

# Assessment of genetic variation between some Libyan date palm (*Phoenix dactylifera* L.) cultivars using molecular markers

# A THESIS SUBMITTED FOR THE AWARD OF THE Ph.D. DEGREE IN BOTANY (GENETICS)

## By

Abdullah Farag Mohamed Abogmiza Lecturer Assistant, Biology Department, Fac. Of Science, El-Asmarya University, Libya

## SUPERVISED BY

Professor Dr. Maher Mohamed Shehata Professor of Molecular Biology, Botany Department Faculty of Science, Ain Shams University

Professor Dr. Ahmed Fahmy Houssien Abo Doma Professor of Molecular Biology, Genetics Department Faculty of Agriculture, Ain Shams University

Dr. Noha Sayed Farag Khalifa Associate Professor of Molecular Biology, Botany Department, Faculty of Science Ain Shams University



## **List of Contents**

No.	Title	Page
I	Introduction	1
II	Literature Review	3
1	Morphological and biochemical study	3
2	Molecular study	6
III	Materials and Methods	24
1	Plant materials	24
2	Morphological traits of the fruits	24
3	Chemical components	25
4	Polysaccharide content	25
5	Total tannin contents	25
6	DNA extraction	26
7	DNA resolving buffer	26
8	ISSR analysis	27
9	Sequence-related amplified polymorphism (SRAP)	28
10	AFLP Florescent dye primer protocol	29
IV	Results and Discussions	31
1	Morphological study	31
2	Chemical contents of the fruit	35
3	Molecular studies	40
4	Inter Simple Sequences Repeats (ISSR)	40
5	Primer 807	41
6	Primer 844a	42
7	Primer 844b	43
8	Primer 17898a	44
9	Primer 17898b	45
10	Primer 17899b	47
11	Primer HB-1	48
12	Primer HB-4	49
13	Primer HB-8	50
14	Primer HB-10	52
15	Primer HB-15	53
16	Similarity indexes	56
17	Sequence-related amplified polymorphism (SRAP)	58
18	Combination Me10 x Em13	58
19	Combination DN6 x Em18	60

20	Combination Me9 x Em18	<i>4</i> 1
20		61
21	Combination me10 x Em18	62
22	Combination me8 x Em19	63
23	Combination me10 x Em19	64
24	Combination me 4 x Em 6	65
25	Combination me11 x Em10	66
26	Combination DN11 x Em20	67
27	Combination me5 x Em20	68
28	Similarity indexes	71
29	Amplified Fragment Length Polymorphism (AFLP)	74
30	Combination I	75
31	Combination II	78
32	Combination III	81
33	Combination IV	86
34	Combination V	89
35	Combination VI	93
V	REFERENCES	105
VI	SUMMARY	103
VII	ABSTRACT	III
VIII	ARABIC SUMMARY	1

## **LIST OF TABLES**

No.	Title	Page
1	the eight palm tree cultivars under investigation and the three	24
2	The used eleven ISSR primers and their sequences	27
3	the used SRAP primers and their sequences	28
4	Sequences of the six used AFLP primer pairs	30
5	The results of the six traits in the investigated eight palm date genotypes	31
6	Total reducing, non-reducing sugars and tannin contents	36
7	banding patterns of palm date collected from different locations in Libya using ISSR primer (807) recorded as 0 for absent and 1 for present	42
8	banding patterns of palm date collected from different locations in Libya using ISSR primer (844a) recorded as 0 for absent and 1 for present	43
9	banding patterns of palm date collected from different locations in Libya using ISSR primer (844b) recorded as 0 for absent and 1 for present	44
10	banding patterns of palm date collected from different locations in Libya using ISSR primer (17898a) recorded as 0 for absent and 1 for present	45
11	banding patterns of palm date collected from different locations in Libya using ISSR primer (17898b) recorded as 0 for absent and 1 for present	46
12	banding patterns of palm date collected from different locations in Libya using ISSR primer (17899b) recorded as 0 for absent and 1 for present	47
13	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-1) recorded as 0 for absent and 1 for present	49
14	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-4) recorded as 0 for absent and 1 for present	50
15	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-8) recorded as 0 for absent and 1 for present	51
16	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-10) recorded as 0 for absent and 1 for present	52
17	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-15) recorded as 0 for absent and 1 for present	53
18	Similarity indexes among the eight palm genotypes under investigation on the base of ISSR banding patterns	56
19	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em13) recoded as 0 for absent and 1 for present	59
20	banding patterns of palm date collected from different locations in Libya using SRAP primer (DN 6 x Em18) recoded as 0 for absent and 1 for present	60
21	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 9 x Em18) recoded as 0 for absent and 1 for present	61
22	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em18) recoded as 0 for absent and 1 for present	62
23	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 8 x Em19) recoded as 0 for absent and 1 for present	63
24	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em19) recoded as 0 for absent and 1 for present	65
25	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 4 x Em 6) recoded as 0 for absent and 1 for present	66
26	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 11 x Em10) recoded as 0 for absent and 1 for present	67
27	banding patterns of palm date collected from different locations in Libya using SRAP primer (DN 11 x Em 20) recoded as 0 for absent and 1 for present	68
28	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 5 x Em 20) recoded as 0 for absent and 1 for present	69

