An-Najah National University

Faculty of Graduate Studies

Genetic diversity among Palestinian fig varieties (*Ficus carica* L.) using ISSR, and RAPD markers

By

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Dedication

To best parents anyone can have ever, my father and my mother, the best friends ever my brothers and my sister and to my little sweet Shama.....

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I wish to express my deepest gratitude to my supervisor Prof. Dr. Mohammed Saleem Ali-Shtayeh, for his encouragement to start and complete this work and for the opportunity to be a member of the inspiring research group. His endless support has been vital and precious during these years.

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My beloved father and mother I could not imagine any kind of expressions I can say here, for all my life you were there for me in all types of easing and support. MAY ALLAH BLESS YOU FOR EVER.

My brothers and my sister best friends I can have ever for endless support and encouragement.

For last my little lovely energetic Shama, having you was a major bless in my life and my motive to go on .....

اقرار

انا الموقعة ادناه مقدمة الرسالة التي تحمل العنوان :

Genetic diversity among Palestinian fig varieties (*Ficus carica* L.) using ISSR, and RAPD markers

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Declaration

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List of Abbreviations

-DNA: Deoxyribose Nucleic Acid

-PCR: Polymorpism Chain Reaction

-RAPD: Random Amplification of polymorphic DNA

-ISSR: Inter Simple Sequence Repeats

-SDS: Sodium Dodecyl Sulfate

-EDTA: Ethylenediamine Tetraacitic Acid

-TAE: Tris / acetate/ EDTA

-BME:Beta Mercapto Ethanol

- UPGMA **:**Unweighted Pair Group Method with Arithmetic Mean

-BERC: Biodiversity and Environmental Research Center

-PA: PalestinianAuthority

-m: Meter

-M: Molar

-Rp: resolving power

-Ib: band informativeness

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Abstract

The genetic diversity in Palestinian fig (*Ficus carica* L.), was studied using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. Twenty three fig accessions (20 common fig cultivars, and two more wild types and one San pedro type (Dafor) crossponding to the main cultivated varieties in Palestine were analyzed. The cultivars were assessed using the randomly amplified polymorphic DNA (RAPD) technique. The 21 out of 25 screened markers showed reproducible polymorphic profiles. The generated 1518 data entries were analyzed among which 420 entries were for present bands and 1098 for absent bands. After determining Jaccard similarity index, some genotypes showed high genetic similarity (77%) was recorded between Zraqi and Ghzali, while other were less similar (0% between Ajloni and Qraee). Moreover, the primers were evaluated for their resolving power, where primer OPH08 achieved the highest power 3.16 meanwhile the weakest power was shown by primer OPA03 (0.15).

Furthermore , dendrogram was elaborated by cluster analysis according to the UPGMA algorithm . The genotypes were clustered into seven clades .

The mean number of amplification RAPD bands (6.7) was little more than that of ISSR (5.57). Moreover, the total number of polymorphic bands (161) detected by RAPD primers was much higher than that of the ISSR primers (69), which suggested that the RAPD markers were more powerful compared to ISSR markers in the capacity of revealing more informative bands in a single amplification.

CHAPTER ONE

Introduction

Common fig (Ficus carica L.) is considered as one of the oldest cultivated fruit tree in the east of the Mediterranean regions (Turkey, Syria, Saudi Arabia) from which it expanded for all Mediterranean regions. The first fig cultivation was recorded in Arabia and Egypt ~1600 B.C, and records from ~1250 A.D indicate commercial fig trade was already established (Ferguson et al., 1990 .)

The tree was mentioned in all Holy books as a blessed sacred tree and it was Prophet Mohammad (peace be upon him) who said "If I should wish a fruit brought to paradise, it would be certainly fig ". The tree is mentioned in folkloric songs, sayings and stories and it has deep relation with healthy food in our heritage (Shtayeh et al., 1991).

1.1 Natural History of the Fig Tree

The Ficus carica, which produces the common fig, is a tree belonging to Order Urticaceae, the Moraceae family, which includes also the banyan, the Indian rubber fig-tree, the sycamore fig and other useful plants. Wild fig trees usually rather shrubs than trees, occur also everywhere; they are usually barren and are described as "male" trees. Fig trees are usually of medium height, 3 –9m for full-grown trees, The summer foliage is thick and surpasses other trees of its size in its cool and dense shade. (Shtayeh et al., 1991)

In Palestine, fig trees are grown all over the country and mostly located on the marginal lands, in mixture with other fruit trees (mainly olive and

grapes), or scattered at the periphery of orchards, and in home gardens. The importance of the fig tree lies in the fact that it can withstand severe climatic conditions in arid and semi arid regions with a low rainfall resulting in low soil humidity, high temperature and high soil calcium carbonate content (Shtayeh et al., 1991). Figs are deciduous subtropical trees whose growth is more limited by winter low temperatures than by summer heat. The typical fig-producing regions are characterized by hot dry summers, low relative humidity, and mild winters. The fig tree has a low chilling requirement. Winter temperatures are a limiting factor particularly with young trees that may be damaged by frosts at temperatures between -10 and 50 oC (Ferguson et al., 1990).

Horticultural requirements for fig production have been described by many authors and were summarized by Ferguson et al. (1990).

1.2Fig Leaf

Ficus carica is well 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. The sap of the green parts of the tree is considered as irritant to human skin, although it has some traditional uses as tendering tough meat tissues prior or during cooking, and as a local treatment of skin warts and (Flaishman et al., 2008; Shtayeh et al., 1990).

1.3Fig Fruit

The fruit of the fig-tree is peculiar. The floral axis, instead of expanding outward, as with most flowers, closes, as the flower develops, upon the small internal flowers, leaving finally but a small opening at the apex; the axis itself becomes succulent and fruit-like. The male flowers lie around the opening, the female flowers deeper in; fertilization is brought about by the presence of small hymenopterans insects (Crane, 1985.)

The fig is an unusual tree as it may produce multiple crops of fruits each year, The breba crop, which is not produced in all cultivar, is borne laterally on the growth of the previous season from buds produced in leaf axils. These buds develop in the following spring, and the fruit matures between June and July. The main crop of figs is produced laterally in the axils of leaves on shoots of the current season. Fruit maturation starts at July and may last until temperature drops between October and December (Flaishman et al., 2008). At the end of the growth period, the leaves fall and the tree enters the dormancy period. Reproductive buds that do not produce fruit during the growing season remain dormant over the winter to give rise to the first spring breba crop. In some cultivars and in appropriate environments, largely developed main crop figs may remain on the tree over the winter and complete development in early spring (Flaishman et al., 2008).

1.4 Fig Varieties

Four types of fig are described based on cropping and pollination characteristics. The type known as common fig (e.g., brown turkey, Mission, and Adriatic), requires no pollination to set a commercial crop. These types are refered to as "persistent" rather than parthhenocapric since fig is not a true fruit. The allele for persistence is dominant but is lethal in the ovule, and can be only conferred by the pollen parent (Saleeb & Sorty, 1975).The flowers in the common fig are all long-styled pistillate flowers and need no pollination for continued growth and maturity. Common fig produces one to two crops each year. Pollination (called caprication in fig) common-type figs sometimes markedly increases fig size, changes the color of both skin and pulp, increases the tendency to split, and enhances fruit taste (Condit, 1947).The other two types of edible figs require pollination by the wasp to set the main crop of figs.

Botanically, these non-persistent types are classified as " caduceus" and are classified as Smyrna types (e.g., Sarilop, Marbout, Zidi, and San Pedro types (e.g., Dauphine, King and San Pedro). The San Pedro types are distinguished by setting a persistent early crop, known as breba fruit, but require caprification to set the main crop.This unique combination in which the same branch persistent and non-persistent fruits develop in the same season. While San Pedro types are in part defined by the setting of breba crop, some common figs also produce brebas. (Flaishman et al., 2008) .

The fourth type serves as a source of pollen for commercial plantings of the cauducous types and known as caprifig. The caprifig is generally termed male or goat fig, reflecting lack of value as human food and with a few exceptions, is inedible. However the caprifig is not only male and the syconium usually contains both staminate and short styled pistillate flowers. The staminate flowers are located in a limited area surrounding the ostiole, while the short-styled pistillate flowers occupy most of the interior surface of the syconium. The short-style pistillate flowers are adapted to ovipostion by symbiotic fig wasp Blastophaga psense, which has coevolved with the fig (Kjellberg et al., 1987).

In Palestine more than 50 varieties of fig were identified (Shtayeh et al.,1991) , with unique characters for each which make it suitable for a purpose but not another. Briefly the most common varieties (which are growing in the BERC-Til Botanic Gardens) are classified according to their fruit colors and origin as follows:

A.Green or yellow varieties, e.g.: Khdari, Biadi, Shunari –abied, Mowazi , Ajloni , Na'emi, Kbari, Klaibi and Sfari , Slati.

B.Greenish violet varieties, e.g., Khortmani, Enaqi, Hmadi and Ghzali

C.Blakish violet to black colored varieties, e.g, Kharobi , Swadi, Odicy and Zraqi (Shtayeh et al ., 1991).

1.5Nutrition

The Food and Agriculture Organization, FAO (2009) estimates that figs are harvested from 427,000 hectares worldwide, producing yearly over I million metric tons of figs around the world. The Mediterranean, around which most of the fig growing countries are located, has been the most important region of fig production from time immemorial (Aljane et al., 2008), representing more than 82 % of the total world annual production (FAO, 2009). According to the data base of FAO, about 30% of the crop is produced by Turkey (280,000 Ton). Other major producers in descending order are Egypt (190,000 T), Greece (80,000 T), Morocco (67,000 T), Spain (61,000 T), Algeria (60,000 T), USA (45,000 T), Syria (43,000 T), Italy (18,000 T), Tunisia (13,000 T), Lebanon (9,000 T), Palestine (7,500T) and Jordan (3,600 T) (FAO, 2009).

Nowadays global awareness of fig nutritional value is increased and can be considered as one of the (functional food group) which refers to food that is in virtue of physiological active components provide benefits beyond basic nutrition and may prevent disease or promote health. Fig is fat free, sodium free, and like other plant foods cholesterol free (Vinson,1999). The fruits have a very high sugar content accompanied by high levels of protein, fat, mineral content and vitamins, thus resulting in a high nutritive value. Its high fiber content helps in the digestive system (Shtayeh et al., 1991). Fig provides more fiber than all of the common fruits, in both soluble and insoluble forms which help in decreasing cholesterol and

lowering blood sugar. Recent studies shows that soluble forms of fibers which is high in fig can control weight loss, which make it an excellent additive for kids food as it fibrous and naturally sweet (Vinson,1999) .

Fig also contains several medical components such as flavone and rutin. The plant has anticancer properties and quercetin, which can be used in cardiovascular disease medicine production (Duke and Ayensu, 1985). Fig fruits are transformed into several processing products, such as dried fig, preserved fruits, jam, juice, wine, powder, and the others, but most popular ones are preserved fruits and jam (Shtayeh et al., 1991) .

1.6Methods of Characterization

The fig trees grown in Palestine especially in the north and middle parts of the country where wild fig types are abundant with high morphological diversity particularly in fruit color. The edible fig is a currently widespread species with a large number of local cultivators whose identity is poorly studied; however plant characterization is an important aspect because the quality of the crop is depending on the varieties. Fig varieties names were mainly given based on skin ground color, internal color, and maturity date (Aljane & Ferchechi, 2009, Basheer-Salimia et al.,2013). In such conditions, a large number of homonymous and synonymous designations and the occurrence of misnamed genotypes exist. However, these genotypes have not yet been investigated and their identity is unknown. At present there are modest

morphological and genomic studies on fig, consequently reliable techniques are required to verify the distinctiveness of these cultivators and clones that constitute a germplasm resource that is unique in the world (De Masi et al., 2003).

Fig trees are locally grown in sites with contrasting climates and soils. However, for several decades, the cultivated area has significantly decreased due to: first, the fig tree is a marginal fruit crop and it is vulnerable to biotic and abiotic stresses; second, the reduction of the number of genotypes selected and maintained since ancient time; and third, the intensive urbanization. As a consequence of these trends, severe genetic erosion is threatening the local germplasm. Moreover, the actual number of cultivars is at present difficult to estimate since synonymy constitutes error sources in cultivar identification (Shtayeh et al., 1991; Basheer- Slaimia et al.2013). Hence, it is imperative to establish a research program aiming at the preservation and the evaluation of the Palestinian germplasm. It is also a crucial necessity for discrimination between these landraces for conservation of plant genetic resources and improvement purposes (Sadder & Ateyyeh, 2006) .

Prospecting and collecting actions have been initiated in Palestine and led to the identification of more than 40 ecotypes (Shtayeh et al., 1991). Some of these are ex situ maintained in a collection at the Biodiversity and Environmental Research Center (BERC), Til, Nablus. On the other hand, data based on the use of morphological traits, particularly those concerning

the fruits, have been reported and involved the main cultivars that are locally predominant (Shtayeh et al., 1991). However, these characters are either sensitive to the environmental conditions or limited to the fruit production season .

On the other hand, surveys of molecular diversity of fig genotypes in Palestine are scarce (Basher-Salimia et al ., 2013). Such studies are imperative to survey the genetic diversity and to have a deeper insight of the genetic organization in this germplasm. This could be made possible throughout the analysis of the polymorphisms in fig accessions preserved in the fig collection at BERC-Til Botanic Gardens, Til, Nablus. Various molecular markers have been successfully designed and used widely to identify various fig cultivars, landraces, as well as population diversity and relatedness. Among these markers, Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR).

1.7Characterization using RAPD and ISSR

One of the most widely tested techniques seems to be the random amplified polymorphic DNA (RAPD) method of Williams et al. (1990). Due to its high sensitivity, this procedure constitutes a powerful method to distinguish genotypic variants (Santoni et al., 2000; Trifi et al., 2000; Amadou et al., 2001; Al-Khalifa & Askari, 2003; Khadari et al., 2003; Rajora et al., 2003). This method is suitable for determining genetic diversity, ge¬notyping, phylogenetic analysis, genetic relationships, etc. RAPD, among other

molecular marker methods, has considerable advantages because it is fast and inexpensive and because the development of RAPD markers does not require prior knowledge of the genome sequence. These markers have been widely used in the molecular analysis of figs (Elisiario et al., 1998; Galderisi et al., 1999; Cabrita et al., 2001; Papadopoulou et al., 2002; Aka-Kaçar et al., 2003; De Masi et al., 2003; Khadari et al., 1995, 2003; Sadder & Ateyyeh, 2006).

Inter Simple Sequence Repeats (ISSRs) are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18 bp). About 10-60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size. The main advantage of ISSRs is that no sequence data for primer construction are needed. Because the analytical procedures include PCR, only low quantities of template DNA are required. Furthermore, ISSRs are randomly distributed throughout the genome. Because of the multilocus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species. In addition, ISSRs are considered useful in gene mapping studies (Godwin et al., 1997; Zietkiewicz et al., 1994; Chatti et al., 2010).

1.8Aim of the Study

Plant identification and estimation of their relationships and diversity are traditionally established on the basis of morphological and agronomic characteristics. Since there are substantial intra-species variations in vegetative traits, it is difficult to differentiate genotypes only on the basis of their external structure (phenotype), especially for leaf and fruit characters, because these may vary according to development conditions. This could lead to misidentification; consequently the lack of a way for safely assessing genetic relationships makes it necessary to use stable markers .

Therefore, the aim of this study was to produce suitable markers and to assess the molecular polymorphism in a set of Palestinian fig cultivars from BERC-BGs fig collection originated from diverse environments of the West Bank in the PA, using RAPD and ISSR. In addition, the study aims to investigate the genetic relationships among the sampled cultivars

CHAPTER TWO

Materials and Methods

2.1 Plant Material:

Young leaf samples from 23 fig accessions preserved at the fig collection at BERC–Botanic gardens in Til village south west Nablus were collected in this study. Among these samples 20 were common fig genotypes corresponding to the main cultivated varieties in Palestine, two caprifig varieties from different sites (Til and Nablus), and one San Pedro type (dafour)accession. The collected samples were classified according to their fruit skin color into three main categories; green or yellow, black or purple brass- colored violet and green bronze or brass -colored or light purple (Table 2.1)

Healthy green young leaf samples were collected in duplicates from adult trees of each variety in April and May .they were kept chilled during transportation then stored frozen for DNA analysis.

