequency of the i allele. This Heterozygosity is expected if the population is in Hardy–Weinberg equilibrium (population is not evolving). However, the observed Heterozygosity is direct counts of heterozygotes over sample size.

The observed heterozygosity ranged from 0.158 at locus LMFC12 to 0.895 at locus FCUP68-1 with mean of 0.496.The highest observed heterozygosity was at locus FCUP68-1(table 4.9).This high heterozygosity is attributed to high number of alleles, and low level of inbreeding. The expected heterozygosity ranged from 0.148 at locus LMFC12 to 0.724 at locus FCUP68-1 with mean of 0.494, the highest expected heterozygosity was at locus FCUP68-1 as shown in (Table 3.9).

A deficit of heterozygosity (excess of homozygotes (Hobs< Hexp) was observed in loci (MFC9, MFC3), where the fixation index was in positive value (Table 4.9). This fixation index is called the coefficient of inbreeding, which is known as the deviation of the observed heterozygosity of an individual relative to the heterozygosity expected under random mating (Hardy-Weinberg equilibrium).Here the inbreeding coefficient was >0,so that means that inbreeding(production of offspring from individuals genetically have the same alleles) is more than expected at random.

However, A heterozygote excess (Hobs > Hexp) was observed for in loci (MFC4, LMFC12, FCUP0082, FCUP68-1, LMFC30) suggesting the absence of null alleles, where the fixation index was in negative value as shown in (table 4.9). So here, the inbreeding coefficient was < 0, which means that inbreeding occurred less often than it would be expected at random. Overall, based to the total average of Ho (0.496) and the total average of He 0.494, the F value (-0.004), which suggesting that violation of the Hardy–Weinberg equilibrium.

Expected and observed heterozygosity values were compared by using Fixation index (inbreeding coefficient) which had an average of -0.053, with values between -0.310 at locus FCUP0082 and 0.446 at locus MFC3 (table 4.9).For two loci which are MFC3,MFC9, the

fixation index was positive (excess of homozygotes observed) and for five loci the fixation index was negative (excess of Heterozygotes observed), as shown in (table 4.9).

Tests for deviation from Hardy–Weinberg equilibrium were conducted by using Genelex 6.503 version and power maker version 3.25. Hardy–Weinberg equilibrium is used to compare population's actual genetic structure over time with the genetic structure we would expect if the population was in Hardy-Weinberg equilibrium (i.e., not evolving).The application of Hardy-Weinberg equilibrium is important to know if the population is in equilibrium for a particular loci that has two alleles. In general, the higher the Chi-Square value, the greater the difference between the observed versus the expected frequencies. The Chi-Square test for Hardy-Weinberg equilibrium assumes the "null hypothesis" - that is, the observed genotype frequencies are not significantly different from those predicted for a population in equilibrium. A probability value, or p-value, is used to evaluate the significance of a Chi-Square, by setting the cutoff for significance at p-value of 0.05 (5%) or less. So, if p value for Chi-Square is < than 0.05 or <than 0.01, "null hypothesis "is rejected and there is deviation from the Hardy Weinberg equilibrium. Significant deviation from the Hardy Weinberg equilibrium is observed at three loci (Table 4.10) All loci but 3 loci (MFC9, MFC3, FCUP68-1) showed HW P<0.01. Deviation from Hardy Weinberg equilibrium in loci (MFC3and MFC9) related to excess of homozygotes excess where the observed Heterozygosity <expected Heterozygosity significantly. However, Deviation from HW in FCUP68-1 related to the heterozygote excess where (Hobs > Hexp), as shown in (table 4.10).

Table 3.11: Diversity parameters for each SSR loci

Primer	Na	Ne	I	Ho	He	F	PI
MFC9	4	2.155	0.987	0.526	0.536	0.018	0.268
MFC3	5	4.173	1.493	0.421	0.760	0.446	0.098
MFC4	3	2.947	1.090	0.684	0.661	-0.036	0.189
LMFC12	3	1.174	0.326	0.158	0.148	-0.065	0.731
FCUP0082	2	1.566	0.547	0.474	0.361	-0.310	0.473
FCUP68-1	5	3.628	1.434	0.895	0.724	-0.235	0.117
LMFC30	2	1.362	0.436	0.316	0.266	-0.187	0.574
Mean	3.429	2.429	0.902	0.496	0.494	-0.053	
SE	0.481	0.444	0.179	0.091	0.090	0.094	

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / Ne = Expected Heterozygosity = 1 - Sum pi^2, F = Fixation Index = (He - Ho) / He = 1 - (Ho / He) Where pi is the frequency of the ith allele for the

population & Sum pi^2 is the sum of the squared population allele frequencies, PI = Probability of Identity at a Locus = 2*[Sum (pi^2)^2]-Sum(pi)^4, Where pi is the frequency of the i-th allele at a locus. PI over multiple loci is calculated as the product of the individual locus PI's.

Table 3.12 Hardy-Weinberg Equilibrium

Locus	DF	ChiSq	p-value	Significance
MFC9	6	17.593	0.007	**
MFC3	10	31.901	0.000	***
MFC4	3	7.111	0.068	ns
LMFC12	3	0.140	0.987	ns
FCUP0082	1	1.830	0.176	ns
FCUP68-1	10	26.074	0.004	**
LMFC30	1	0.668	0.414	ns

ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

In general, the higher the Chi-Square value, the greater the difference between the observed versus the expected frequencies. The Chi-Square test for Hardy-Weinberg equilibrium assumes the "null hypothesis" - that is, the observed genotype frequencies are not significantly different from those predicted for a population in equilibrium. Expected and observed heterozygosities and Hardy–Weinberg equilibrium (HWE) were conducted using GenAlex version 6.51 (Peakall and Smouse, 2006) and pwermaker version 3.25 (Liu and Muse 2005) Significant deviation from the Hardy Weinberg equilibrium is observed at three loci (Table).

3.8.2 PIC (polymorphism information content), PI (a probability of identification) and RP (Resolving power) for each SSR locus

The usefulness of SSR markers is not just influenced by the number of detected alleles but the most important factor is their frequency and distribution .The way how to find out their effectiveness is to calculate Polymorphic information content (PIC), Resolving power and Probability of identity (PI) which corresponds to the probability of two random individuals displaying the same genotype.

Calculated PIC was varied between 0.142 at locus LMFC12 to 0.719at locus MFC3 with mean of 0.448,as shown in (Table 4.11).The two loci MFC 3, Fcup68-1 are considered the highly informative loci where PIC > 0.5.

Resolving power was calculated for each SSR loci to identify primer powerfulness in detecting the variation among the tested fig genotype, as shown in (table 4.11).The resolving power of

the 7 SSR primers ranges from 0.3 at locus LMFC12 to2.8 at locus MFC3with mean of 1.6.The highest Resolving power was calculated for primerMFC3, which means that, this locus is highly informative.However, the lowest Resolving power was calculated for LMFC12 locus.