## **LIST OF TABLES**

No.	Title	Page
1	the eight palm tree cultivars under investigation and the three	24
2	The used eleven ISSR primers and their sequences	27
3	the used SRAP primers and their sequences	29
4	Sequences of the six used AFLP primer pairs	30
5	The results of the six traits in the investigated eight palm date genotypes	31
6	Total reducing, non-reducing sugars and tannin contents	36
7	banding patterns of palm date collected from different locations in Libya using ISSR primer (807) recorded as 0 for absent and 1 for present	42
8	banding patterns of palm date collected from different locations in Libya	43
9	using ISSR primer (844a) recorded as 0 for absent and 1 for present banding patterns of palm date collected from different locations in Libya	44
9	using ISSR primer (844b) recorded as 0 for absent and 1 for present	44
10	banding patterns of palm date collected from different locations in Libya	45
10	using ISSR primer (17898a) recorded as 0 for absent and 1 for present	15
11	banding patterns of palm date collected from different locations in Libya	46
	using ISSR primer (17898b) recorded as 0 for absent and 1 for present	_
12	banding patterns of palm date collected from different locations in Libya using ISSR primer (17899b) recorded as 0 for absent and 1 for present	47
13	banding patterns of palm date collected from different locations in Libya	49
	using ISSR primer (HB-1) recorded as 0 for absent and 1 for present	
14	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-4) recorded as 0 for absent and 1 for present	50
15	banding patterns of palm date collected from different locations in Libya	51
10	using ISSR primer (HB-8) recorded as 0 for absent and 1 for present	
16	banding patterns of palm date collected from different locations in Libya using ISSR primer (HB-10) recorded as 0 for absent and 1 for present	52
17	banding patterns of palm date collected from different locations in Libya	53
1 /	using ISSR primer (HB-15) recorded as 0 for absent and 1 for present	23
18	Similarity indexes among the eight palm genotypes under investigation on	56
1.0	the base of ISSR banding patterns banding patterns of palm date collected from different locations in Libya using	<b>7</b> 0
19	SRAP primer (me 10 x Em13) recoded as 0 for absent and 1 for present	59
20	banding patterns of palm date collected from different locations in Libya using	60
21	SRAP primer (DN 6 x Em18) recoded as 0 for absent and 1 for present banding patterns of palm date collected from different locations in Libya using	61
	SRAP primer (me 9 x Em18) recoded as 0 for absent and 1 for present banding patterns of palm date collected from different locations in Libya using	
22	SRAP primer (me 10 x Em18) recoded as 0 for absent and 1 for present	62
23	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 8 x Em19) recoded as 0 for absent and 1 for present	63
24	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em19) recoded as 0 for absent and 1 for present	65
25	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 4 x Em 6) recoded as 0 for absent and 1 for present	66
26	banding patterns of palm date collected from different locations in Libya using	67
	SRAP primer (me 11 x Em10) recoded as 0 for absent and 1 for present	07
27	banding patterns of palm date collected from different locations in Libya using SRAP primer (DN 11 x Em 20) recoded as 0 for absent and 1 for present	68
28	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 5 x Em 20) recoded as 0 for absent and 1 for present	69

29	Similarity indexes among the eight palm genotypes under investigation on	72
	the base of SRAP banding patterns	
30	banding patterns of palm date collected from different locations in Libyausing	76
50	florescent dye labeled AFLP primer (combination 1)	70
31	banding patterns of palm date collected from different locations in Libyausing	79
31	florescent dye labeled AFLP primer (combination I1)	1)
32	banding patterns of palm date collected from different locations in Libyausing	83
32	florescent dye labeled AFLP primer (combination II1)	03
33	banding patterns of palm date collected from different locations in Libyausing	87
33	florescent dye labeled AFLP primer (combination 1V)	07
34	banding patterns of palm date collected from different locations in Libyausing	90
Эт	florescent dye labeled AFLP primer (combination V)	70
35	banding patterns of palm date collected from different locations in Libyausing	94
33	florescent dye labeled AFLP primer (combination V1)	71
36	Total number of unique finger prints generated by primer pair	97
50	combinations with each palm genotype	)