2.2 Characters Used in Determining the Classification of Fig Varieties:

Plant identification is traditionally established on the basis of morphological characteristics as it shown on table (2.1) and examples in figures (2.1)and (2.2).

Table 2.1 : Palestinian fig varieties grown at BERC-Til Botanic gardens and their morphological discription

Vaiety name	Arabic common name	Accession number	Skin color	Fruit size	Bulb color	Flavor	Tree size	Shape of the fruit	Leaves shape	Leaves size (length*width) (mm)
black or purple brass- colored violet										
Ghzali, GH	غزالي	BERC-BG-F.carica -GH-1	Violet	(40-46)*(35-38)	Brownish	Semi sweet	Very large	Pear shaped	3 loops	(154-213)*(146-189)
Kharobi, KHR	خروبي	BERC-BG-F.carica -KHR-1	Dark purple	(33-40)*(33-41)	Deep red	Very sweet	Moderate	Globose	---	(171-209)*(155-109)
Qaisi, QI	قيسي	BERC-BG-F.carica -QI-1	Dark purple	(38-40)*(37-39)	Pale pink	Sour	Moderate	Globose	3 loops	(164-193)*(177-195)
Swadi, S	سوادي	BERC-BG-F.carica -S-1	Dark purple	(37-42)*(37-41)	Bright red	Slightly sweet	Moderate	Globose	5 deep loops	(183-117)*(174-208)
Zraqi, Z	زراقي	BERC-BG-F.carica -Z-1	Black	(32-40)*(34-44)	Dark red	Semi sweet	Moderate	Wide globose	3-5loops	(186-223)*(166-200)
green or yellow										
Ajloni, AG	عجلوني	BERC-BG-F.carica -AG1	Light green	(28)*(38)	Honey	Sweet	Moderate	Oblong	---	(106-127)*(130-136)
Blati, BL	بلاطي	BERC-BG-F.carica -BL-1	Yellow green	(47-52)*(39-47)	Light pink	Semi sweet	large	Oblong	--	--
Borqawi, BR	برقاوي	BERC-BG-F.carica -BR-1	Green	---	Reddish	Sweet	Large	Globose	---	----
Hmadi Biadi, HB	حماضي-بياضي	BERC-BG-F.carica -HB-1	Yellow green	(35-40)*(37-40)	Pink to red	Sweet and sour	Moderate	Globose	5 loops	(184-220)*(174-98)
Khdari, KHD	خضاري	BERC-BG-F.carica -KHD-1	Yellow green	(30-39)*(28-40)	Pink to red	Sweet	Large	Globose	3-5 loops	(114-119)*(68-96)
Mwazi, MO	موازي	BERC-BG-F.carica -MO-1	Yellow green	(33-39)*(48-51)	Red	Sour to sweet	High	Oblonge	5 loops	(137-160)*(26-140)
Neami, NEA	نعيمي	BERC-BG-F.carica -NEA-1	Yellow green	35*35	Red	Very sweet	Moderate	Oblate	Deaply looped	Large
Qraee, Q	قراعي	BERC-BG-F.carica -Q -1	Yellow green	(47-52)*(39-47)	Red	Very sweet	Large	Oblong	3-5 loops	(163-200)*(132-189)
Sfari, SF	صفاري	BERC-BG-F.carica -SF-1	Yellow		Pink	Sweet	Moderate	Pear shaped	Barly looped	---
green bronze or brass colored or light purple										
Adloni, AD	عدلوني	BERC-BG-F.carica -AD-1	--------	---------	--------	----------	----------	----------	---------	---------
Hmadi, H	حماضي	BERC-BG-F.carica -H-1	Purple green	(38-48)*(36-43)	Browny	Sweet - sour	Large	Globose	3-5 loops	(147-179)*(114-144)
Hmari, HA	حماري	BERC-BG-F.carica -HA-1	---------	---------	--------	---------	----------	----------	---------	--------
I'naqi, AN	عناقي	BERC-BG-F.carica -AN-1	Green purple	(44-46)*(36-48)	Browny	Sweet	Moderate	Oblong	3- 5 loops	--------
Khurtmani,KH	خرطماني	BERC-BG-F.carica -KH -1	Green purple	(38-48)*(23*41)	Browny	Very sweet	Large	Oblong	3 loops	(143-173)*(151-176)
Qrawi, KR	قراوي	BERC-BG-F.carica -KR-1	--------	----------	----------	--------	--------	----------	---------	----------

Variety name	Arabic common name	Accession number	Skin color	Fruit size	Bulb color	Flavor	Tree size	Shape of the fruit	Leaves shape	Leaves size (length*width) (mm)
Others										
Dafour, DA	دافور	**BERC-BG-F.carica -DA-1**	-------	-------	-------	-------	-------	-------	-------	-------
Wild type	بري	**NABLUS -F.carica –A**	-------	-------	------	------	------	------	------	------
Wild type	بري	**NABLUS -F.carica-- B**	-------	-------	-------	-------	-------	-------	-------	------

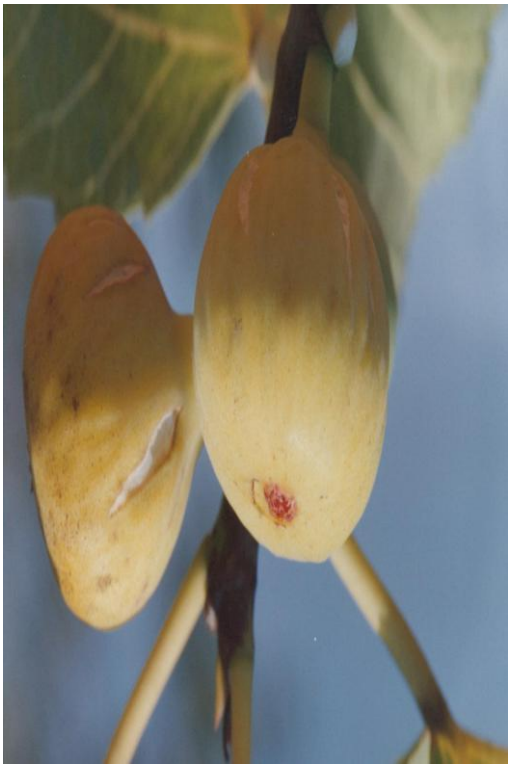
AN1

AN3

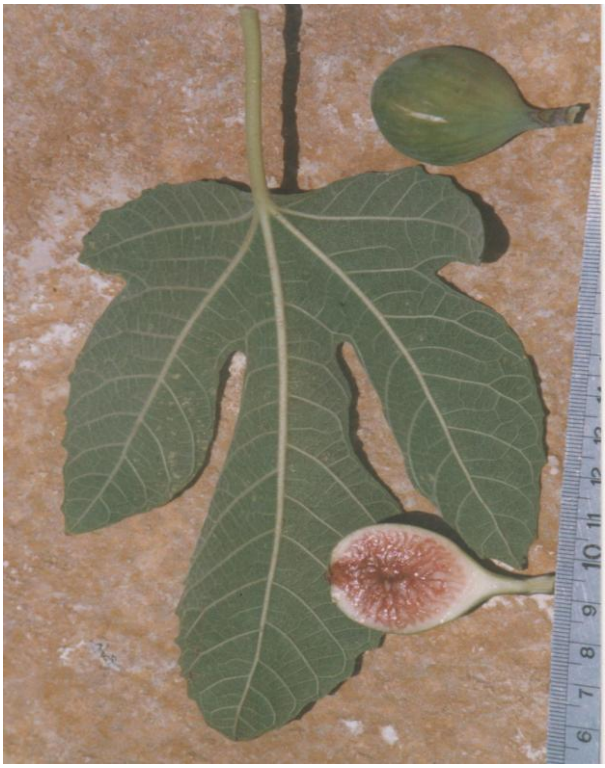
QI1

QI3

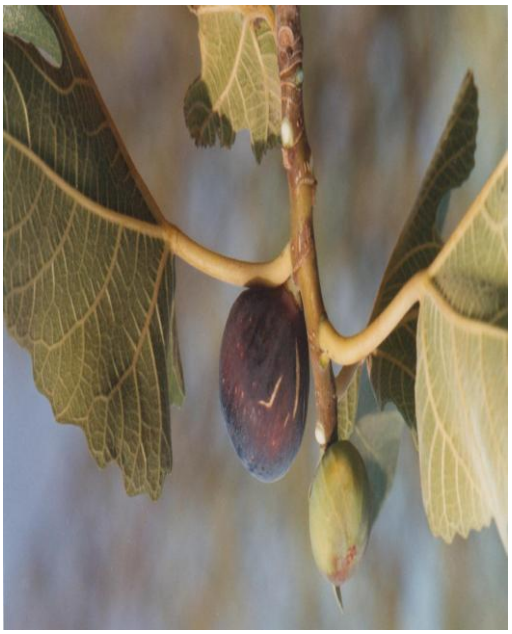
Figure 2.1 : Some characters used for determining the classification of fig varieties. AN1: I'naqi Fruit skin colour , AN3: I'naqi leaf , QI1: Qaisi fruit colour , QI3:Qaisi leaf



SF1



SF2 & 3



KHR1

KHR2

Figure 2.2 :Example (2): Characters used for determining the classification of fig varieties: SF1: Sfari fruit colour, SF2&3: Sfari bulb colour and leaf, KHR1: Kharobi fruit skin colour, KH2: Kharobi bulb colour

2.3 DNA Extraction:

Genomic DNA was extracted from powdered preparation (ground in liquid nitrogen) acoording to modified Dellaporta method described by (Lin *et al.*, 2001).

Leaf material (100mg) were transferred to1.5 ml microfuge tube with, a 500 µl of dellaporta extraction buffer (100 mM Tris-Hcl, pH=8, 50mM EDTA, 500mM NaCl, 10 mM BME) and 33 µl of 20 % SDS The tube was mixed well, and incubated 10 minutes at 65 ° C with shaking, 160 µl of 5 M of KAc (potassium acetate) was added and the mixture was mixed and centrifuged for 10 minutes at 10,000 x g.The supernatant was collected and transferred to new tube.

-Two µl of RNAase were added and the mixture was incubated for 10 minutes at 37°C. 500 µl of phenol / chlorophorm / isomethyl alcohol (25: 24:1) was added to the supernatant, the mixture was mixed and centrifuged for 10 minutes at 10,000 x g.

The supernatant was transferred to 1.5 new tube, and 2 volume of cold ethanol were added before incubated for 20 minutes at -20°C. The tube was centrifuged for 10 minutes at 10,000 x g, the pellet was washed with 500 µl 70% ethanol, centrifuged for 10 minutes at 10,000 x g rpm and the supernatant was discarded. The palate was air dried ,the DNA was resuspended in 40 µl sterile distilled deionized H2O.

The quantity and the quality of DNA samples were analysed by running 1 µl of DNA sample on 1% TAE gel.

2.4 Molecular Marker Analysis

2.4.1 Primers and PCR Assays:

Twenty five universal oligonucleotides purchased from Operon technologies were used to perform RAPD, and 12 primers that are complementary to microsatellites motives and are arbitrary defined were used in order to perform ISSR (Chatti et al.,2010) (Table 2.3).

Table 2.3:Primers used for detection of molecular polymorphism in Palestinian fig cultivars (chatti *et al.*, 2010)

Method	Primer	Sequence 5'- 3'	Temperature ^{o}C
RAPD	**OPH02**	TCGGACGTGA	35
	OPT10	CCTTCGGAAG	35
	OPT20	GACCAATGCC	35
	OPA01	CAGGCCCTTC	35
	OPA02	TGCCGAGCTG	35
	OPA05	AGGGGTCTTG	35
	OPA11	CAATCGCCGT	35
	OPA16	AGCCAGCGAA	35
	OPA18	AGGTGACCGT	35
	OPA03	AGTCAGCCAC	35
	OPA04	AATCGGGCTG	35
	OPA07	GAAACGGGTG	35
	OPA08	GTGACGTAGG	35
	OPA09	GGGTAACGCC	35
	OPA10	GTGATCGCAG	35
	OPA12	TCGGCGATAG	35
	OPA13	CAGCACCCAC	35
	OPA14	TCTGTGCTGG	35
	OPA15	TTCCGAACCC	35
	OPA17	GACCGCTTGT	35
	OPA19	CAAACGTCGG	35
	OPA20	GTTGCGATCC	35
	OPH05	AGTCGTCCCC	35
	OPH08	GAAACACCCC	35
	OPH12	ACGCGCATGT	35
	OPH16	TCTCAGCTGG	35
ISSR	**UBC807**	AGAGAGAGAGAGAGAGT	45
	UBC808	AGAGAGAGAGAGAGAGC	49
	UBC810	GAGAGAGAGAGAGAGAT	45
	UBC811	GAGAGAGAGAGAGAGAC	49
	UBC812	GAGAGAGAGAGAGAGAA	45
	UBC814	CTCTCTCTCTCTCTCTA	45
	UBC815	CTCTCTCTCTCTCTCTG	49
	UBC816	CACACACACACACACAT	45
	UBC817	CACACACACACACACAA	45
	UBC818	CACACACACACACACAG	49
	UBC823	TCTCTCTCTCTCTCTCC	49
	UBC861	ACCACCACCACCACCACC	57

2.4.2 DNA Amplification and Agarose Gel Electrophoresis

2.4.2.1 RAPD PCR

The RAPD marker is powerful to differentiate even between clones of the same cultivar (Papadopoulou *et al.*, 2002; Sadder & Ateyyeh, 2006), and specially for fig tree, it was demonstrated to be applicable for genotype identification in various gene pool, e. g., Tunesia (Zehdi *et al.*, 2004) Greece (Papadopoulou *et al.*, 2002), Jordan (Sadder & Ateyyeh, 2006), Italy (Galderisi *et al.*, 1999; De Masi *et al.*, 2003) and in Turkey (Cabrita *et al.*, 2001) and Japan (Ikegami *et al.*, 2009).

In RAPD analysis the polymorphisms of cultivars are a direct consequence of the differences existing at genomic DNA level. Since RAPD markers are transmitted by inheritance because they are associated with DNA (Luo *et al.*, 2002), they represent a better model than those based on phenotypic determinations.

RAPD PCR reactions were performed as described by Hanachi *et al.*, (2006) in a total reaction mixture of 25 µl containing:

1 µl DNA template, 2 µl of 20 mM of MgSO4, 2.5 µl of 10 X Taq reaction buffer (Hylabs), 0.9 µl of 10 mM of dNTP Mix (GeneDirex), 0.3 µl of 1 U of Taq DNA polymerase (Hylabs) and 1.5 µl of 10 µM of primers.

A negative control with the reaction mixture excluding DNA was also included in each experiment. DNA was amplified using polymerase chain

reaction (PCR) in a Gene Amp thermocycler (Gene Amp PCR system 9700, applied biosystems) machine with heated led, the PCR program was, 1 cycle at 94°C for 5 min; followed by 40 cycles of: 94°C for 1 min, 35°C (1 min), and 72°C (1 min); then a final 5-min extension at 72°C, and cooling down to 4°C.

2.4.2.2 ISSR PCR

ISSR PCR reactions were performed as described by Zehdi *et al.*, (2004), in a total reaction mixture of 25 µl containing:1 µl DNA template, 2.5 µl of 20 mM of MgSO4, 2.5 µl of 10 X Taq reaction buffer (Hylabs), 1.5 µl of 10 mM of dNTP Mix (GeneDirex), 0.3 µl of 1 U of Taq DNA polymerase (Hylabs) and 1.5 µl of 10 µM of primers. A negative control with the reaction mixture excluding DNA was also included in each experiment. The ISSR amplifications were performed in a Gene Amp thermocycler (Gene Amp PCR system 9700, applied biosystems) with the following conditions: 1 cycle of (5 min at 94 °C) followed by 35 cycles of (30 sec at 94 °C for denaturation, Annealing for 1 min, elongation for 2 min at 72 °C); followed by a final 5-min extension at 72°C, and then cooling down to 4°C.

PCR products (25 µl) were separated by electrophoresis on 1.5% (w/v) agarose gel at 120 V for 60 minutes in 1X TAE buffer (See Appendix A for recopies)Tris acetic acid, Na2 EDTA (pH 8)). DNA ladder marker (GeneDirex) was used as marker. DNA fragments were stained with

ethidium bromide (Sigma), and generated bands were screened and digitally photographed using Nikon camera (D5000) under UV light (Tl-2000 Ultraviolet translinker, UVP, USA). All reactions were repeated twice and only bands that were bright and reproducible were scored for analysis (Zehdi *et al*.,2004).