With a probability of identification (PI) which means probability of two cultivars sharing the same genetic Profile by chance, also there are only two loci (MFC3, Fcup68-1) are considered to be highly informative because PI value were low (0.09, 0.11) respectively, However, the maximum PI value was at locus LMF30, as shown in (table 4.9), with mean of probability of identity of 0.35.

Table 3.13: powerfulness and efficiency of each SSR primer

Marker	MAF	Genotype no	PIC	Rp
MFC9	0.6316	5	0.4830	1.8
MFC3	0.3158	9	0.7198	2.8
MFC4	0.3947	5	0.5868	2.6
LMFC12	0.9211	3	0.1423	0.3
FCUP0082	0.7632	2	0.2962	1.0
FCUP68-1	0.4211	6	0.6838	2.4
LMFC30	0.8421	2	0.2306	0.6
Mean	0.6128	4.571	0.4489	1.6

MAF: major allele frequency.

Overall, the loci show low to moderate information content with observed heterozygosities.

3.9 Correlation among the two genetic matrices

As shown in (Table 4.12) and Figure9, a high fit between the Nei72 and the Jaccard matrices was revealed by Mantel test where a high correlation Cophenetic coefficient (**r = 0.95570**) was achieved. Note that a value of 1 means identical matrices.

Table 3.14 MxComp Matrix correlation for fig cultivars SSR clustering analysis generated by NTSYS.PC v 2.10e.

2-way Mantel test - Mantel (1967) method	Tests for associations: Matrix correlation: **r = 0.95570**
N = 171 points Mean X = 0.4633, SSx = 9.8624	(= normalized Mantel statistic Z) Approximate Mantel t-test: t = 7.9722
Mean Y = 0.4963, SSy = 8.7575	Prob. random Z < obs. Z: p = 1.0000

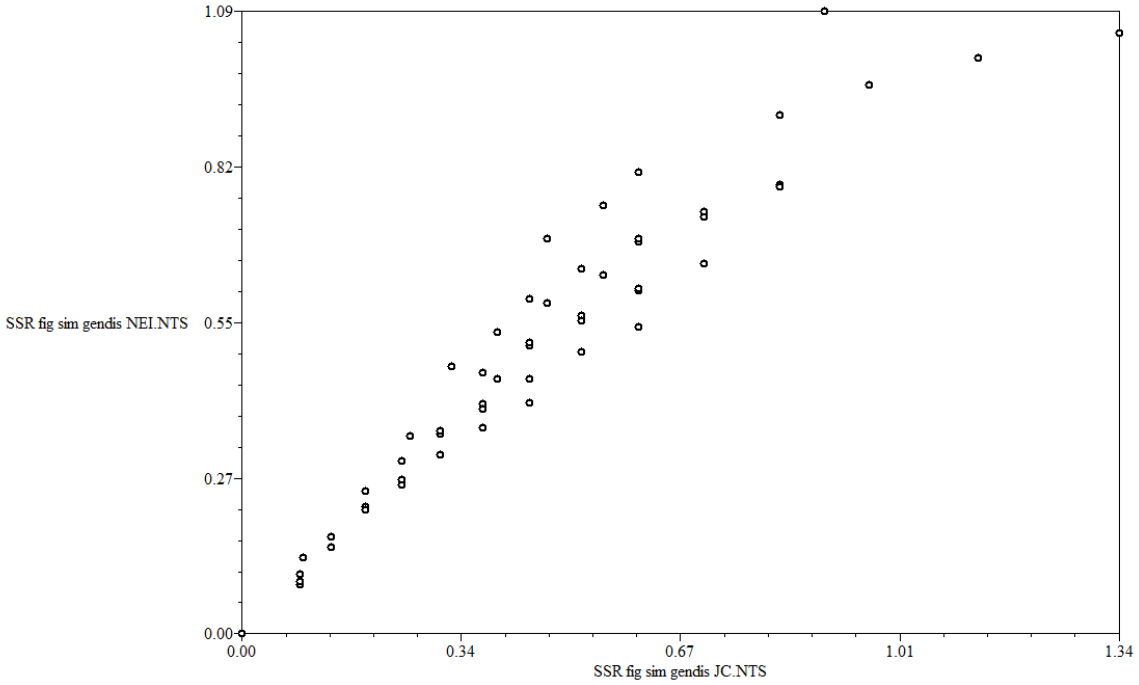

Figure 3. 9: Shows Nei and JC matrix comparison plot following Mantel test

3.10 SSR Cluster Analysis

The genetic relationships among the fig genotypes are illustrated by two trees; the first is a dendrogram based on UPGMA analysis following a similarity matrix generated by Jaccard's coefficient, and the second is a phylogram based on Neighbor Joining analysis following a similarity matrix generated by Jaccard's coefficient, with bootstrap value 1000. However, phylogram tree and dendogram tree that are obtained by two different methods (UPGMA, NJ), respectively were almost similar. There was difference in grouping between two dendograms trees that are obtained by ISSR and SSR methods. However, some common groupings were observed (khurtumani, Inaki), (Quwari, Smari),(Thalami,Hemadi), (Biadi,Hemari)by both ISSR based and SSR based dendograms (Figure 4),(figure 10). Dafor cultivar in dendogram obtained by SSR marker was separated and identified as distant genotype. Whereas Mwazi cultivar was considered as distant genotype by ISSR method, as shown in (Figure 4).

In addition, the analysis of data by principle coordinate of analysis (PCA) was used to confirm the rigidity of the phylogenetic trees. As shown in Figure12, the 3 - dimensional plot is in support with clustering patterns seen in both trees of SSR marker. The phylogram of 19 fig

cultivars based on SSR marker was made of two clusters, the first cluster was made of five sister groups (khurtumani, Inaki), (Quwari, Smari),(Thalami,Hemadi), (Biadi,Hemari)and four verities were member subgroups (khdari, Shehami, Ruzi,QurawiB),this cluster is made of 15cultivars that originated from different regions of Palestine. The second cluster was made of two sister groups (swadi, Dafor), (Baqawi, Sebai).In dendogram tree of SSR markers regarding race type, Dafor cultivar is separated alone and was considered as distant genotype. This cultivar is sanpedro cultivar differ from common edible cultivars based to race.