2.4.3 Bands Scoring and Statistical Analysis

Amplification products were scored as either present (1) or absent (0). To compare the efficiency of primers in identifying different fig genotypes, the resolving power (Rp) and band informativness (Ib) were calculated. Band informativness is a measure of closeness of a band to be present in 50% of the genotypes, resolving power (Rp) is the sum of Ib values of all the bands amplified by a primer (Guasmi *et al*., 2010). Ib and Rp were calculated as reported by Prevost and Wilkinson (1999):

Band informtivness of a given band (Ib)= $1-(2\times|0.5-P|)$ where P is the proportion of the total genotypes containing the band.

Resolving power of a primer is the sum of band informativnes for the same primer.

A data matrix was prepared to determine Jaccard's similarity indices using the software package SPSS version 16. Similarity index is defined as: [similarity index = (number of electrophoretic bands in common)/(number of bands not in common + number of bands in common)] (Sadar *et al*.,

2006).The Jaccard similarity were further subjected to dendro UPGMA program to construct trees using un-weighted pair group method with arithmetic mean (UPGMA).

CHAPTER THREE

Results and Discussion

3 .1 Molecular DNA markers

To assess the genetic variability among studied common fig genotypes, RAPD and ISSR analysis were applied. Initially 25 different RAPD and 12 ISSR short oligo primers were screened. All markers yielded reproducible and clear polymorphic band profiles after running in agarose gel. Amplified DNA fragments ranged between 300 and 2000 bp in size for all tested RAPD and ISSR primers.

3.2 Data Analysis and Interpretation

3.2.1 RAPD PCR Analysis of Fig Varieties

RAPD –PCR was used in this study to determine the genetic relatedness of 23 fig varieties to one another and their relatedness to the wild type variety.

In general different banding patterns were reported in most fig varieties in this study. Ajloni (AG) cultivar produced the lowest number of bands with all primers (29 bands), while Qrawi (KR) cultivar produced the highest number of bands (71 bands) .

Some primers didn't produce any band in some varieties, as in primers (OPA01,OPA05, OPA11), where no bands produced among Ajloni (AG) and Khdari (KHD) varieties (Figures 3.1-3.3) and in primer (OPA01), Qraee (Q) variety did not produce any band (Figure 3.1).

Total 1518 data entries were analyzed, among which 420 data entries were for present bands (1) and 1098 for absent bands (0). Different number of bands were scored for each primer out of total number of entries for this primer, e. g., primer (OPH12) 125 present bands were scored out of 252 entries, in primer (OPH08)73 present bands were scored out of 138 entries. (Tables 3.1-3.5).

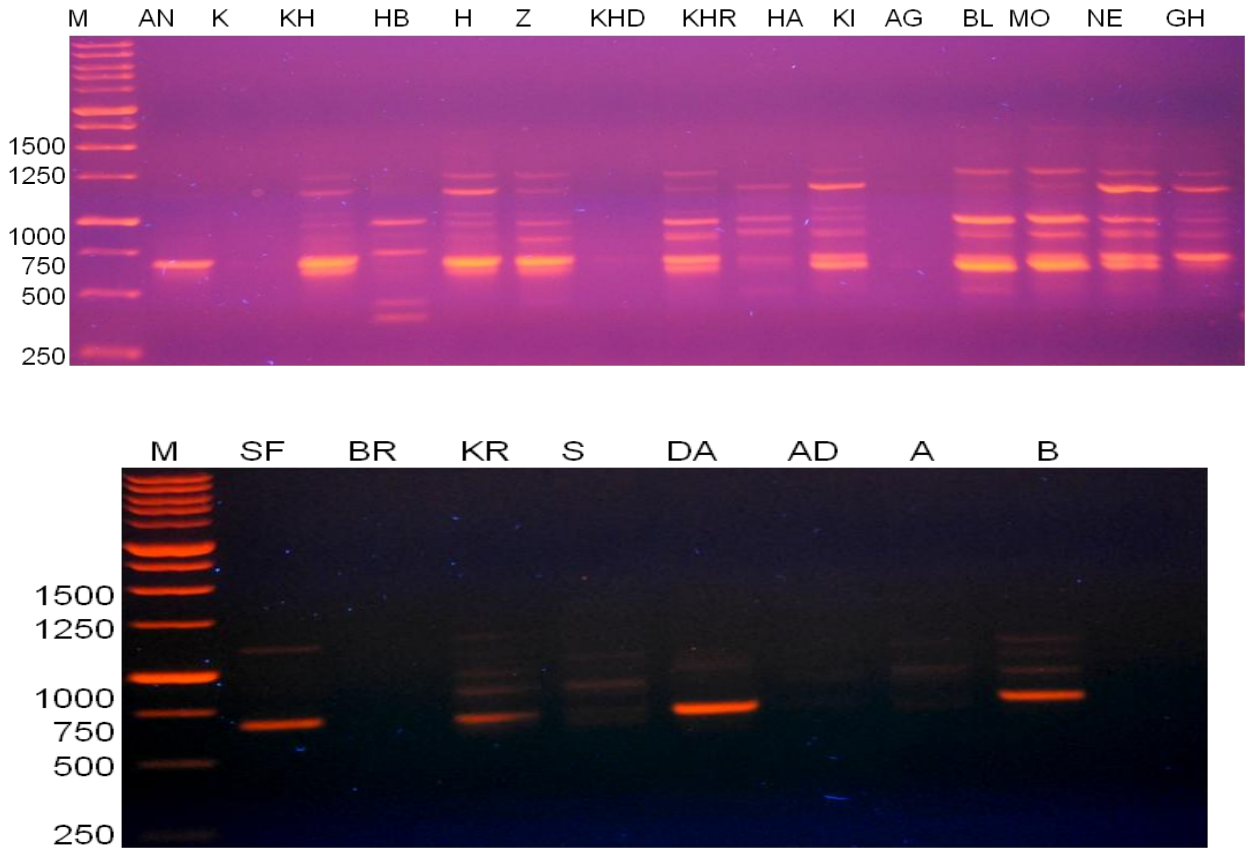


Figure 3.1 : A RAPD Pattern of RAPD primer (OPA01) with all fig genotypes AN:I'naqi,Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, ,NEA:Neami,GH:Ghzali,SF:Sfari,BR:Barqai, KR:krawi, S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari KHR . :Kharobi, HA:Hmari ,QI:Qaisi ,AG:agloni ,BL:Blati,MO:Mwazi A &B:Wild type fig.

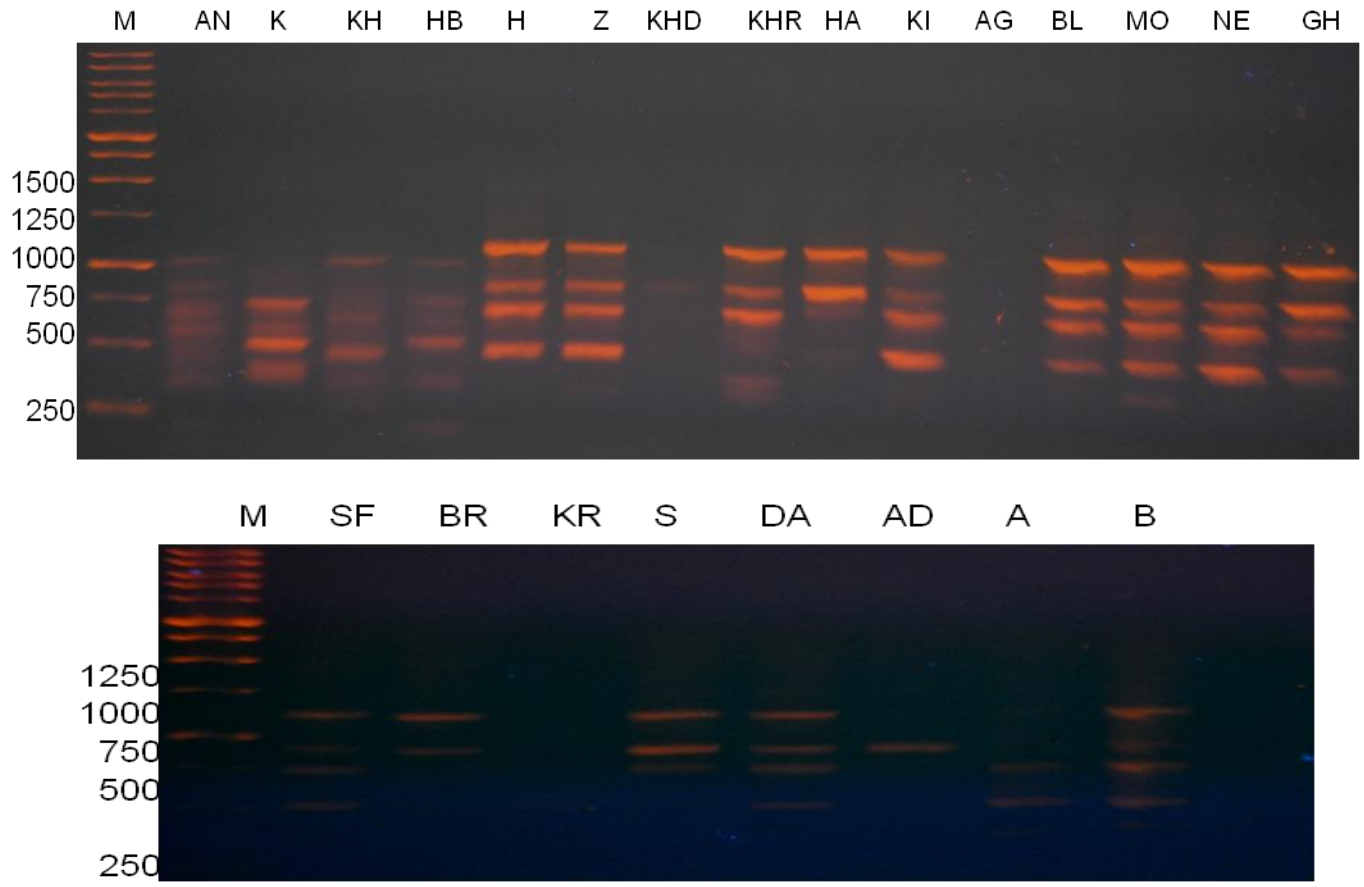


Figure 3.2 : A RAPD Pattern of RAPD primer (OPA05) with all fig genotypes AN:I'naqi,Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, ,NEA:Neami,GH:Ghzali,SF:Sfari,BR:Barqai, KR:krawi, S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari KHR . :Kharobi, HA:Hmari ,QI:Qaisi ,AG:agloni ,BL:Blati,MO:Mwazi A &B:Wild type fig.

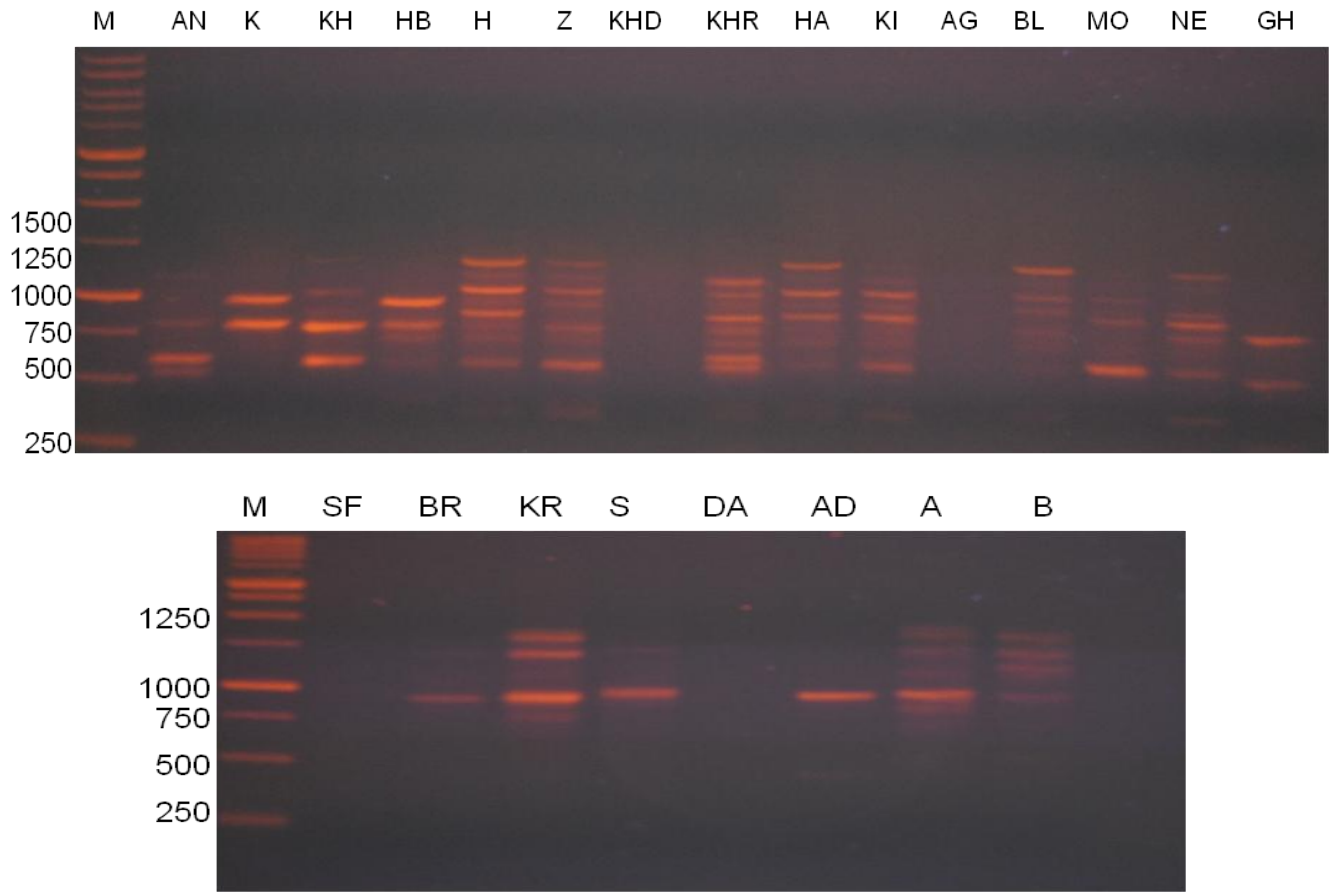


Figure 3.3 : A RAPD Pattern of RAPD primer (OPA11) with all fig genotypes AN:I'naqi,Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, ,NEA:Neami,GH:Ghzali,SF:Sfari,BR:Barqai, KR:krawi, S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari KHR . :Kharobi, HA:Hmari ,QI:Qaisi ,AG:agloni ,BL:Blati,MO:Mwazi A &B:Wild type fig.

Table 3.1: Amplified DNA fragments using RAPD (OPH12) primer AN:I'naqi,Q:Qraee, KH:Khurtmani,, HB:HmadiBiadi,H:Hmadi,Z:Zraqi,NEA:Neami,GH:Ghzali,SF:Sfari,BR:Barqai,KR:krawiS:swadi,DA:Dafor,A D:Adloni,KHD:Khdari, KHR:Kharobi,HA:HmariQI:Qaisi,AG:agloni,BL:Blati,MO:Mwazi A &B:Wild type fig.

Primer (OPH12)	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A (N)	B (T)
3000	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
2000	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**1**
1800	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**1**	**1**
1500	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**1**	**0**	**1**	**1**
1300	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1100	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1000	**0**	**0**	**1**	**1**	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**0**	**1**	**0**	**0**
750	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**0**	**1**	**1**
700	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
400	**0**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
300	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table 3.2: Amplified DNA fragments using RAPD (OPH12) primer AN:I'naqi,Q:Qraee, KH:Khurtmani,, HB:HmadiBiadi,H:Hmadi,Z:Zraqi, NEA:Neami, GH:Ghzali SF:Sfari,BR:Barqai, KR:krawi S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,H:Hmari,QI:Qaisi,AG:agloni,BL:Blati,MO:Mwazi A &B:Wild type fig.