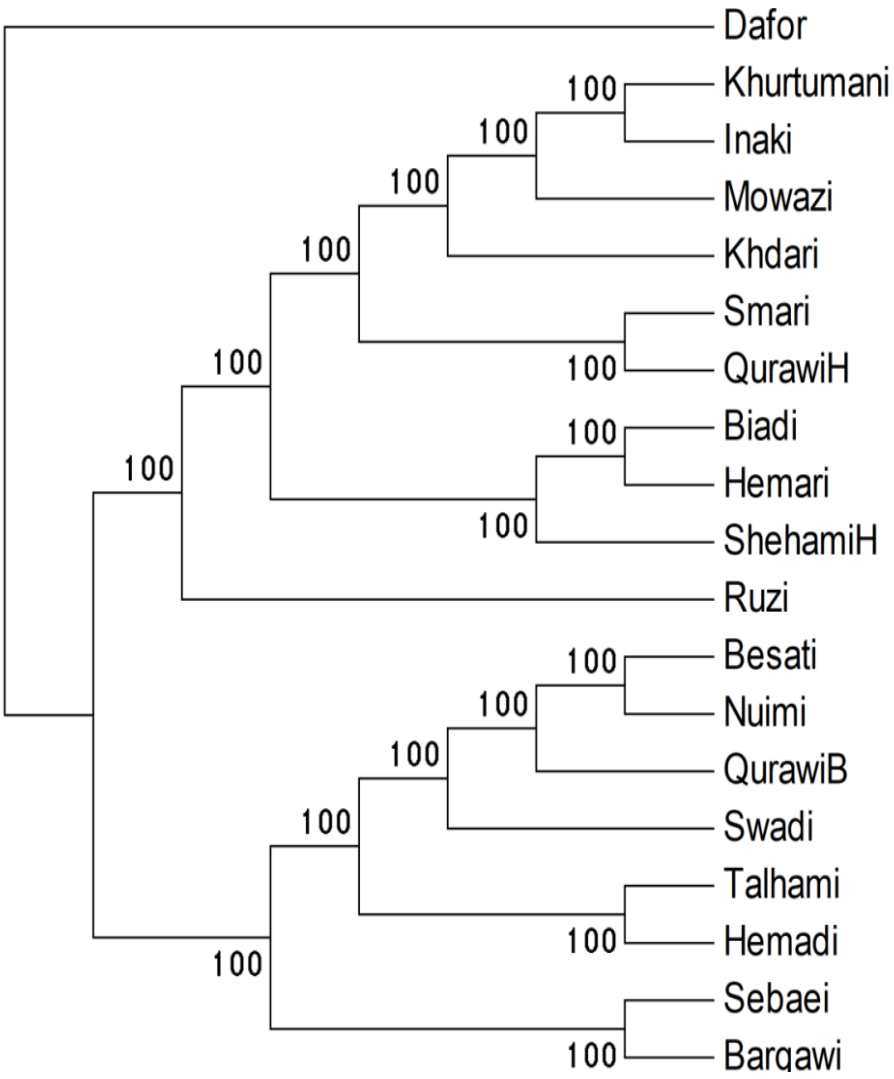

Figure 3.10: Dendogram of 19 Palestinian fig cultivars based SSR data, based on UPGMA analysis using the maximum similarity (Jaccard's coefficient) method generated by FAMD. Bootstrap values are shown on the nodes, 1000 replicates. The tree is displayed by MEGA4.

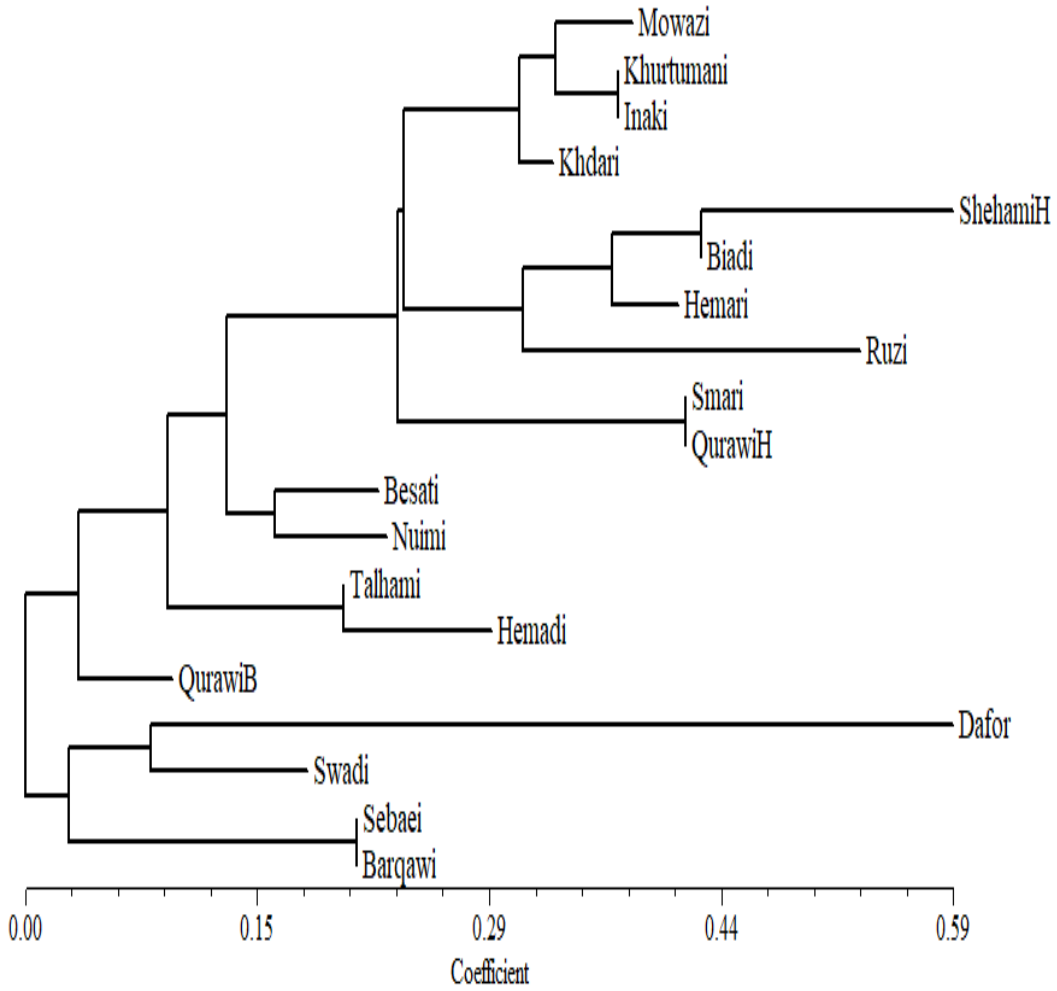

Figure 3.11: Phylogram of 19 Palestinian fig cultivars based on weighted Neighbor Joining NJ analysis and SSR DATA using the similarity matrix generated by Jaccard.

In addition, the analysis of data by principle coordinate of analysis (PCA) was used to confirm the rigidity of the phylogenetic trees. As shown in Figure12, the 3-dimensional plot is in support with clustering patterns seen in both dendogram and phylogram trees. APCA analysis from combined data also showed similar results using cluster analysis.

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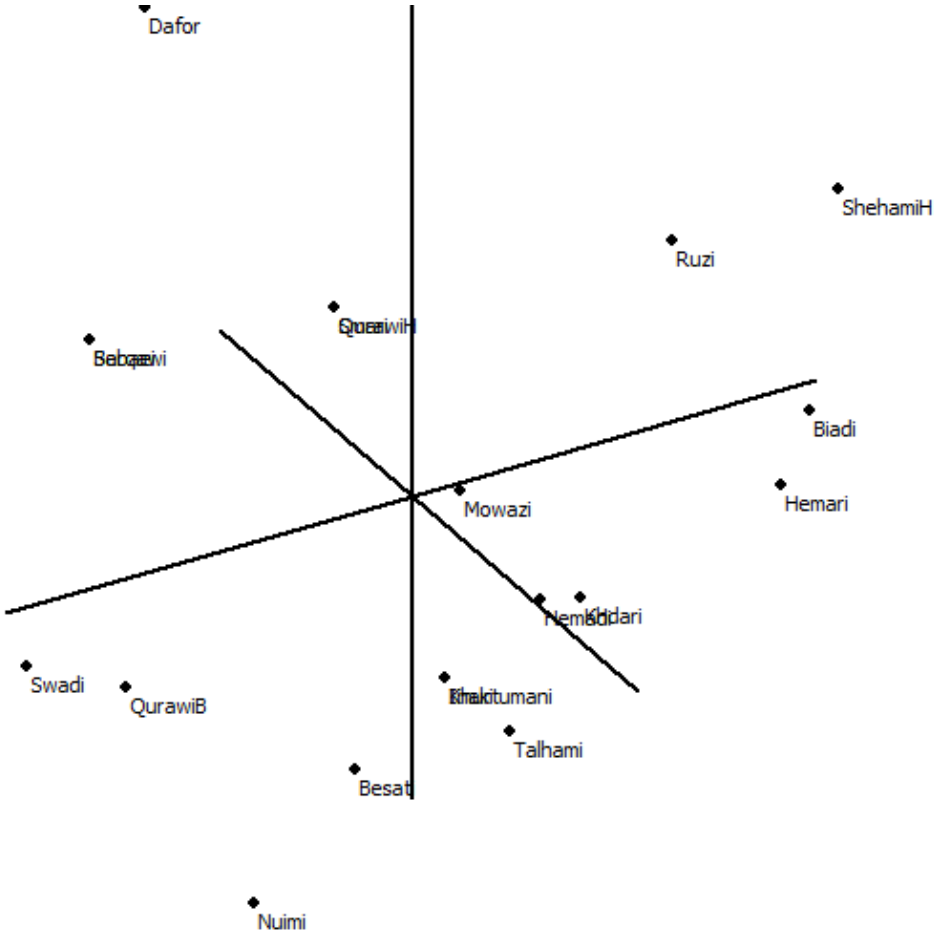

Figure 3.12**:** 3-D Plot of the principal coordinate analysis of SSR Data of the 19 fig cultivars.

3.11 Cluster analysis joining both ISSR and SSR matrices

The genetic relationships among the fig genotypes are illustrated by dendogram based on combined SSR and ISSR data (Figure 13) and by phylogram based on combined SSR and ISSR data (Figure 14).The dendogram was made of twoMain clusters and Mwazi cultivar is branced separatly and considered as distant genotype (Figure 13). The first cluster was the largest one was made of 13 cultivars that are originated from different regions of palestine (North,middle,south).This Mains that cluster was further divided into two subclustersthis main cluster made of four sister groups (khurtumani, Inaki), (Quwari, Smari), (Thalami,Hemadi), (Biadi,Hemari).These sister groups were observed also in dendogram based ISSR data and in dendogram based SSR data,they were common and stable between two established marker trees.In addition, there was a sister group (Qurawi B,Besati) was observed in dendogram based combined ISSR and SSR data and in dendogram based ISSR data (Figure 13).

The second main cluster was made of six varities,originated from hebron and Bethlehem regions.This main cluster was further divided into two subclusters,these subclusters comprise two sister group (Sebai,Barqawi)and (khdari,sheamiH).This sister group(Sebai,Barqawi) was observed in dendogram based SSR data and in dendogram based combined ISSR,SSR data(Figure 13). (khdari,sheamiH) sister group was observed in dendogram based ISSR data and in dendogram based combined ISSR,SSR data (Figure 13) (figure 3)

Based on the genetic relationship among 19 fig cultivars that are based on combined (SSR and ISSR) Data by UPGMA the following five genotypes,Mwazi,Dafor,swadi,nuimi and Ruzi may be considered as distinct cultivars .Swadi and Mwazi were also distinct genotypes in dendogram based ISSR data and in dendogram based SSR data.Dafor,Ruzi were distinct genotypes in dendogram based SSR data.The remaining genotypes may be considered as synonymous groups (Sebai,Barqawi) (khurtumani, Inaki), (Quwari, Smari), because the genetic distance is small,or closely related lik,(Thalami,Hemadi), (Biadi,Hemari), The pylogram tree based ISSR and SSR data differs slightly from dendogram tree in clustering few genotypes like swadi,Nuimi and khdari.This difference is attributed to different method has been used.

The clustering pattern obtained with two types of marker data showed almost mutually independentResults,Different dendograms were obtained by two markers,However there are four common groups btween three dendograms obtained.(Figure 3), (Figure 10).