Primer (OPH08)	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A (N)	B (T)
1500	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0
1100	1	0	1	0	1	1	0	1	1	1	0	0	0	1	1	0	1	1	1	1	0	1	1
1000	0	1	0	0	1	1	0	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1
750	1	1	1	1	1	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1	1	1	1
600	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1
500	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0

Table 3.3: Amplified DNA fragments using RAPD (OPH05) primer AN:I'naqi,Q:Qraee, KH:Khurtmani,, HB:HmadiBiadi,H:Hmadi,Z:Zraqi, NEA:Neami, GH:Ghzali SF:Sfari,BR:Barqai, KR:krawi S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,H:Hmari,QI:Qaisi,AG:agloni,BL:Blati,MO:Mwazi A &B:Wild type fig.

Primer (OPH05)	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A (N)	B (T)
2000	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
1100	0	0	0	0	1	1	0	0	1	0	0	1	1	1	1	1	0	1	1	1	0	0	0
1000	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1
850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
750	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0

Table 3.4: Amplified DNA fragments using RAPD (OPH14) primer AN:I'naqi, Q:Qraee, KH:Khurtmani,, HB:HmadiBiadi,H:Hmadi,Z:Zraqi, NEA:Neami, GH:Ghzali SF:Sfari,BR:Barqai, KR:krawi S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,HA:Hmari,QI:Qaisi,AG:agloni,BL:Blati,MO:Mwazi A &B:Wild type fig.

Primer (OPA14)	AN	Q	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A (N)	B (T)
1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
1000	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1
850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
750	1	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	1
500	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

Table 3.5: Amplified DNA fragments using RAPD (OPA10) primer AN:I'naqi,Q:Qraee, KH:Khurtmani,, HB:HmadiBiadi,H:Hmadi,Z:Zraqi, NEA:Neami,GH:Ghzali SF:Sfari,BR:Barqai, KR:krawi S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,H:Hmari,Q I:Qaisi,AG:agloni,BL:Blati,MO:Mwazi A &B:Wild type fig.

Primer (OPA10)	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A (N)	B (T)
1500	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1300	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
750	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
600	1	1	1	0	1	1	1	1	1	0	0	0	1	0	1	1	0	1	0	1	0	1	0
500	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

The number of reproducible polymorphic bands that could be assessed varied among the selected 24 RAPD primers. The RAPD primer (OPH12) produced the maximum number of 9 different polymorphic bands out of 12, while the RAPD primers (OPA20) and (OPH16) produced the lowest number of only one polymorphic band out of 2 bands. (Table 3.6)

The total number of present or absent bands assessed per polymorphic RAPD primer, likewise, varied among the 24 tested primers and was accordingly proportional to the number of polymorphic bands. In total 137 polymorphic bands out of 161 (85.1%) were assessed for all 24 RAPD primers.

To identify which primer is more powerful in distinguishing the tested fig genotypes resolving power (Rp) was calculated for each primer (Table 3.6). The calculated Rp was the highest for primer OPH08 (3.16) while the lowest resolving power was (0) for primers OPA15, OPA16 ,OPA07,OPA08.

Table 3.6 Characteristics of RAPD banding profiles produced in figs

primer code	*primer sequence*	*PCR Bands scored*	*monomorphic DNA fragments*	*Polymorphic DNA fragments*	*poly morpic DNA fragments %*	*resolving power*
OPA01	CAGGCCCTTC	9	1	8	88.89%	1.33
OPH02	TCGGACGTGA	8	0	8	100.00%	1.25
OPA05	AGGGGTCTTG	9	0	9	100.00%	0.83
OPA11	CAATCGCCGT	10	1	9	90.00%	2.91
OPA16	AGCCAGCGAA	8	0	8	100.00%	0
OPA18	AGGTGACCGT	5	1	4	80.00%	1.08
OPH02	TCGGACGTGA	9	1	8	88.89%	0.5
OPT20	CCTTCGGAAG	8	3	5	62.50%	2.16
OPA03	AGTCAGCCAC	8	1	7	87.50%	0.166
OPA04	AATCGGGCTG	8	0	8	100.00%	1.66
OPA07	GAAACGGGTG	6	0	6	100.00%	0
OPA08	GTGACGTAGG	7	0	7	100.00%	0
OPA09	GGGTAACGCC	7	2	5	71.43%	1.58
OPA10	GTGATCGCAG	6	0	6	100.00%	0.833
OPA12	TCGGCGATAG	5	0	5	100.00%	0.9166
OPA13	CAGCACCAC	2	2	0	0.00%	0.416
OPA14	TCTGTGCTGG	5	0	5	100.00%	0
OPA15	TTCCGAACCC	3	2	1	33.33%	0.833
OPA17	GACCGCTTGT	5	1	4	80.00%	1.5833
OPA19	CAAACGTCGG	4	2	2	50.00%	0.75
OPA20	GTTGCGATCC	2	1	1	50.00%	0.25
OPH05	AGTCGTCCCC	6	2	4	66.67%	0.666
OPH08	GAAACACCCC	6	0	6	100.00%	3.166
OPH16	TCTCAGCTGG	2	1	1	50.00%	0.41
OPH12	ACGCGCATGT	12	3	9	75.00%	1.08
Total		160	24	136	85%	

3.2.1.2 RAPD Similarity Matrix and Dendrogram

Similarity Matrix. Similarity levels between studied genotypes ranged between (0-77 %). Some genotypes showed very low or no genetic similarity, i.e. 0% between Ajloni and Qraee which indicating the absence of any genetic relations; 4% between Khdari and Adloni; while Adloni was genaticly closer to Mwazi with alittle higher similarity matrix (around 5%). The highest similarity (77%) was recorded between Zraqi and Ghzali varieties which may indicate their strong geneticly similarities . However, both fig wild types A and B showed a range of (10-56)% similarity with the tested varieties (Table 3.7), while similarity level between the two wild types was 68% giving evidences of moderately high genetic similarities to each other. Similar results were recorded in Jordan, where low similarity levels (3%) were recorded between local genotypes (Sadder & Ateyyeh, 2006).

Dendrogram. To further illustrate the genetic relatedness among the studied common fig genotypes, they were clustered to form unrooted tree dendrogram (Fig3.4). The unrooted tree consists of seven major groups. The first group contains (I'naqi, AN; Wildtype figs A, B; Qrawi, KR and Barqawi, BR). (wildfig , A and B) varieties both were caprifig collected from different sites, they appear in the same cluster and show similarity (37-56%) with other verities Barqawi; Qrawi; I'naqi, while the two caprifig

varieties were relatively highly similar to each other (68%), I'naqi and Baqawi had 32% similarity index and have same sweet taste.

The second group includes (Zraqi,Z; Ghzali, GH; Qaisi,QI) which is characterized by black to purple fruit skin color ,Ghzali and Zraqi have similarity index(77%) and share similer taste. It also includes (Blati, BL; Neami, NEA). Blati and Ghzali have (51%) genetic similarity and share similertaste, while Blati is closer to Ne'ami (69%) with both having similar green skin colour.

Third group contains (Hmadi, H; Kharobi, KHR; Swadi, S). Swadi and Kharobi both have black to purple skin color, while Hmadi have brass green to purple color which correlate with the fact that Swadi is more similar to Kharobi (45%) than to Hmadi (40.%), while Kharobi and Hmadi similarity was (50%) and share both leafe and fruit shape.

The fourth group (Hmari, HA; Dafor, DA; Sfari, SF). Dafor is a foreign variety which has green skin colour, the similarity matrix between Dafor and Hmari (57%) was higher than with Sfari (33 %), Sfari and Hmari have relatively low similarity index (26%) and share moderate tree size.

The fifth group contains (Khurtmani,KH; Hmadi Biadi HB) with similarity matrix of (45%) however, the fruit is green brass to violet colour while the other having the yellow to green skin colour and have relatively close fruit size.

The sixth group contains (Ajloni, AG; Adloni, AD), with about (30%) similarity which does not indicate that both are synonyms to the same variety, which might ensure the common local idea which consider them as two different varieties, adding to this the fact that they don’t share any morphological characters.

The last group contains the three green to yellow colored skin varieties (Qraee, Q; Khdari, KHD; Mwazi, MO) with relatively low to moderate similarity matrix (12-40%) and share same red bulb colour.

Table 3.7 : jaccard's similarity index as percentages generated for 23 common fig genotypes' RAPD data AN:I'naqi,Q:Qraee, KH:Khurtmani, HB:HmadiBiadi ,H:Hmadi,Z:Zraqi, NEA:Neami,GH:Ghzali SF:Sfari,BR:Barqai, KR:krawi S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,HAH:mari,Q I:Qaisi, AG:agloni,BL:Blati,MO:Mwazi A &B:Wild type fig.

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NEA	GH	SF	BR	KR	S	DA	AD	A	B
AN	1																						
Q	0.21	1																					
KH	0.32	0.17	1																				
HB	0.24	0.29	0.45	1																			
H	0.20	0.17	0.06	0.13	1																		
Z	0.39	0.18	0.25	0.28	0.42	1																	
KHD	0.21	0.40	0.17	0.16	0.17	0.14	1																
KHR	0.18	0.09	0.14	0.22	0.50	0.42	0.19	1															
HA	0.19	0.13	0.20	0.14	0.29	0.24	0.21	0.22	1.00														
QI	0.25	0.15	0.19	0.31	0.33	0.40	0.11	0.37	0.22	1.00													
AG	0.14	0.00	0.27	0.11	0.00	0.15	0.00	0.05	0.15	0.08	1.00												
BL	0.27	0.16	0.16	0.29	0.39	0.53	0.13	0.47	0.22	0.49	0.10	1.00											
MO	0.22	0.20	0.09	0.15	0.33	0.22	0.15	0.21	0.18	0.21	0.00	0.41	1.00										
NEA	0.33	0.19	0.22	0.29	0.39	0.50	0.14	0.39	0.30	0.41	0.11	0.69	0.37	1.00									
GH	0.42	0.17	0.28	0.27	0.37	0.77	0.13	0.36	0.28	0.39	0.14	0.51	0.28	0.58	1.00								
SF	0.26	0.19	0.13	0.13	0.27	0.36	0.19	0.25	0.38	0.25	0.13	0.21	0.09	0.23	0.34	1.00							
BR	0.32	0.09	0.28	0.22	0.26	0.38	0.09	0.24	0.27	0.37	0.15	0.27	0.11	0.34	0.36	0.36	1.00						
KR	0.43	0.16	0.26	0.29	0.31	0.51	0.16	0.41	0.18	0.29	0.13	0.40	0.18	0.31	0.43	0.27	0.45	1.00					
S	0.17	0.17	0.08	0.21	0.40	0.33	0.24	0.45	0.26	0.32	0.00	0.30	0.23	0.30	0.28	0.37	0.23	0.22	1.00				
DA	0.30	0.19	0.18	0.17	0.32	0.41	0.19	0.30	0.57	0.25	0.21	0.28	0.17	0.32	0.39	0.33	0.20	0.27	0.30	1.00			
AD	0.35	0.10	0.26	0.15	0.09	0.37	0.05	0.14	0.19	0.15	0.33	0.22	0.06	0.25	0.35	0.23	0.22	0.29	0.16	0.35	1.00		
A	0.52	0.20	0.28	0.27	0.22	0.48	0.20	0.28	0.22	0.31	0.22	0.33	0.10	0.37	0.47	0.35	0.37	0.56	0.19	0.35	0.46	1.00	
B	0.53	0.18	0.38	0.26	0.21	0.55	0.18	0.28	0.32	0.27	0.26	0.33	0.11	0.42	0.53	0.48	0.48	0.44	0.27	0.35	0.48	0.68	1.00

One of the interesting observations was that Qrawi and Agloni having similarities in their green skin and red bulb colour, while the similarity matrix for those varieties were 13%, on the other hand they have (51%)similarity with Zraqi with dark violet skin .

The second was that Barqawi and Hmadi which have similar skin and bulb colour. However they were not genetically closely related since their similarity matrix value less than 3%.

Our results show the potential use of those - particularly the more distant genotypes- for future breeding programs. Moreover, fig genotypes present in the BERC show variable fruit colors (e.g., yellow, green, purple, violet, and light and dark violet). Fruit weight is also variable among genotypes. Therefore, this rich and variable genetic pool could be potentially incorporated into local or regional breeding programs.

RAPD analysis can be an invaluable tool for safeguarding and improving figvarieties. In fact, the biodiversity of the *F carica* L species can be safeguarded efficiently only after characterizing the genetic diversity of the present fig population with RAPD markers or other means. Selection assisted by RAPD markers may also be helpful to produce new cultivars with improved productivity, organoleptic aspects, and pest resistance (De Masi *et al*., 2005).

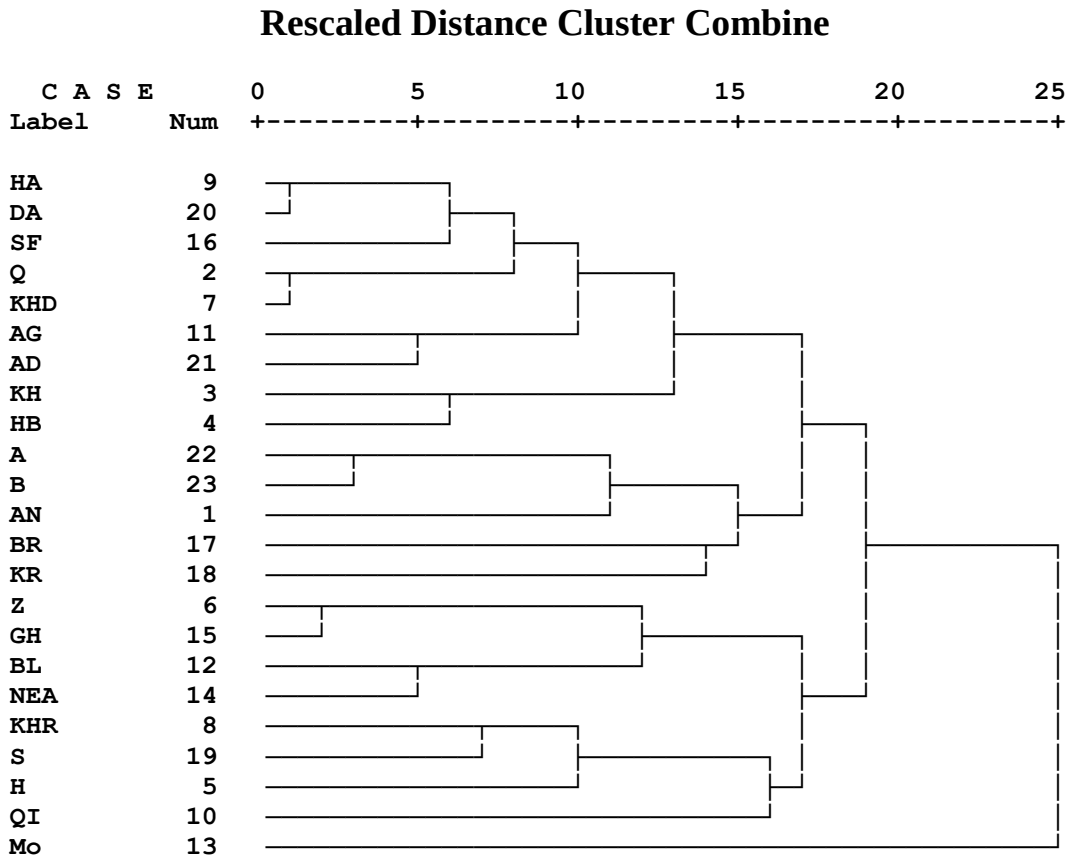
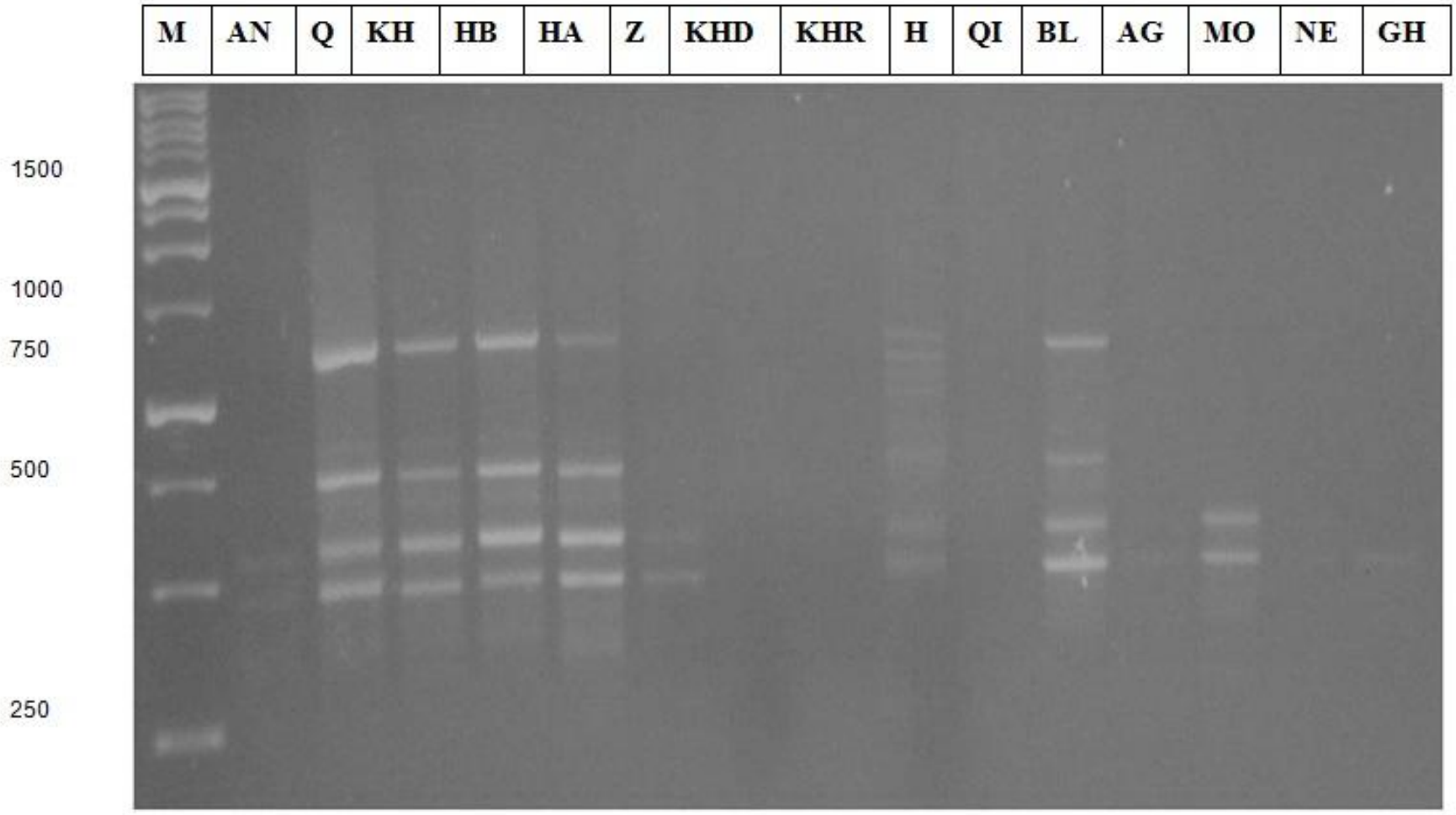