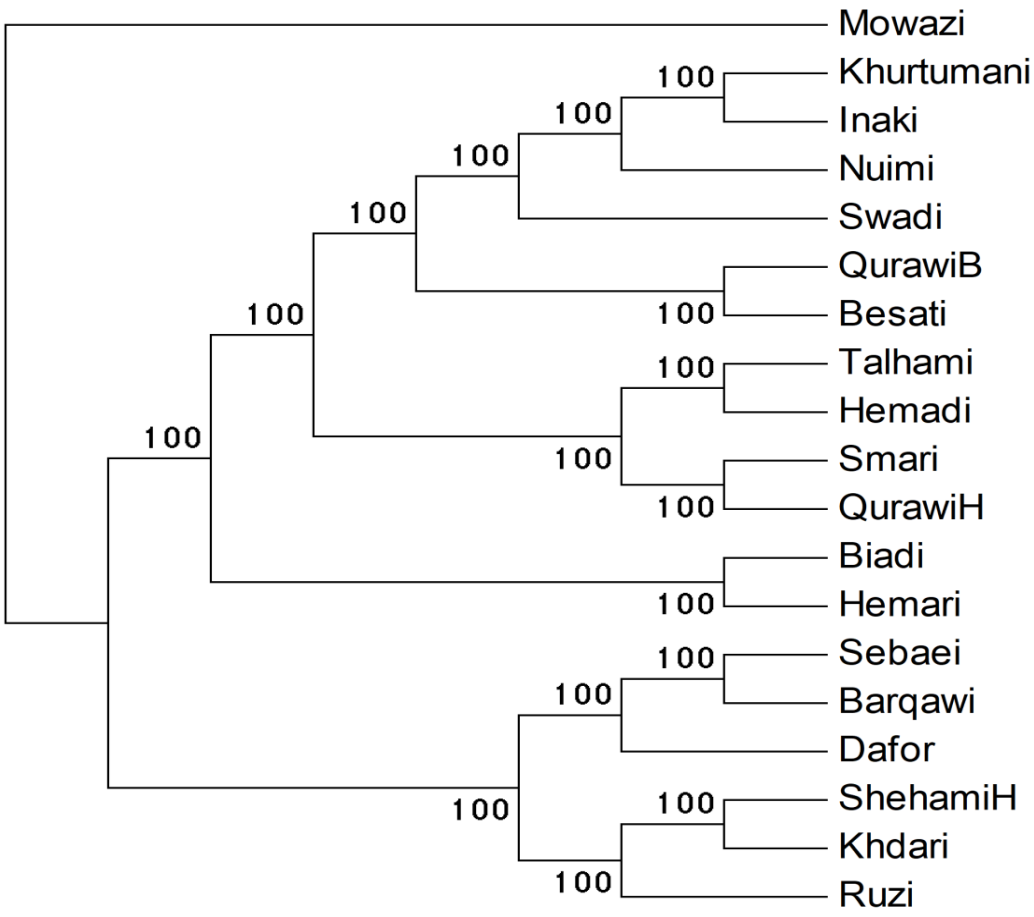

Figure 3.13: Dendogram of 19 Palestinian fig cultivars based on combined ISSR and SSR data, based on UPGMA analysis using the maximum similarity (Jaccard's coefficient) method generated by FAMD. Bootstrap values are shown on the nodes, 1000 replicates. The tree is displayed by MEGA4.

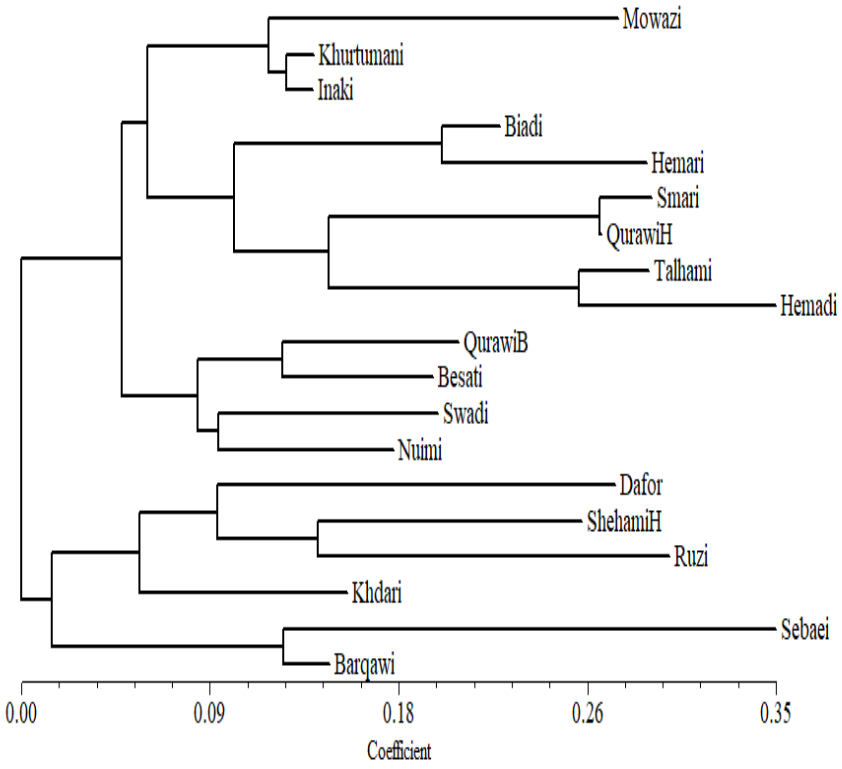

Figure 3.14: Phylogram of 19 Palestinian fig cultivars based on weighted Neighbor Joining NJ analysis, based on combined SSR, ISSR Data using the similarity matrix generated by Jaccard.

APCA analysis from combined data (SSR, ISSR) also showed similar results using cluster analysis.

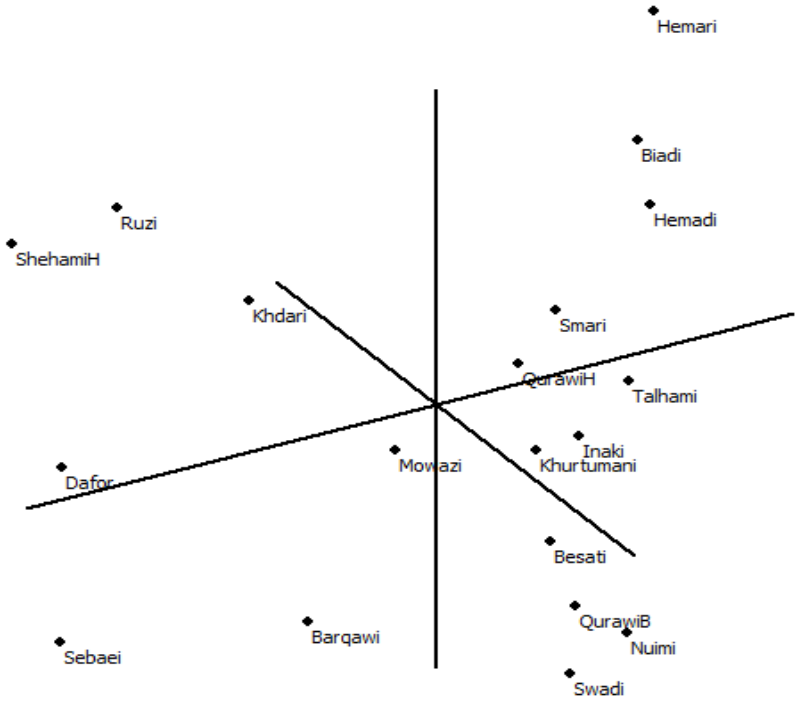

Figure 3.15: 3-D Plot of the principal coordinate analysis of combined data (SSR, ISSR) of the 19 Palestinian fig cultivars.

3.11.1 Correlation of the genetic distances among two marker systems

A comparison was made of the genetic distances obtained with the two types of molecular markers (SSR and ISSR) by Mantel test.The comparison between marker combinations resulted low correlation coefficient(0.0246) which indicates that two markers showed no correspondence with others at the variety level and ISSR marker can not be alternative to SSR marker.,we can not reject the null hypothesis which is (The matrices are not correlated) In fact, the clustering pattern obtained by ISSR Data and SSR Marker showed somewhat close clustering in some fig landraces, but on the other hand also showed some difference in other fig landreces.A correlation coeeficent r will be statistically significant at 0.01 at probability level if the taxnomic units exceeds 15 (Lapointe and Legendre 1992).

3.12: Comparison between ISSR and SSR markers:

Acomparitive table btween ISSR and SSR marker is iullustrated in(Table 3.12).Among the two markers,ISSR marker produced the highest number of bands 56 bands.ISSR (6primer),SSR (7 primer pairs)techniques produced polymorphic bands 46, 21, respictively.The average no polymorphic bands produced per primer or primer pair was 7.6, 3 for ISSR,SSR respictively.Among the two markers,ISSR produced 82% polymorphic marker.In contrast,SSR marker exhibted 88% polymorphism. PIC (polymorphic information content) and Rp (Resolving power) were calculated for ISSR marker and SSR marker,Both of these parameters measure the discriminatory power of each marker to differentiate between fig cultivars.The average of PIC was the highest for SSR marker, So,that means that SSR marker are most discriminative than ISSR marker,since SSR marker is multialleliec whereas ISSR marker is biallelic marker.

Table 3.15 Level of polymorphism detected by ISSR and SSR markers with Fig cultivars

Component	**ISSR**	**SSR**
Total no of primers	6	**7**
Total no of bands per primer	56	**24**
Average no of bands per locus	9.3	**3.4**
No of polymorphic bands	46	**21**
Maximum no of bands amplified by a single primer	10	**5**
Percentage of polymorphic marker	82	**88**
Average PIC	0.272	**0.44**
Average Resolving power	10.8	**1.6**
average polymorphism per primer	7.6	**3**

CHAPTER FIVE

Discussion and Conclusions

Discussion

The determination of genetic variations in local fig cultivars is an important step towards the improvement of this traditional plant through breeding programs and the conservation of plant genetic resources (Ali-Shtayeh, Jamous et al. 2014). Several molecular techniques have been used to assess the genetic diversity among fig cultivars and other crops worldwide including ISSR, SSR and RAPD markers (Ikegami, Nogata et al. 2009); (Khadari, Hochu et al. 2003); (Guasmi, Ferchichi et al. 2006). Those microsatellite-based markers are more informative than the random, non-reproducible RAPD markers. In Palestine, few studies aiming at the determination of the genetic diversity of some local fig cultivars were conducted. All of these studies have employed the RAPD dominant marker (Ali-Shtayeh, Jamous et al. 2014), (Basheer-Salimia, Awad et al. 2012). Our current study is the first in Palestine to employ ISSR dominant markers and SSR co-dominant marker to assess the genetic diversity among 20 local fig cultivars.

The mean percentage of polymorphic DNA marker that was obtained by Salimia et al (2012) study was 77% and that was conducted by Shtayeh et al (2014) was 73% where both researchers used RAPD markers. In our study the mean percentage of polymorphic DNA marker is 82% using ISSR marker and 88% using SSR marker. Regarding the discriminative power of primers to differentiate between the fig cultivars, the total value of RP parameter of RAPD markers was 27 with a mean value of 0.98 (Ali-Shtayeh, Jamous et al. 2014). For the Salimia results the total RP value of RAPD markers was 18.8 with a mean value of 2.09 (Basheer-Salimia, Awad et al. 2012). In our results the total RP value of the ISSR markers is 64 with a mean value of 10.8. These results indicate that ISSR method is more discriminative than RAPD method.

Dendrograms that were based on RAPD markers by both Salimia and Shtayeh were not similar as expected with such markers. For example, according to Salimia, khurtumani and Inaki were classified as two sister groups and so are Swadi and Biadi. Whereas, in the dendrogram generated by Shtayeh group, these cultivars clustered separately and distantly.

These results are in support with the fact that RAPD markers, in contrast to ISSR and SSR markers, are highly unreproducible and are no longer employed for such research purposes ISSR are dominant molecular markers known for their powerfulness genotype discrimination and in revealing genetic diversity of plants including fig germplasm (Guasmi, Ferchichi et al. 2006; (Khadari, Hochu et al. 2003; Ikegami, Nogata et al. 2009). For example, ISSR markers have been used successfully for the characterization of genetic polymorphism in maize, figs and olive tree (Kantety, Zeng et al. 1995; Blair and Curran 1999; Zhang, Liu et al. 2005). The ISSR discriminative values in our results are comparable to those obtained by Haddou et al (2012). In our case, 56 loci with an average value of 9.3 loci per ISSR primer and a percent of polymorphism of 82% were obtained using sex ISSR Primers (Table 3.2). Haddou et al (2012) detected 54 loci using seven ISSR primers in 22 Moroccan fig cultivars with an average value of 8 loci per primer and a percent of polymorphism of 80%. Moreover, a total of 52 loci, 13 loci per primer with 90% of polymorphism, were detected in 18 Tunisian fig cultivars using 4 ISSR markers (Salhi-Hannachi, Trifi et al. 2004). Furthermore, a total of 71 DNA loci, 5.5 per primer, with 62.4% polymorphism, were scored for 19 fig cultivars from Japan, 4 of which are breeding lines of European and American origin, using 13 ISSR markers in 19 fig cultivars (Ikegami, Nogata et al. 2009). These results indicate the relatively moderate to high degree of polymorphism present among the studied Palestinian fig cultivars is comparable to fig cultivars of other countries. These results are in support with the reliability and informativeness of ISSR markers as a tool for revealing the molecular polymorphism in the fig germplasm.

Primers having high Rp along with high PIC values are more powerful for analysis of genetic diversity (Singh, Sharma et al. 2007). PIC has been known to provide an estimate of the discriminatory power of a locus or loci. In our results, the most useful ISSR primers are UBC812, UBC817 which detected 11 loci with RP values of 13.4 and 11.3 and PIC values of 0.313 and 0.25, respectively. The results of UBC812 primer are comparable and similar with a study that was conducted on Asian and European cultivars by Ikegami (Ikegami, Nogata et al. 2009). Additionally, the PIC values of our study which range from 0.196 to 0.296 (Table 3.2) are considered to be Similar to PIC values that were obtained in a Jordanian study which aimed to investigate the genetic diversity among 30 fig cultivars (Abdel-Ghani and Migdadi 2012). ISSR produced a unique fingerprint for some fig cultivars examined in this study as shown in Table 3.4. This presents the powerfulness of ISSR markers not only for

genetic diversity studies and differentiation among fig cultivars but also as a tool for genotype identification (Khadari, Hochu et al. 2003).

Generally, more loci would be detected when more ISSR markers are used and this would increase the reliability of the interpretation of the results. However, the number of scored bands can be highly affected by the type of analyzed plant species and the type of ISSR employed. In general, most studies screen many ISSR markers and recommend the use of 6-10 of these markers for further studies based on the RP and PIC values of these markers. For example, Pradeep et al. (2005) used seven primers, while twelve markers were used by Wiesner and Wiesnerova (2003). Furthermore, the primers that we used in this study are dinucleotide repeat. This is because previous studies showed that di-nucleotide repeat are more efficient than the primers tri-nucleotide repeat in detecting loci in the fig genome (Haddou, Charafi et al. 2012). This mainly because tri -nucleotides are less frequent and their use in ISSRs is lesser than the di-nucleotides so the di-nucleotides would be more informative than tri- nucleotides (Reddy, Sarla et al. 2002).