Figure 3.4: Unrooted tree constructed for the investigated 23 common fig genotypes using UPGMA method. AN:I'naqi, K:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi ,NEA:Neami ,GH:Ghzali, SF:Sfari, BR:Barqai, KR:krawi, S:swadi,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi, HA:Hmari, KI:Qaisi ,AG:Ajloni ,BL:Blati ,MO:Mwazi A &B:Wild type fig).

3.2.2 ISSR PCR Analysis

The ISSR analysis was demonstrated to be available for the phylogenetic study and cultivar identification. ISSR markers contribute with a significant number of polymorphic markers which could be useful in identifying fig cultivars, contributing to saturate genetic maps, in marker assisted selection, but which also could contribute useful data in phylogenetic analyses (Vurall *et al.*,2010).

In general different banding patterns appeared in most fig varieties using the ISSR. Some of fig varieties (Ghzali,GH; Khdari, KHD; Hmari,H; Qaisi, KI; Sfari,S; Barqawi, BR; Swadi, S; Dafor, Da; Adloni, AD) did not produce any band with all ISSR primers (Figures 3.8-10), thus they were excluded from statistical data analysis. Khdari produced the highest number of bands (52 bands).


M
AN
Q
KH
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Z
KHD
KHR
H
QI
BL
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NE
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1500
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250

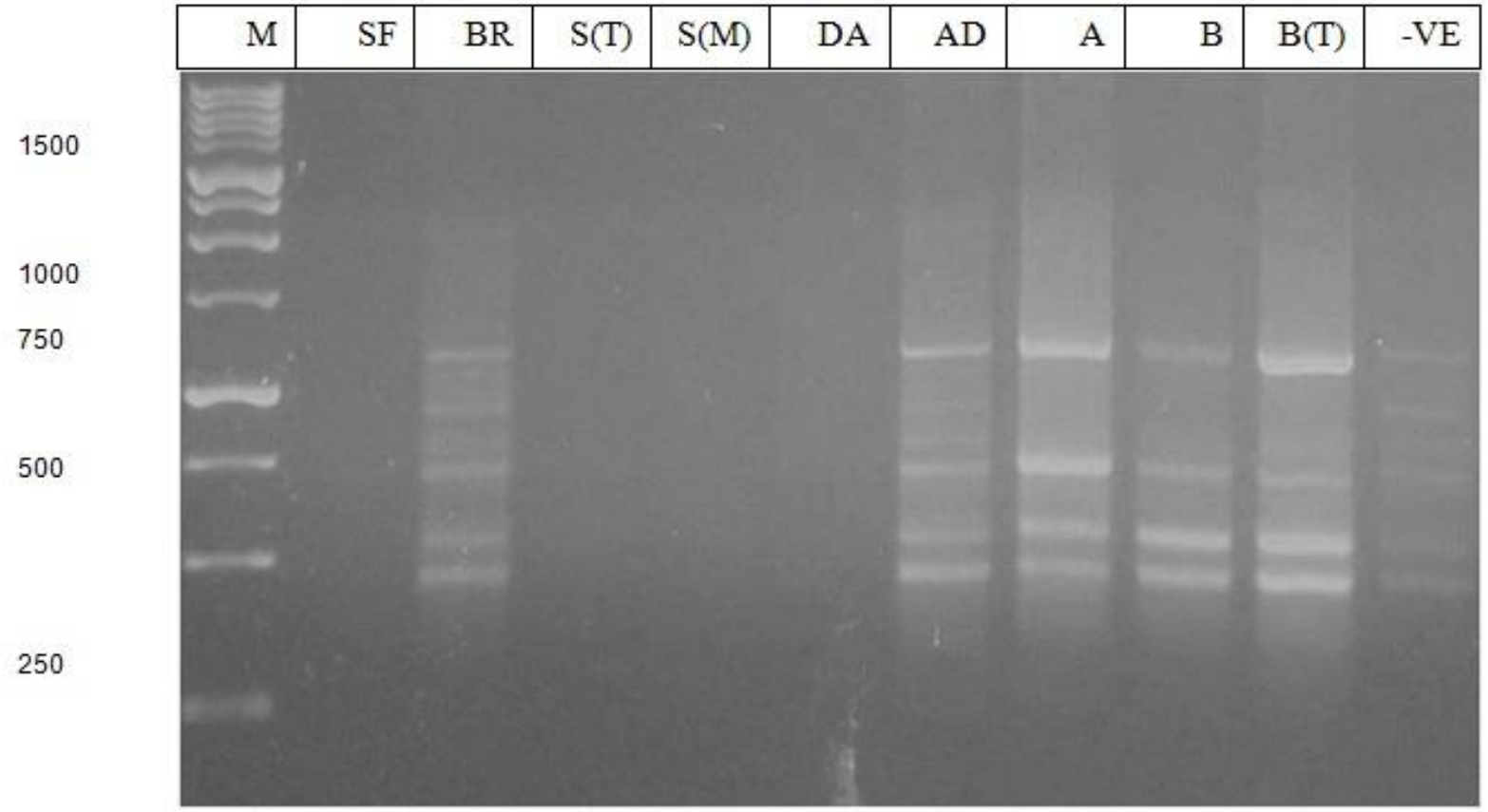
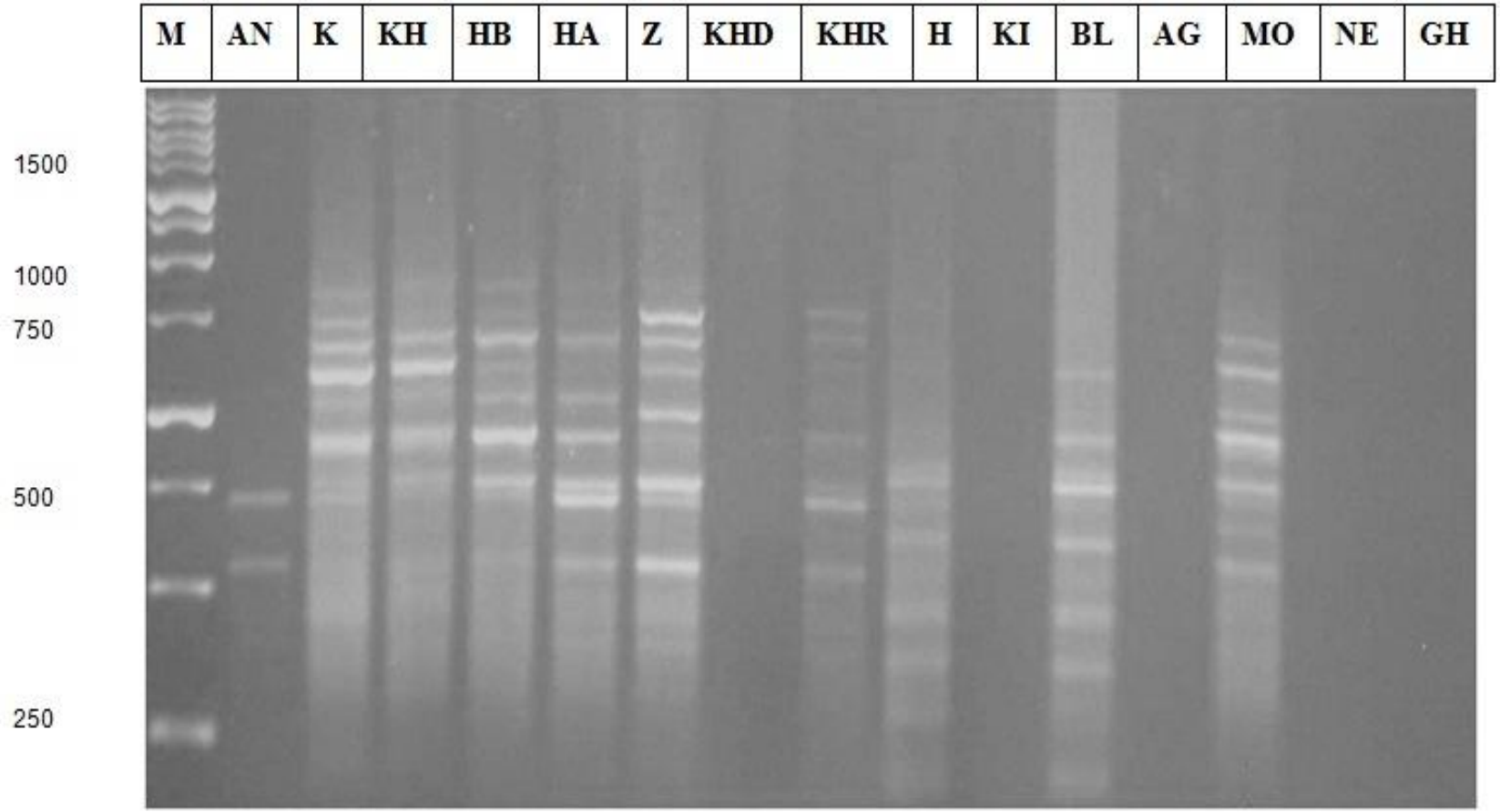


Figure 3.5 : An ISSR Pattern of primer (UBC823) with al l fig genotype AN:I'naqi, Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi , NEA:Neami,GH:Ghzali, SF:Sfari, BR:Barqai, KR:krawi, S(T) Swadi tree S(M) Swadi from nursery ,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,, HA:Hmari, KI:Qaisi, AG:Ajloni ,BL:Blati,MO:Mwazi, A &B:Wild type fig.


M
AN
K
KH
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Z
KHD
KHR
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KI
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1500
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750
500
250

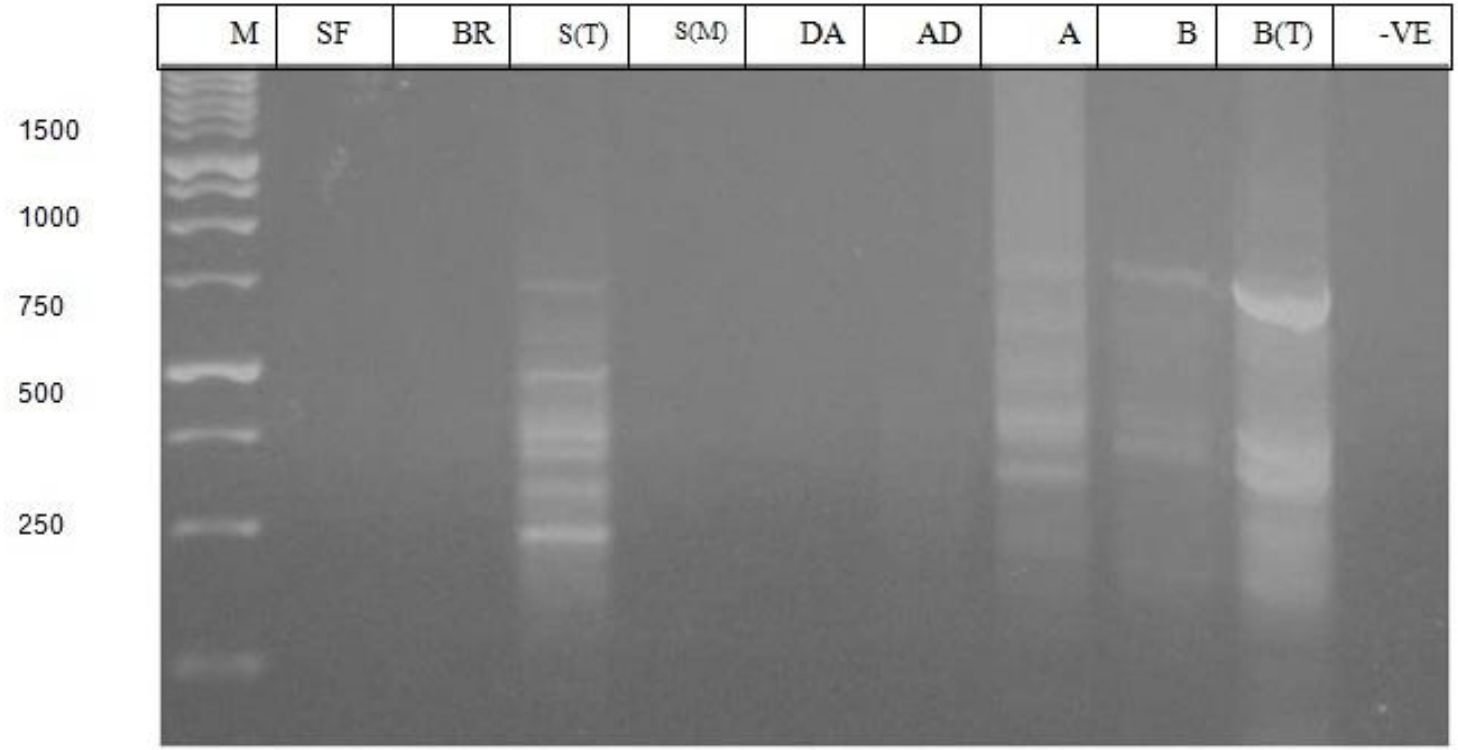
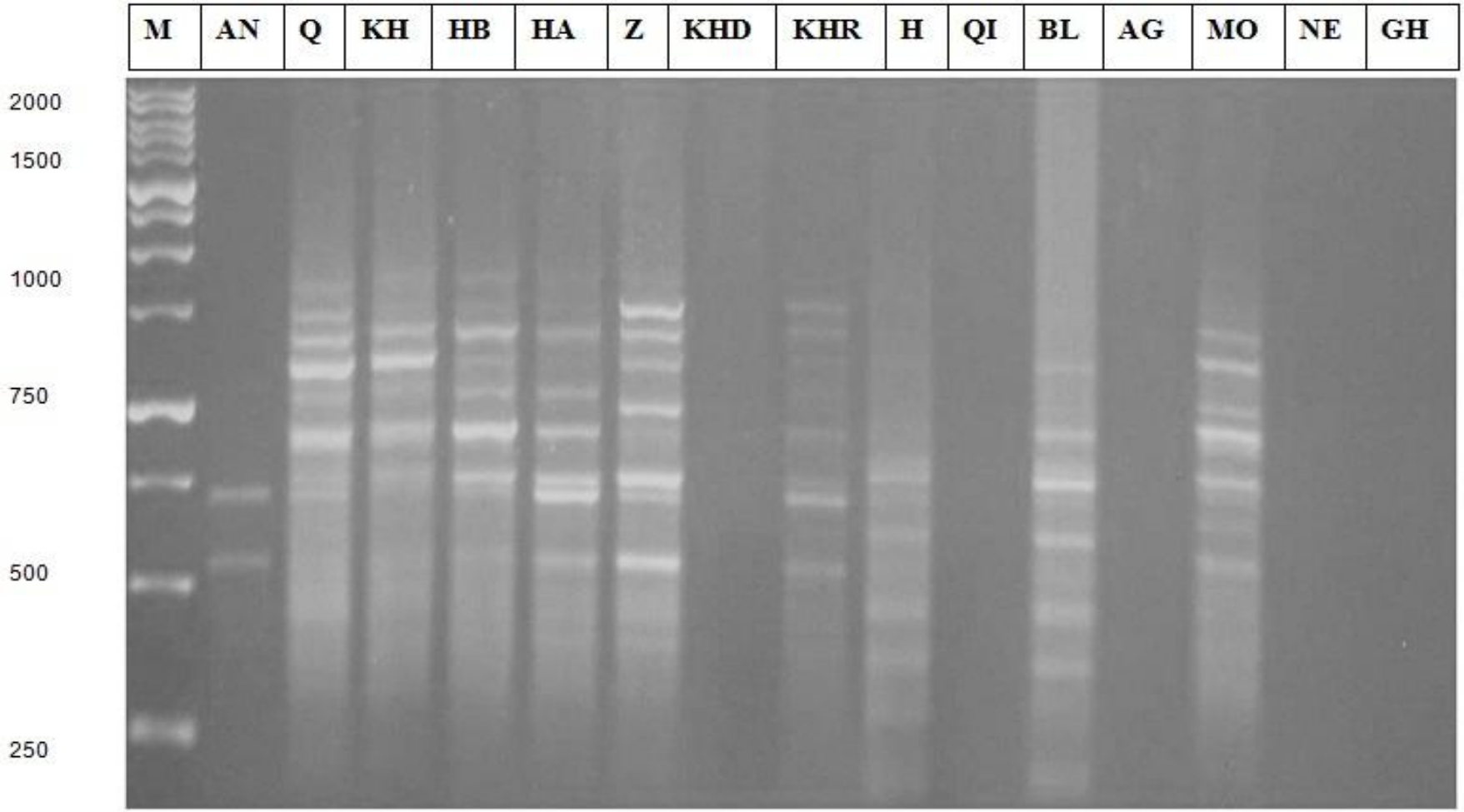