The ISSR markers used in our research generated polymorphic profiles with variable PIC value. For dominant markers as ISSR, the maximum PIC value is 0.5 (De Riek, Calsyn et al. 2001). The mean PIC value obtained in this study was 0.27 indicating relatively good degree of polymorphism. This PIC value is the result of the relatively high average number of loci detected by the used ISSR markers (9.3) of which 7.6 are polymorphic. These values are sufficient to reveal variations among the cultivars (Khadari, Lashermes et al. 1995). The genetic relationships among the 20 cultivars are illustrated by dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) and phylogram based on the Neighbor Joining Method (Khakabimamaghani, Najafi et al.). UPGMA is a simple and fast method but uses algorithm that do not aim to reflect evolutionary relationship and assumes that the evolutionary rate is the same in all cultivars. For this reason, the NJ algorithm has been the choice for generating phylogenetic trees (Huelsenbeck and Ronquist 2001). The ISSR genetic distance between Palestinian fig cultivars ranges from 0.05 to 0.64, this range is higher than that genetic distance of RAPD Data detected by Shtayeh (0.238 - 0.477) (Ali-Shtayeh, Jamous et al. 2014. That result reveled that ISSR marker is more discriminative than RAPD. In this study, the ISSR genetic distance was lower than genetic distance between 30 Jordanian fig cultivars that were analyzed by 5 ISSR primers (0.00 to 0.830). This

difference is attributed to different genetic materials. So, these results indicate the presence of genetic divergence in the Palestinian fig cultivars. Needless to say that the lowest the genetic distance is, the more related are the cultivars. As an example, the low genetic distance (0.05) between Smari and Qurawi indicates that these two cultivars are closely related. There are some differences in the clustering patterns of the dendrogram and the phylogram. This might be because of the different methods used to construct such trees. However, for studies based on binary matrices, the dendrograms are more reliable than the phylograms. Despite these clustering differences, many of the cultivars clustered with each other regardless of the tree type (Figurers 3.3 and 3.4). These cultivar duets (sister cultivars) are (Smari and Quwari), (Hemadi and Talhami) (Inaki and khurtumani), and (Hemari and Biadi). These cultivars might be more closely related to each other than those who clustered differently. The close relatedness of some fig cultivars was also revealed by a phenogram generated in a previous study (Salimia et al., 2012) where both Khortomani and Inaki cultivars showed common morphological characters. The correlation coefficient between the ISSR dendrogram and the phenogram was insignificant, ($r = -0.08$, $p < 0.07$), which could be explained by the absence of linkage between the used ISSR markers and the loci that control the studied morphological characters (Abdel-Ghani and Migdadi 2012).

Synonymies and homonymies among fruit trees have been widely reported (Condit 1955, Khadari, Roger et al. 2005). Different examples of synonymies are available in fig species (Giraldo, Viruel et al. 2005). For example, Fig cultivars are very common in Jordan and their denomination is very complicated because of the morphological similarity. Homonymy is a problem in Jordan since denominating cultivars is solely based on common morphopomological traits. Therefore, different cultivars could have the same name because they share same morphopomological traits (Abdel-Ghani and Migdadi 2012). Discriminating of homonymous cultivars could be achieved by genotyping.

Homonymies cases were noticed in our study. Examples include (QurawiH, Qurawi B) (Shehami H, ShehamiB). Though, these cultivars have the same name, our results showed they have different genotypes. Such cases of homonymies and synonymies are probably a consequence of mislabeling due to sole dependence on the morphological characters for naming fig cultivars. Moreover, the inconsistency in naming might be also due to the ease of transport of plant materials between different regions where slight phenotypic differences

could arise due to artificial or natural selection with the accumulation of mutations (Galderisi, Cipollaro et al. 1999). Also, the harshest weather condition in the Mediterranean region where fig can grow might lead to phenotypic modifications (Sadder and Ateyyeh 2006). Therefore, these results would help establishing a reference collection of well-characterized and identified fig cultivars based on reliable DNA markers.

Furthermore, the clustering profiles generated by both trees showed no obvious correlation between the cultivars and their geographical distribution. This result is suggesting a common genetic basis (common origin of population) or can be explained by the occurrence of gene flow among cultivars (Ikegami, Nogata et al. 2009).

As a result of their high reproducibility, locus specific polymorphism, and transferability, microsatellites have become the markers of choice for fingerprinting and fruit cultivar identification (Achtak, Oukabli et al. 2009; Khadari, Roger et al. 2005). This is the first study that applies SSR markers for revealing genetic polymorphism among the Palestinian fig cultivars. Employing seven SSR markers, in this study, a total of 24 alleles were detected (Table3.5) with a mean of 3.4 allele per locus. Similar average number of detected alleles per locus was detected by Bandelj, et al. (2007), where 15 SSR markers were used to assess the genetic variability in 19 fig individuals. They detected a total of 59 alleles with a mean of 3.9 alleles per locus. Compared to other cited literature, higher average numbers of alleles per locus were detected. For example, in a total of 42 alleles detected from 22 fig cultivars using 9 SSR markers, the average number of alleles per locus was 5 (Haddou, Charafi et al.) This difference could be attributed to the number and type of SSR primers used and the plant material studied.

A relatively high polymorphism was obtained among the analyzed Palestinian fig cultivars in this study using SSR markers. This reflects the presence of biodiversity and rich genetic resources for future breeding programs. The percent of polymorphism of SSR marker in our study is 88% compared with 82% obtained by the ISSR markers (Table 3.12). The genetic diversity parameters of SSR markers like observed heterozygosity and expected heterozygosity were calculated and compared to check whether the cultivars follow HardyWeinberg equilibrium or not.

In general, moderate observed heterozygosity values were observed in the studied Palestinian fig cultivars (0.49). These results are comparable to those obtained by Bandelj et al., 2007 and

Knap et al., 2015 but less than those observed for Moroccan fig cultivars where the Ho was 0.79 (Haddou, Charafi et al.2012) and higher than those observed for the Tunisian caprifig accessions where the Ho was 0.33 (Essid, Aljane et al. 2015) . 0.33 The explanation of these differences might be due to the different numbers of amplified alleles in each study and the different genetic structure of the analyzed plant material (Knap, Jakše et al. 2015). Five of the seven SSR loci show an excess of observed heterozygosity in regard to the expected heterozygosity which mean deviation from Hardy Weinberg equilibrium for population under random mating (Table 3.11). The negative fixation index reflects violation of Hardy-Weinberg equilibrium. This means the presence of forces that would induce evolution through selection pressure suggesting that gametes may conjugate not randomly (Haddou et al., 2012). In the other two SSR loci, a heterozygosity deficiency was calculated and supported with positive values of their fixation index probability (Table 3.11). This deficiency was significant in locus MFC3. These results are comparable with those reported the Moroccan (Khadari, Oukabli et al. 2005) and Tunisian fig cultivars (Saddoud, Baraket et al. 2011). This heterozygosity deficiency could be explained due to excess of homozygotes that might be due to the occurrence of more inbreeding than expected at random where inbreeding results in a decrease in genetic variability (Falconer and Mackay 1996). Chi-Square test was conducted to assure the presence of deviations from Hardy-Weinberg equilibrium (Guo and Thompson 1992). Hardy-Weinberg Equilibrium is used to compare allele frequencies in a given population for a particular locus over a period of time. HardyWeinberg Equilibrium provides a baseline against which locus evolution in a given population is measured. The Hardy-Weinberg Principle states that the amount of genetic variation in a population (allele and genotype frequencies) will remain constant from one generation to the next in the absence of evolutionary forces. The evolutionary forces include mutations that occur and lead to allele changes, migration of individuals either into or out of the population, nonrandom mating, genetic drift, and natural selection. If any of these forces occurred, the population would not be in Hardy-Weinberg Equilibrium. Hardy-Weinberg Equilibrium allows a comparison of frequencies between expected (ideal conditions) and observed (with evolutionary forces) in the population (Wigginton, Cutler et al. 2005). In three of the seven SSR loci (MFC3, MFC9, and FCUP68-1) a significant deviation from HardyWeinberg equilibrium was recorded (Table 3.12). Actually, the Hardy-Weinberg

equilibrium cannot exist in real life; most populations are under the influence of natural selection, mutation and migration, gene flow (Wigginton, Cutler et al. 2005).

Whereas the deviation from Hardy-Weinberg equilibrium in loci MFC3 and MFC9 is due to excess of homozygotes, in locus FCUP68-1 it is due to excess of heterozygotes and different genotypes developed (Table3.11). Such violations could be explained due to mutation effect. Since fig is propagated mainly by vegetative means, developing of different genotypes could have been occurred by engagement of mutations through development. This violation also, could be explained due to selection effect (Sadder and Ateyyeh 2006), (Haddou, Charafi et al.).

SSR primers with high PIC and low PI values are considered to be the most discriminative (Korkovelos, et al. 2008). In this study, the most informative SSR markers were MFC3 and FCUP68-1 with PIC values of 0.71 and 0.68 and PI values of 0.09 and 0.11, respectively (Tables 3.11 and 3.13). The RP value of MFC3 was 2.8 while that of FCUP68-1 was 3.8. Both markers generated five alleles. Similar results were reported in the literature regarding the PIC value and PI value of FCUP68-1 (Bandelj, JAVORNIK et al. 2007) and MFC3 (Ikegami, Nogata et al. 2009).

The genetic relationships among 19 Palestinian figs cultivars were illustrated by dendrogram and phylogram trees as shown in (Figures 3.10 and 3.11). The clustering patterns of the cultivars in both trees are highly similar in contrast to the different clustering patterns of the denedrogram and phylogram generated by the ISSR markers. This might indicate that the codominant SSR markers are more powerful than the dominant ISSR markers in revealing genetic relatedness. The dendrogram based on SSR data is different from that generated by the ISSR data. However, there are four genotype pairs (cultivar duets) that maintain the same clustering pattern types of trees and markers indicating that these genotype pairs are closely related. These are (smari and Qurawi H) (Khurtumani and Inaki), (Biadi and Hemari), and (Talhami and Hemadi). The genetic distances for these pairs are very low supporting their close relatedness. Additionally, it is obvious from the phylogram (Figure 3.11) that Dafor cultivar is distantly related to the other cultivars. This might be attributed to the origin of Dafor, race type sanpedro, which differs from the common fig cultivars in the caprification process. The Principal coordinate analysis of SSR Data supports the SSR phylogenetic analysis.

An SSR and ISSR combined dendrogram and phylogram (Figures 3.13 and 3.14) provide a reliable reflection of the genetic relationship among the local fig cultivars. Both trees showed highly similar clustering profiles. In the dendrogram, Mwazi genotype is distantly branched from the other cultivars, which is in agreement with the results Salimia et al., (2012). However, in the phylogram, this cultivar clustered with Inaki and Khurtumani cultivars, though with a high genetic distance.

The different clustering profiles revealed by ISSR and SSR markers might be explained by the fact that these two DNA markers target different parts of the genome (Powell et al. 1996). Statically this result is supported by insignificant correlation between ISSR and SSR markers. Little correlation between ISSR and SSR markers was also reported in wheat (Pejic, AjmoneMarsan et al. 1998) and maize (Bohn, Utz et al. 1999).

Table3.15 summarizes the differences between the SSR and ISSR markers in revealing genetic differences among the local fig cultivars. Overall, the percentage of polymorphism, PIC and PI values are in favor of the SSR markers. While ISSR markers yield multilocus, biallelic products, SSR markers yield multi-allelic products of a single locus. Therefore, SSR markers are highly discriminative. This superiority of SSR marker over ISSR in detecting polymorphism was frequently reported (Teulat, Aldam et al. 2000)

Conclusion

Molecular markers are efficient tools for cultivar identification and estimation of relatedness through DNA analysis. In our study, for first time in Palestine two types of molecular markers SSR and ISSR were used to analyze the genetic polymorphism among Palestinian fig cultivars.

The results of percentage of polymorphic marker and discriminatory powers of primers showed that SSR and ISSR markers appear to be a powerful and informative technique for the analysis of genetic diversity of fig cultivars. The results of diversity index indicate and elucidate known primers for future genetic diversity analysis of fig cultivars for the identification of available varieties. The six ISSR and seven SSR primers showed moderate level of polymorphism among Palestinian fig cultivars due to the ease transportation of selected plant materials between regions.

The mean percent of polymorphism obtained by SSR primers was the highest compared with ISSR marker, indicating that SSR method is the most informative and polymorphic technique for the analysis of genetic diversity among fig cultivars. Also, the mean value of polymorphic information content was the highest for SSR marker indicting that SSR marker has highest discriminatory power compared with ISSR marker for obtaining polymorphism among Palestinian fig cultivars.

Variability that was obtained by local Palestinian fig cultivars can be used in breeding programs for F. carica L improvement and serve as target traits for selection by fig growers and breeders and also can be available as genetic resource for conservation and preserve for further breeding program. ISSR and SSR screening revealed the presence of similar genetic profile and genotypic identity for fig cultivars that would save time needed for screening different genotypes for breeding program and this provide evident that ISSR and SSR markers provide the basic information necessary to conserve materials from different genetic background.

Moreover, the analysis of clustering based on ISSR and SSR data suggest the interrelation of fig cultivars despite their phenotypic difference and suggest the ungrouping of fig cultivars have same denomination in spite of their phenotypic similarity. These suggestion of homonymies cases indicate the need to establish a reference collection well-characterized with molecular tools and clarify the limitation of traditional method that based on morphology in discriminating fig cultivars.

The variability that was obtained by local Palestinian fig cultivars indicates low similarities between Palestinian fig cultivars, however, and while low similarities are also present among Palestinian fig cultivars, a further study including more foreign fig genotypes is required to find out more about their relatedness or uniqueness.

The study demonstrates variability and genetic diversity obtained among Palestinian fig population which might be caused by the fact that figs have been cultivated for a long time in the Mediterranean Basin, resulting in a high number of cultivars developed by empirical selection.

Low correlation between the ISSR marker and SSR marker suggesting that one marker cannot be used as alternative to another marker, both ISSR and SSR markers may be combined in the assessment of fig genetic diversity for use in fig breeding and conservation.

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