Figure 3.6 : An ISSR Pattern of primer (UBC818) with al l fig genotype AN:I'naqi, Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi , NEA:Neami,GH:Ghzali, SF:Sfari, BR:Barqai, KR:krawi, S(T) Swadi tree S(M) Swadi from nursery ,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,, HA:Hmari, KI:Qaisi, AG:Ajloni ,BL:Blati,MO:Mwazi, A &B:Wild type fig**.**


M
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2000
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500
250

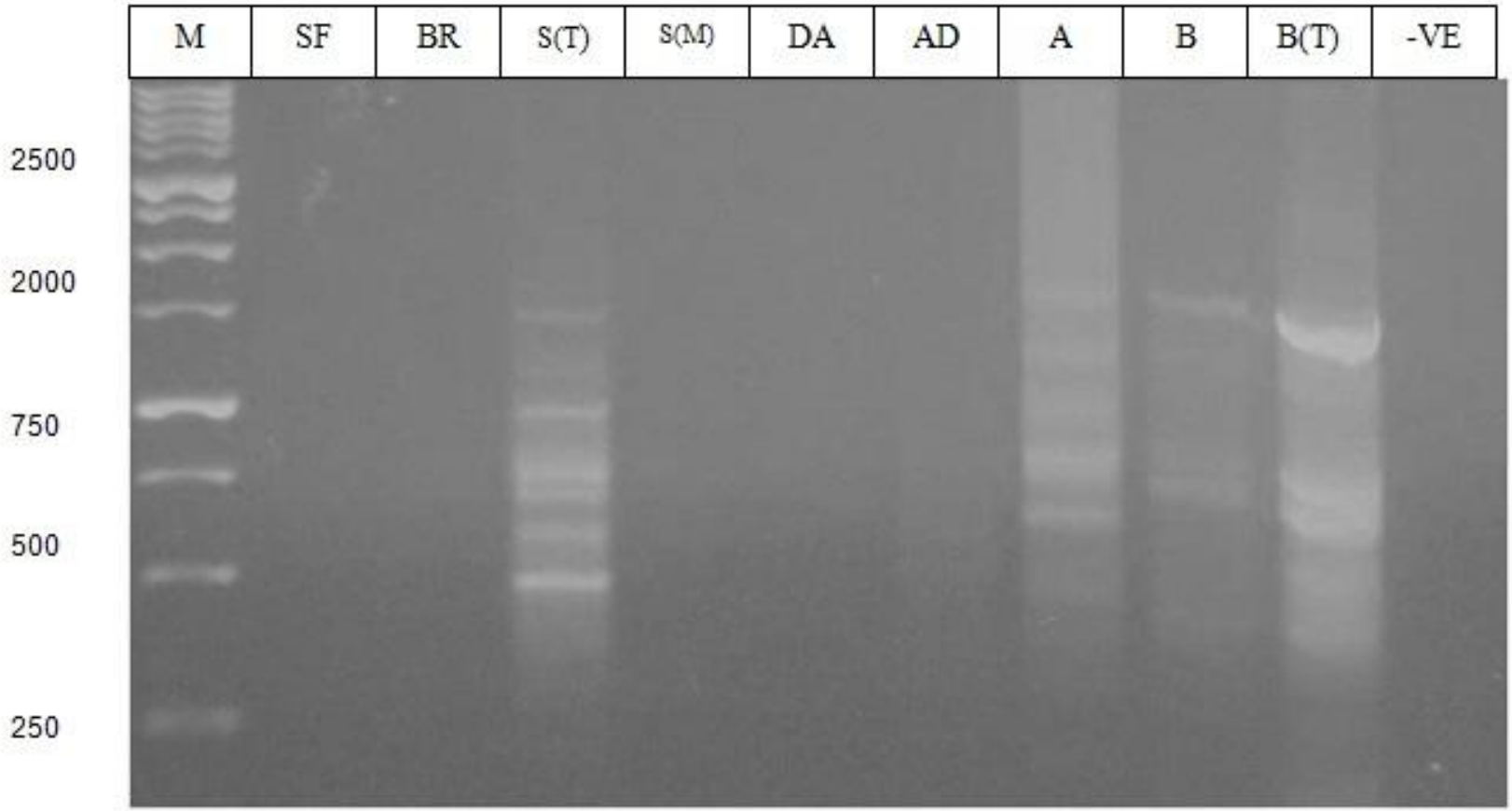

Figure 3.7: An ISSR Pattern of primer (UBC813) with al l fig genotype AN:I'naqi, Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi , NEA:Neami,GH:Ghzali, SF:Sfari, BR:Barqai, KR:krawi, S(T) Swadi tree S(M) Swadi from nursery ,DA:Dafor, AD:Adloni, KHD:Khdari, KHR:Kharobi,, HA:Hmari, KI:Qaisi, AG:Ajloni ,BL:Blati,MO:Mwazi, A &B:Wild type fig.

Total 1518 data entries were analyzed, among which 420 data entries were for present bands (1) and 1098 for absent bands (0). Different primers represent different banding patterns e.g., in primer (UBC 818) 76 present bands were scored out of 165 entries, in primer (UBC 817) 54 present bands were scored out of 150 entries, in primer (UBC 810) 52 present bands were scored out of 105 entries, in primer (UBC 861) 62 present bands were scored out of 105entries, in primer (UBC 807) 47 present bands were scored out of 75 entries (Tables 3.8-3.12).

Table 3.8 :Amplified DNA fragments using ISSR primer (UBC818) AN:I'naqi, Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi ,NEA:Neami,GH:Ghzali, , A (N)B(N), B(T):Wild type fig.

Primer(UBC818)	AN	Q	KH	HB	H	Z	KHR	AG	BL	MO	NE	GH	A (N)	B (N)	B (T)
1700	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0
1500	0	1	1	1	0	1	1	0	1	0	0	0	0	0	0
1400	0	1	1	1	0	1	1	0	1	1	0	0	1	1	1
1200	0	1	1	1	0	1	1	0	1	1	0	0	1	1	1
1100	0	1	1	1	0	1	1	0	0	1	0	0	1	0	0
900	0	1	1	1	0	1	1	1	1	1	0	0	1	0	0
750	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1
700	0	1	0	1	1	1	1	1	1	1	0	0	0	1	1
650	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
550	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0
500	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Table 3.9: Amplified DNA fragments using ISSR primer (UBC817) AN:I'naqi, K:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi ,NEA:Neami,GH:Ghzali, , A (N)B(N), B(T):Wild type fig.

Primer (UBC 817)	AN	K	KH	HB	H	Z	KHR	AG	BL	MO	NE	GH	A (N)	B (N)	B (T)
1400	0	1	1	0	0	0	0	1	0	0	0	0	0	1	1
1100	0	1	1	1	0	1	0	1	0	0	0	0	1	1	1
900	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0
750	0	1	1	1	0	1	0	1	0	0	0	0	1	1	1
600	0	1	1	0	0	1	0	0	0	0	0	0	1	1	1
550	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1
500	0	1	1	1	0	1	0	0	0	0	0	0	1	0	0
450	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0
400	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0
350	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0

Table 3.10 :Amplified DNA fragments using ISSR primer (UBC810) : AN:I'naqi ,Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi ,NEA:Neami,GH:Ghzali, , A (N,)B(N), B(T):Wild type fig.

primer (UBC 810)	AN	Q	KH	HB	H	Z	KHR	AG	BL	MO	NE	GH	A (N)	B (N)	B (T)
1250	0	1	1	0	1	1	1	1	1	1	1	0	0	0	0
1150	0	1	1	0	1	1	1	1	1	1	1	0	1	1	1
1000	0	1	1	0	1	1	0	1	1	1	1	1	0	0	0
700	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1
650	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
600	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
500	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1

Table 3.11:Amplified DNA fragments using ISSR primer (UBC861) AN:I'naqi, Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi ,NEA:Neami,GH:Ghzali, , A (N)B(N), B(T):Wild type fig
.

Primer (UBC861)	AN	Q	KH	HB	H	Z	KHR	AG	BL	MO	NE	GH	A (N)	B (N)	B (T)
1150	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1
900	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0
800	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0
700	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1
650	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1
400	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1
300	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1

T**able 3.12: Amplified DNA fragments using ISSR primer (UBC807): AN:I'naqi,Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi ,NEA:Neami,GH:Ghzali, , A (N)B(N), B(T):Wild type fig**
.

Primer (UBC 807)	AN	Q	KH	HB	H	Z	KHR	AG	BL	MO	NE	GH	A (N)	B (N)	B (T)
1500	0	1	1	0	0	1	0	1	1	1	1	0	1	1	1
1100	0	0	1	0	0	1	0	1	1	1	1	0	1	1	1
750	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1
650	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1
450	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0

The number of reproducible polymorphic bands that could be assessed varied among the selected 12 chosen ISSR primers. The ISSR primer (UBC818) produced the maximum number of 10 different polymorphic bands out of 11, while the primer UBC816 produced the lowest number of only 2 polymorphic band out of 2.

The total number of present or absent bands assessed per polymorphic ISSR primer, likewise, varied among the 12 tested primers and was accordingly proportional to the number of polymorphic bands. In total 55 polymorphic bands out of 69 (80%) were assessed for all 12 ISSR primers (Table 3.16)

To identify which primer is more powerful in distinguishing the tested fig genotypes resolving power was calculated (Table 3.13). The calculated Rp was the highest for primer UBC818 (3.2) while the lowest resolving power was (0.933) for primers UBC818.

Table 3.13 Characteristics of ISSR banding profiles produced in figs

primer No	*primer code*	*primer sequence*	*PCR Bands scored*	*monomorphic DNA fragments*	*Polymorphic DNA fragments*	*polymorpic DNA fragments %*	*resolving power*
51	UBC807	AGAGAGAGAGAGAGAGT	5	2	3	60%	2.26
52	UBC808	AGAGAGAGAGAGAGAGC	4	1	3	75%	1.33
53	UBC810	GAGAGAGAGAGAGAGAT	7	2	5	71%	2.266
54	UBC811	GAGAGAGAGAGAGAGAC	3	1	2	67%	1.066
55	UBC812	GAGAGAGAGAGAGAGAA	5	2	3	60%	2.266
56	UBC814	CTCTCTCTCTCTCTCTA	6	2	4	67%	2.4
57	UBC815	CTCTCTCTCTCTCTCTG	6	1	5	83%	0.933
58	UBC816	CACACACACACACACAT	2	0	2	100%	1.73
59	UBC817	CACACACACACACACAA	10	0	10	100%	1.866
60	UBC818	CACACACACACACACAG	11	1	10	91%	3.2
61	UBC823	TCTCTCTCTCTCTCTCC	4	2	2	50%	2
62	UBC861	ACCACCACCACCACCACC	6	0	6	100%	2.133
			69	14	55	80%	

3.2.2.2 ISSR Dendogram and Matrix:

After generating a data matrix, the Jaccard similarity index was determined between each pair of genotypes and presented in Table 3.14.

Similarity matrix Some genotypes showed very low genetic similarity, eg 1% between Inaqi and Neami, 3% between wild type BN and Ghzali and 4% between Hmadi and Qraee.

The highest similarity 80 % was recorded between the varieties wild type BT and wildtype BN. However, all fig wild types, wild type AN1, wild type BN and wildtype BT showed incredible similarity with each other.

Table 3.14 : jaccard's similarity index as percentages generated for 15 common fig genotypes AN:I'naqi ,Q:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi ,NEA:Neami,GH:Ghzali, , A (N,)B(N), B(T):Wild type fig.

	AN	Q	KH	HB	H	Z	KHR	AG	BL	MO	NEA	GH	AN1	BN	BT
AN	1.000														
Q	.000	1.000													
KH	.000	.600	1.000												
HB	.000	.128	.152	1.000											
H	.000	.205	.139	.300	1.000										
Z	.000	.326	.250	.227	.250	1.000									
KHR	.000	.103	.088	.286	.200	.130	1.000								
AG	.000	.256	.143	.053	.045	.423	.056	1.000							
BL	.000	.400	.462	.208	.231	.484	.167	.393	1.000						
MO	.000	.478	.550	.172	.233	.417	.100	.257	.472	1.000					
NEA	.000	.077	.091	.600	.222	.136	.167	.000	.125	.103	1.000				
GH	.000	.026	.000	.000	.000	.045	.000	.067	.042	.034	.000	1.000			
AN1	.000	.396	.386	.100	.200	.250	.067	.162	.405	.357	.069	.036	1.000		
BN	.000	.353	.432	.129	.188	.268	.097	.184	.421	.405	.065	.033	.657	1.000	
BT	.000	.370	.326	.115	.231	.243	.077	.182	.297	.359	.080	.042	.625	.800	1.000

Dendrogram analysis : To further analysis of genetic relatedness among the studied common fig cultivars, results were clustered to form unrooted tree dendrogram (Fig3.8). The unrooted tree consists of six major groups.

First cluster consisted of Ghzali,GH and I'naqi, AN ,both of them shows no genetic similarity to each other .I'naqi didn't show any similarities to any of the varieties .while Ghzali show similarities with some of the varieties in relatively high values as with Ajloni despite the big differences in their skin colors.

The second group contains (Hmadi Biadi,B; Neami,NEA; Hmadi,H; Kharobi,KHR) Hmadi Biadi and Neami have the same green yellow skin color , which correlate with the high similarity index (60%), however Hmadi shows a relatively low similarity with both of them (20-22%) and characterized by violet brass skin colour, Kharobi have dark violet colour which goes with the its low similarity indexes with the other three varieties.

The third one contains (Zraqi,Z; Blati,BL; Ajloni,AG), Blati and Ajloni sharing the green skin color and show relatively low similarity (39%) and share same fruit shape, while Zraqi show higher similarities with both of them; Ajloni and Zraqi (42%) while blati to Zraqi relatively high (48%) and share same taste.

Wildtypes:AN1; BN; BT are caprifig and clustered in the fourth group. They show a high similarities to other varieties (1%- 34%) which indicates

that their genome may differ from common fig cultivars . The wild type BN and BT show incredible similarity to each other (80%).

The fifth group contains (Qraee,Q; Khdari, KH; Mwazi, MO) , Kraee and Mwazi ,three varieties show the same green to yellow skin colour and share same red bulb colour with a relatively high similarity indexs (47-60%).

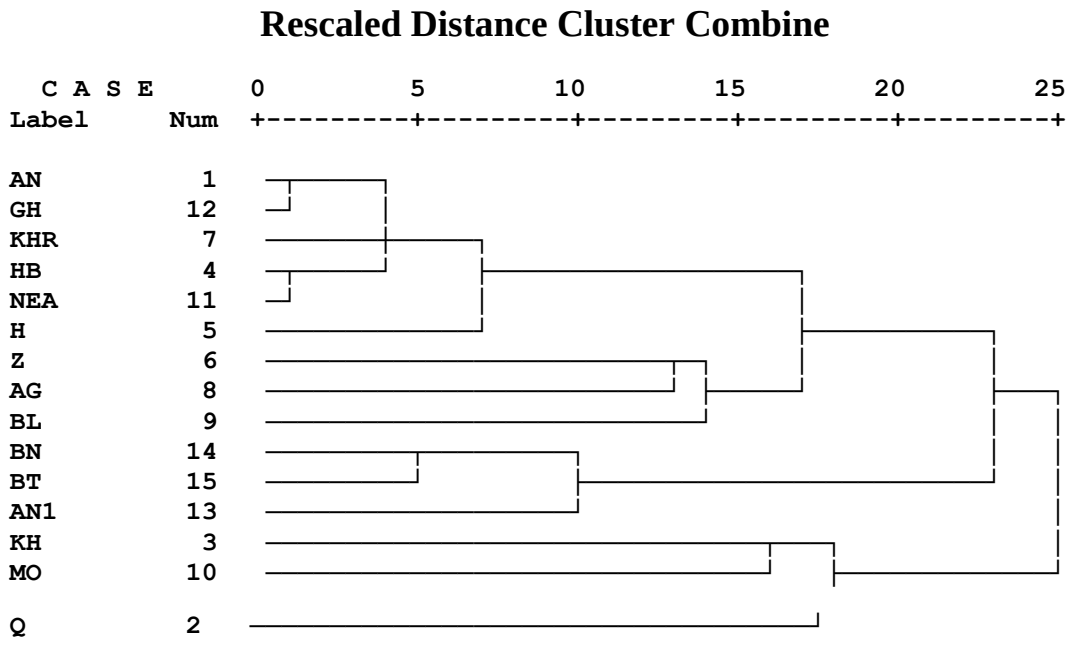

Figure 3.8: Unrooted tree constructed for the investigated 15 common fig genotypes using UPGMA method AN:I'naqi, K:Qraee, KH:Khurtmani , HB:HmadiBiadi, H:Hmadi, Z:Zraqi, BL:Blati, MO:Mwazi . ,NEA:Neami,GH:Ghzali, , A (N)B(N), B(T):Wild type fig.

It is worth mentioning the relatively low genetic similarity between the two varieties Qraee and Blati which slightly exceeded (40%) which may not support the common and local belief that Qraee and Blati were synonyms to same variety.

In this study ISSR shows relatively low similarities present among the Palestinian genotypes.A further study including more fig genotypes is required to find out more about their relatedness or uniqueness .

The mean number of amplification RAPD bands (6.7) was more than that of ISSR (5.57). Moreover, the total number of polymorphic bands (161) detected by RAPD primers was much higher than that of the ISSR primers (69), which may suggested that the RAPD markers were better compared to ISSR markers in the capacity of revealing more informative bands in a single amplification. This may be because of poly-allelic nature of RAPD markers. A possible explanation for the difference in the resolution of RAPD and ISSR is that the two markers techniques targeted different regions in the genome. These differences may also be attributed to marker sampling errors and / or the level of polymorphism detected, reinforcing the importance of the number of loci and their coverage of the whole genome for obtaining reliable estimates of genetic relationships among cultivars (Souframnien *et al*., 2004)

The RAPD markers were marginally more informative than ISSR in the assessment of genetic diversity in common fig cultivars. Similar results are reported in Caldesia grandis (Chen et al., 2006). This may be because of the fact that two marker techniques targeted different regions of the genome. Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions and to be selectively neutral

(Bachmann 1997; Landergott et al., 2001). On the contrary, some other studies have shown that RAPD markers

are distributed throughout the genome and may be associated with functionally important loci (Penner 1996). However, there is little information to indicate that ISSR markers are functionally important (Esselman et al., 1999).

The relatively high genetic relatedness of wild type A with the cultivar Swadi which was 56% using RAPD technique may indicates that these cultivars is descending evolutionary from the same origin while Mwazi may descending from disserent origin, since it is similarity index to wild type and Swadi was10% and 23% respectively. Wild type B had 55% similarity index with Zraqi which show the close evolution origins compared to Mwazi with only 18% and 22% with wild type B and Zraqi respectively which give Mwazi a potential older evolutionary origin than that of the present wild types.

According to ISSR the wild type AN1 have close evolutionary origin to blati since their similarity matrix is relatively high (40.5%) . Wild type BN had (43.2%) similarity matrix with Khdari this result may indicates the close evolutionary origin of both, while wild type BT and Qraee have 37% similarity matrix which give impression of close evolutionary relation between them.

According to ISSR Ghzali may be the oldest cultivar of all we used since it show a very low similarity indexes of all wild types AN1, BN, BT (3.3%, 3.6% , 4.2%) respectively which may give impression of far evolutionary origin from present dominant wild types .

These local genotypes are moreover, adapted to some of the harshest prevailing weather conditions in the Mediterranean basin where fig can grow (precipitation as low as 325 mm). Moreover, they have a wide spectrum of fruit weight, percent of total soluble solids, and percent of titratable acidity ranging from 9.3 to 27.6 g, 21.46 to 26.75%, and 0.28 to 0.59%, respectively. Moreover, they show variable fruit colors (e.g. yellow, green, purple). Therefore, this rich and variable genetic pool could be potentially incorporated for the local or regional breeding programs (Sadder & Ateyyeh, 2006).

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Appendixes

Appendix A

Solution Preparations :

50mM EDTA-0.2% SDS:

- 50mM EDTA (1.46 gm were dissolved in 50 ml SDW at pH 8 using NAOH 1 M)

  -0.2% SDS until dissolved completely (0.2 gm SDS were added then volume was completed to 100 ml)

5 M (Potassium acetate) KA c:

4.9075 gm of potassium acetate were dissolved in 10 ml of sterile distilled water

TAE buffer (1 L) 5X:

2.922g EDTA were dissolved in 20 ml of SDW at pH 8, 54 gm of TRIS_base , and 27.5 gm acetic acid then volume was completed to 1 Liter

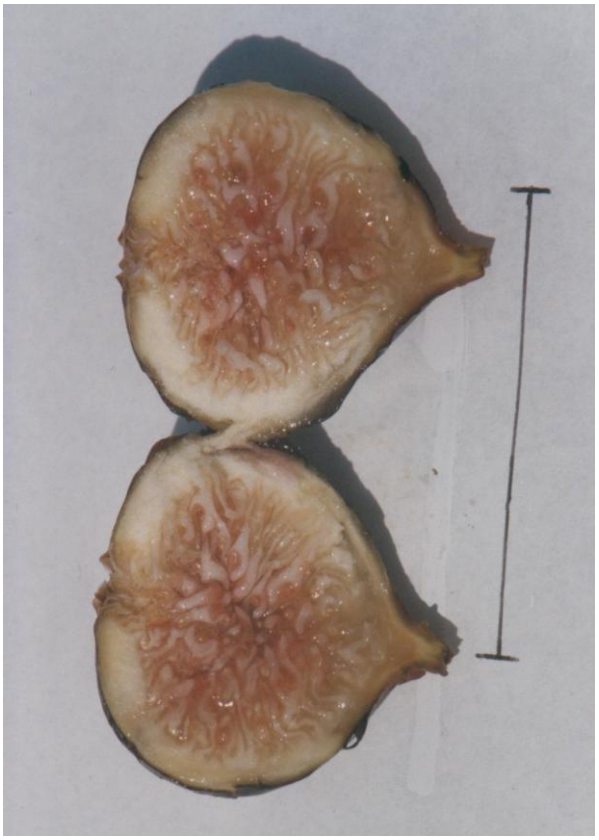
1 Liter of 1X TAE buffer :

200 ml of 5X TAE buffer were added to 800 ml distilled water

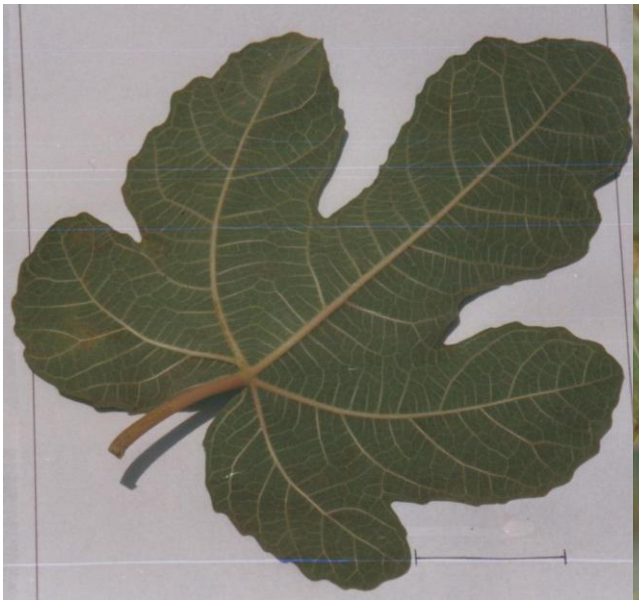
APPENDIX B

Characters used for determining the classification of fig varieties

S1



S2

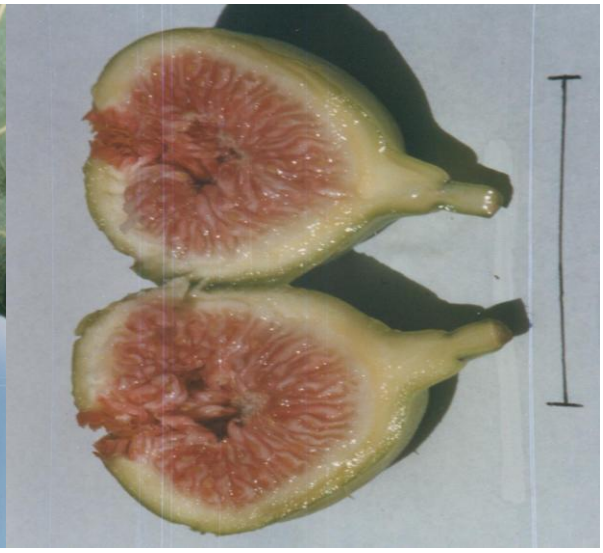


S3

HA1

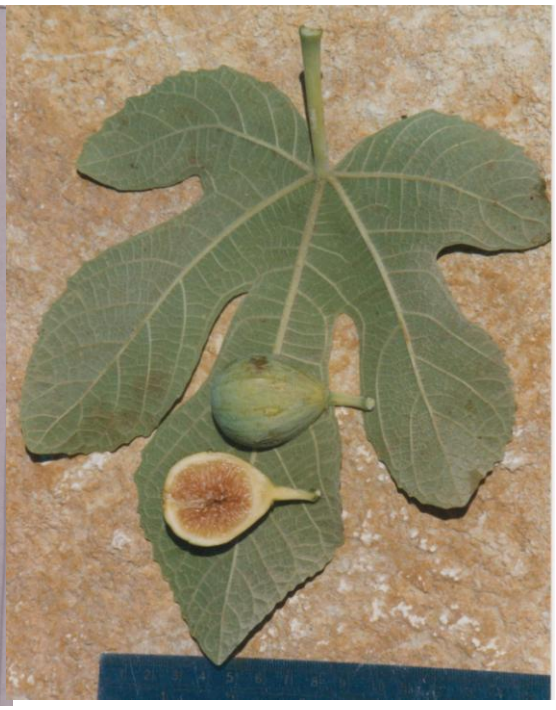
Figure B.1 Examples on characters used for classification fig S1: Swadi Fruit skin colour , S2 : Fruit bulb colour , S3: Swadi leaf, HA1: Hmari Fruit skin colour

HB1



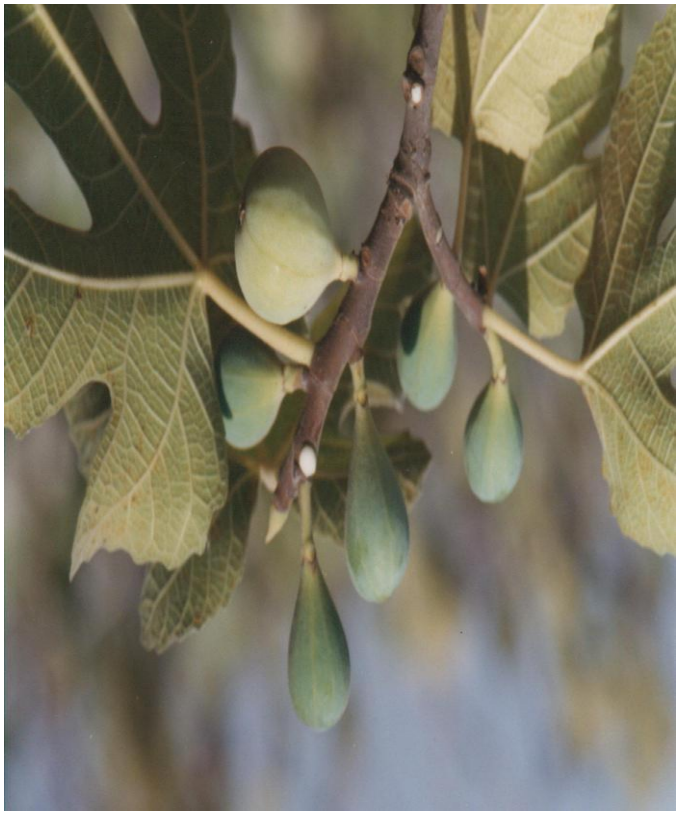
HB2

HB3



BL1

Figure B.2 Examples on characters used for classification fig HB1: Hmadi Biadi Fruit skin colour, HB2 : Fruit bulb colour , HB3:Hmadi Biadi leaf,BL1: Blati Fruit skin colour & leaf



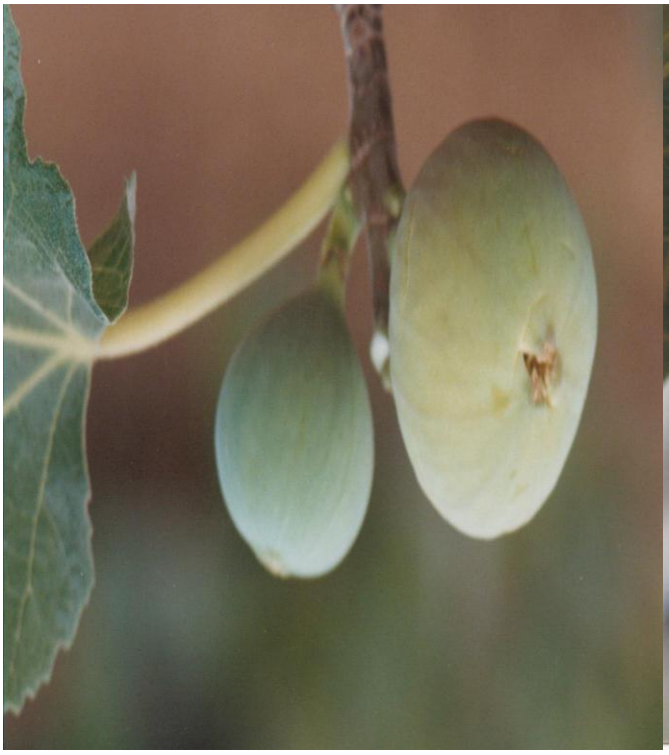
AG1

NEA1

NEA2

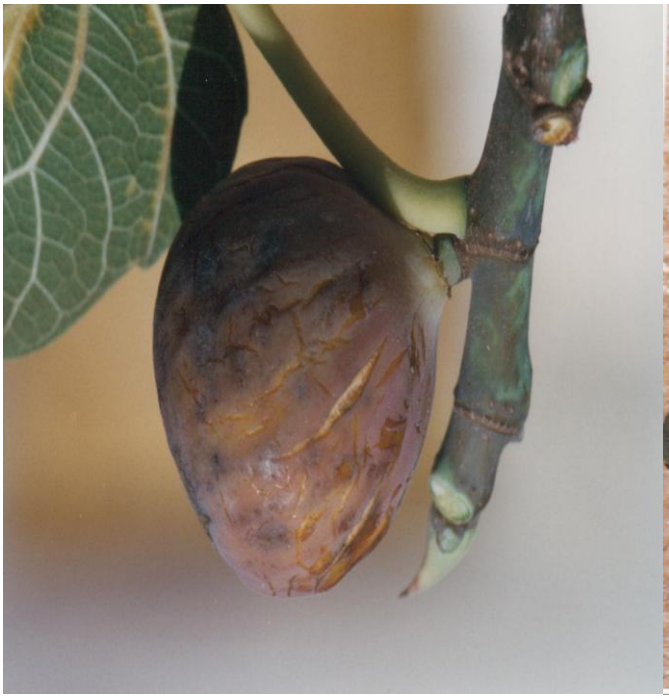
NAE1

Figure B.3 : Examples on characters used for classification fig NEA1: Neami Fruit skin colour,NEA2:Neami Fruit bulb colour & leaf, AG1: Ajloni fruit colour



KHD1

K1

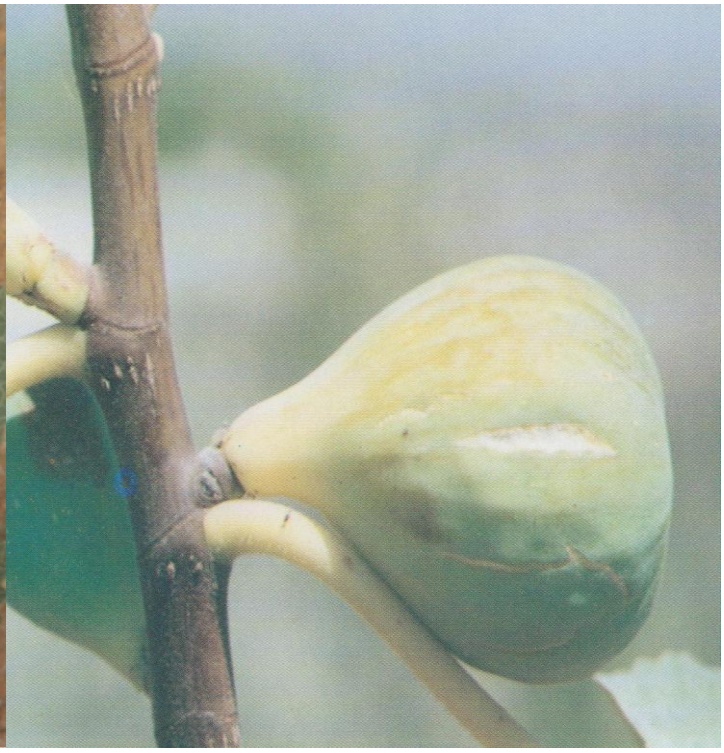


GH1

GH3

Figure B.4 : Examples on characters used for classification fig KHD1: Khdari Fruit skin colour,Q 1: Qraee Sfari Fruit bulb colour , GH1: Ghzali leaf , GH2: Ghzali bulb colour ,GH3: Ghzali leaf

MO2



MO1

Z 1

Z3

Figure: B.5 : Examples on characters used for classification fig: MO1:Mwazi skin colour, Mwazi Bulb colour,Z1:Zraqi skin colour,Z3:Zraqi leaf.

KR1 KR2

H1 H3

Figure B.6 : Examples on characters used for assification fig KH1 : Khurtmani fruit skin and bulb colour,KH2::khurtmani leaf shape,H1: Hmadi fruit colour, H3: Khurtmani leaf shape .

APPENDIX C

C1: RAPD Primers Scores

Table C1.1

Scores of Bands Generated by RAPD Primer OPH02

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	0	0	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0
1300	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
850	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1
750	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
700	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
600	0	0	0	0	1	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
500	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Table C1.2

Scores of Bands Generated by RAPD Primer OPT10

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	0	0	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1
1300	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1100	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	1	1	0	0	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1
850	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
750	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1
600	1	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
500	1	1	1	1	0	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	0	1	1

Table C 1.3

Scores of Bands Generated by RAPD Primer OPA01

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	0	1	0	1	1	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0
1400	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0
1100	0	0	0	0	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	0	0	0	0
1000	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0	1	0	0	1
850	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
750	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
700	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1
450	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0
250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table c.1.4 :

Scores of Bands Generated by RAPD Primer OPA02

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	0	1	0	1	1	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0
1400	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0
1100	0	0	0	0	1	1	0	1	1	1	0	1	1	1	1	1	0	1	0	0	0	0	0
1000	0	0	0	1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0	1	0	0	1
850	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
750	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
700	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1
450	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table C1.5

Scores of Bands Generated by RAPD Primer OPA05

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1300	1	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	0
1100	0	0	0	0	1	1	0	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0
1000	1	0	1	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
850	0	0	0	0	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	0
750	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0
700	0	0	0	0	1	1	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0
500	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0
400	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0

Table C1. 6

Scores of Bands Generated by Primer OPA11

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1300	1	1	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	0	1	1	1	0
1100	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
850	1	1	0	0	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	0
750	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0
700	0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0
400	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Table C1.7
Scores of Bands Generated by RAPD Primer OPA16

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
1500	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	1
1400	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0	0	1	1	0	0	0	1	1
1100	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1
1000	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1
850	0	1	0	0	1	1	0	1	0	1	0	1	1	1	1	0	1	1	0	0	0	0	0
750	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Table C1.8
Scores of Bands Generated by RAPD Primer OPA18

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1000	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
850	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
750	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
600	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
500	0	1	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	0	1	1	1	1	1

TableC1.9

Scores of BandsGenerated by RAPD Primer OPA03

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
750	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table C10

Scores of Bands Generated by RAPD Primer OPA04

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1300	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	
1100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1000	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	1	0	1	0
850	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	1
750	1	1	0	0	0	1	1	0	0	1	0	1	0	0	1	0	1	1	1	1	1	1	1
600	1	1	1	1	0	1	0	0	0	1	0	0	1	0	1	0	1	1	1	1	0	1	1
500	0	0	0	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	1	0	0	0	1
400	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TableC1.11

Scores of Bands Generated by RAPD Primer OPA07

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1100	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
850	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
750	1	1	1	0	1	1	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0
600	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	1	1	1
500	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Table C1.12

Scores of Bands Generated by RAPD Primer OPA08

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1300	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	1	1	0	0	1	0
1000	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
750	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0
400	1	0	0	0	1	0	1	0	1	1	0	1	0	1	1	0	0	0	0	1	0	1	0

Table C.1.13
Scores of Bands Generated by RAPD Primer OPA09

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1300	1	1	0	0	1	0	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1
1000	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
850	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	1
750	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0
600	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1
500	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
400	1	1	0	0	1	1	1	1	0	0	1	0	1	1	1	0	0	1	1	1	1	0	0

Table C1.14
Scores of Bands Generated by RAPD Primer OPA10

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1300	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
750	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
600	1	1	1	0	1	1	1	1	1	0	0	0	1	0	1	1	0	1	0	1	0	1	0
500	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Table C1.15
Scores of Bands Generated by RAPD Primer OPA12

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1300	**1**	**0**	**1**	**0**	**1**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**0**	**1**	**1**	**1**	**1**
850	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
750	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
500	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
400	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**

Table C1.16
Scores of Bands Generated by RAPD Primer OPA14

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
850	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**1**	**1**
750	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**

Table C1.17
Scores of Bands Generated by RAPD Primer OPA15

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
1000	**1**	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**1**
850	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
750	**1**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**1**
500	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**

Table C1.18
Scores of Bands Generated by RAPD Primer OPA17

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
2000	1	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	1	1	0	1	1	1
1000	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
500	1	1	1	0	1	1	0	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1

Table C1.19
Scores of Bands Generated by RAPD Primer OPA19

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
2000	1	1	1	0	1	1	0	0	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1
1500	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1000	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
750	1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	1	1	1	1
600	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Table C1.20
Scores of Bands Generated by RAPD Primer OPA20

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
750	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
700	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	1	1	0	1
500	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1

Table C1.21
Scores of Bands Generated by RAPD Primer OPH05

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1500	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table C1.22
Scores of Bands Generated by RAPD Primer OPH08

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
2000	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
1100	0	0	0	0	1	1	0	0	1	0	0	1	1	1	1	1	0	1	1	1	0	0	0
1000	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1
850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
750	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0

Table C1.23
Scores of Bands Generated by RAPD Primer OP016

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
1500	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0
1100	1	0	1	0	1	1	0	1	1	1	0	0	0	1	1	0	1	1	1	1	0	1	1
1000	0	1	0	0	1	1	0	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1
750	1	1	1	1	1	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1	1	1	1
600	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1
500	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0

Table C1.24

Scores of Bands Generated by RAPD Primer OPH16

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
3000	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0		0	0	0	1	1
2000	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	1
1800	1	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1
1500	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	1
1300	1	1	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0
1100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
1000	0	0	1	1	1	1	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	0	0
750	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1
700	1	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
400	0	1	1	1	1	1	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0
300	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1

Table C1.25

Scores of Bands Generated by RAPD Primer OPH12

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	KR	S	DA	AD	A	B
400	1	1	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1

C2 ISSR PRIMERS SCORES

TableC2.1 :

scores generated by ISSR primer UBC807

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1500	0	1	1	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1
1100	0	0	1	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1
750	0	1	1	0	0	1	0	1	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1
650	0	1	1	1	1	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1
450	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Table C2.2 :

scores generated by ISSR primer UBC808

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
750	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
550	0	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1
450	0	1	1	1	1	1	0	1	1	0	1	1	1	0	0	1	1	1	1	0	1	1	1
400	0	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0

Table C2.3 :

scores generated by ISSR primer UBC810

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1250	0	1	1	0	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
1150	0	1	1	0	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1
1000	0	1	1	0	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
700	0	1	1	0	1	1	0	1	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1
650	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1

Table C2.4 :

scores generated by ISSR primer UBC811

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
950	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
550	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	0	0	0	0	1	1	1
450	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1

Table C2.5 :
scores generated by ISSR primer UBC812

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
850	0	1	1	1	0	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
800	0	1	1	1	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
700	0	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	0	1	0	1	1
650	0	1	1	0	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
500	0	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	0	1	1	1	1

Table C2.6 :
scores generated by ISSR primer UBC814

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1400	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1000	0	1	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
750	0	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1
550	0	1	1	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1
450	0	1	1	1	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
350	0	1	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1

Table C2.7 :
scores generated by primer UBC 815

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
2000	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
1400	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
900	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0
550	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0
500	0	1	0	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	1	0
300	0	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1

TableC2.8 :
scores generated by ISSR primer UBC816

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
550	0	1	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
500	0	1	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1

TableC2.9 :
scores generated by primer UBC817

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1400	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
1100	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1
900	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
750	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1
600	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
550	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
500	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
450	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
350	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TableC2.10 :
scores generated by primer BC818

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1700	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
1500	0	1	1	1	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
1400	0	1	1	1	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	1	1	1
1200	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1
1100	0	1	1	1	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
900	0	1	1	1	0	1	0	1	1	0	1	1	1	0	0	0	0	1	0	0	1	0	0
750	0	1	1	1	1	1	0	1	1	0	1	1	1	0	0	0	0	1	0	0	1	1	1
700	0	1	0	1	1	1	0	1	1	0	1	1	1	0	0	0	0	1	0	0	0	1	1
650	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
550	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0

Table C2.11:
scores generated by ISSR primerUBC823

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1200	0	1	1	1	1	0	0	0	1	0	1	1	1	1	0	0	1	0	0	1	1	1	1
750	0	1	1	1	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	1	1
550	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	0	1	0	0	1	1	1	1
500	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	0	1	0	0	1	1	1	1

Table C2.12:
scores generated by ISSR primer UBC862

	AN	Q	KH	HB	H	Z	KHD	KHR	HA	QI	AG	BL	MO	NE	GH	SF	BR	S	DA	AD	A (N)	B (N)	B (T)
1150	0	0	1	1	0	1	0	0	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1
900	0	0	1	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
800	0	0	1	1	1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
700	0	0	1	1	1	1	0	0	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1
650	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1
400	0	0	1	1	1	1	0	0	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1
300	0	0	1	1	1	1	0	0	1	0	1	1	1	1	0	0	0	0	0	0	1	1	1

جامعة النجاح الوطنية

كلية الدراسات العليا

التنوع الجيني في مجموعة التين الفلسطينية باستخدام تقنيتيّ RAPD و ISSR

اعداد

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اشراف

أ.د.محمد سليم علي اشتية

قدمت هذه الاطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين

2012

التنوع الجيني في مجموعة التين الفلسطينية باستخدام تقنيتي RAPD وISSR

إعداد

ايات خالد راشد مبسلط

اشراف

أ. د. محمد سليم علي اشتية

الملخص

اجريت هذه الدراسة لتحديد التنوع الوراثي في الصفات الجينية ل 23 صنفا من أصناف التين (*Ficus carica* L.) (20 صنفا محليا مشهورا وصنفين بريين وصنف واحد اجنبي) ممثلة للاصناف الموجودة في فلسطين ، باستخدام آليات التضخم العشوائي متعدد الأشكال للحمض النووي الرايبوزي (RAPD)، وجملة تكرار التسلسل البسيط (ISSR). 21 كاشفا وراثيا من اصل 25 كاشف استخدمت في الدراسة اظهرت انماطا مختلفة من حزم مضاعفة DNA .

وصل عدد مدخلات تقنية RAPD التي تم تحليلها نحو 1518، كان من ضمنها 420 لحزم DNA الموجودة (1)، و 1098 لحزم DNA غير الموجودة (0). وبحساب معامل التشاظهرت بعض الاصناف تشابها كثيرا بنسبة 77% بين زراقي وغزالي بينما لم يظهر بعض الاصناف أي نوع من التشابه كما في الصنفين عجلوني وقراعي . قيمت الكاشفات بحساب قيمة Rp لها فأظهر الكاشف OPH08 اعلى قيمة 3.16 بينما كانت15 . اقل قيمة للكاشف OPA03 . كما وتم حساب المسافات الجينية بين الاصناف باستخدام معامل تشابه النرد Dendrogram بالاعتماد على تحليل الكتلةUPGMA.و كانت حزم DNA الناتجة باستخدام RAPD موزعة على سبعة مجموعات اساسية.

وكان متوسط عدد حزم التضاعف باستخدام ألية RAPD (6.7) أكثر قليلا من تلك باستخدام آلية ISSR (5.57). وعلاوة على ذلك، فإن العدد الإجمالي للحزم المتضاعفة متعددة المظهر باستخدام RAPD (161 حزمة) أعلى بكثير من تلك الناتجة باستخدام آلية ISSR

(69 حزمة). الأمر الذي يشير إلى أن ألية استخدام RAPD كانت أكثر كفاءة من آلية ISSR من خلال عدد حزم DNA المتضاعفة في التفاعل الواحد.