## **LIST OF FIGURES**

No.	Title	Page
1	Map of the three different regions of collection in Libya	24
2-a	Representative images show the morphology and size of the fruit and seed	32
	of A: Umfetity, B: Bekrary, C: Alhamraya, D: Sufeer-genab, E: Alsaeedy show, F: Faraj Barameel, G: Majhool Alheelo, H: Alkhadraya	
2-b	Representative images show the trees and the production of A: Umfetity, B:	33
	Bekrary, C: Alhamraya, D: Sufeer-genab, E: Alsaeedy show, F: Faraj Barameel, G: Majhool Alheelo, H: Alkhadraya	
3-a	The fruit length in the eight date palm cultivars	33
3-b	The fruit diameter in the eight date palm cultivars	33
3-c	The fruit weight in the eight date palm cultivars	34
3-d	The flush weight in the eight date palm cultivars	34
3-е	The seed weight in the eight date palm cultivars	34
3-f	The Total fruit production in Kg / year in the eight date palm cultivars	34
4-a	Total sugar contents as percentage of flash dry weight	36
4-b	Reducing sugar contents as percentage of flash dry weight	36
4-c	Non-reducing sugar contents as percentage of flash dry weight	37
4-d	Tannin contents as percentage of flash dry weight	37
5	profile of palm date collected from different locations in Libya using ISSR primer (807)	42
6	profile of palm date collected from different locations in Libya using ISSR primer (844a)	43
7	profile of palm date collected from different locations in Libya using ISSR primer (844b)	44
8	profile of palm date collected from different locations in Libya using ISSR primer (17898a)	45
9	profile of palm date collected from different locations in Libya using ISSR primer (17898b)	46
10	profile of palm date collected from different locations in Libya using ISSR primer (17899b)	48
11	profile of palm date collected from different locations in Libya using ISSR primer (HB-1)	49
12	profile of palm date collected from different locations in Libya using ISSR primer (HB-4)	50
13	profile of palm date collected from different locations in Libya using ISSR primer (HB-8)	51
14	profile of palm date collected from different locations in Libya using ISSR primer (HB-10)	52
15	profile of palm date collected from different locations in Libya using ISSR primer (HB-15)	53
16	Cluster analysis with UPGMA method of related eight Libyan date palm	57
	genotypes using 10 pairs of ISSR primer combinations of data based on Jaccard similarity matrix. S1 (Umfetity), S2 (Bekrary), S3 (Alhamraya), S4	
	(Sufeer-ganab), S5 (Alsaeedy show), S6 (Faraj Barameel), S7 (Majhool	
	Alheelo) and S8 (Alkhadraya)	<b>#</b> 0
17	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em13)	59
18	banding patterns of palm date collected from different locations in Libya using SRAP primer (DN 6 x Em18)	60

19	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 9 x Em18)	62
20	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em18)	63
21	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 8 x Em19)	64
22	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 10 x Em19)	65
23	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 4 x Em6)	66
24	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 11 x Em10)	67
25	banding patterns of palm date collected from different locations in Libya using SRAP primer (DN 11 x Em20)	68
26	banding patterns of palm date collected from different locations in Libya using SRAP primer (me 5 x Em20)	69
27	Cluster analysis with UPGMA method of related eight Libyan date palm genotypes using 10 pairs of SRAP primer combinations of data based on Jaccard similarity matrix. S1 (Umfetity), S2 (Bekrary), S3 (Alhamraya), S4 (Sufeer-ganab), S5 (Alsaeedy show), S6 (Faraj Barameel), S7 (Majhool Alheelo) and S8 (Alkhadraya)	73
28	pseudo gel showing the banding patterns of eight palm date genotypes collected from different locations in Libya using florescent dye labeled AFLP primer (combination 1)	77
29	pseudo gel showing the banding patterns of eight palm date genotypes collected from different locations in Libya using florescent dye labeled AFLP primer (combination I1)	81
30	pseudo gel showing the banding patterns of eight palm date genotypes collected from different locations in Libya using florescent dye labeled AFLP primer (combination II1)	86
31	pseudo gel showing the banding patterns of eight palm date genotypes collected from different locations in Libya using florescent dye labeled AFLP primer (combination 1V)	89
32	pseudo gel showing the banding patterns of eight palm date genotypes collected from different locations in Libya using florescent dye labeled AFLP primer (combination V)	93
33	pseudo gel showing the banding patterns of eight palm date genotypes collected from different locations in Libya using florescent dye labeled AFLP primer (combination V1)	96
34	Cluster analysis with UPGMA method of related eight Libyan date palm genotypes using 6 pairs of AFLP primer combinations of data based on Jaccard similarity matrix. S1 (Umfetity), S2 (Bekrary), S3 (Alhamraya), S4 (Sufeer-genab), S5 (Alsaeedy show), S6 (Faraj Barameel), S7 (Majhool Alheelo) and S8 (Alkhadraya)	98

#### **ACKNOWLEDGMENTS**

First and foremost grateful thanks to "ALLAH" the most beneficent and merciful.

And words would not be sufficient to express my deepest gratitude and appreciation to prodigious efforts of prof. Dr. Maher Mohamed Shehata Professor of Molecular Biology, Botany Department, Faculty of Science, Ain Shams University, for his supervising and illuminating criticism in reading the manuscript. I greatly appreciate his meticulous guidance, emotional support and valuable time.

Words also are not enough to reply Prof. Dr. Ahmed Fahmy Houssien Abo Doma, Professor of Molecular Biology, Genetics Department, Faculty of Agriculture, Ain Shams University, for his generous supervision, and for the critical reading and revision of all details of manuscript. His scientific merit, deep experience, and constant support leading to completion of this thesis.

I am especially indebted to Dr. Noha Sayed Farag Khalifa Associate Professor of Molecular Biology, Department of Botany, Faculty of Science, Ain Shams University, for her great efforts in planning the practical work, scientific help, constructive comments, kind co-operation and participation in revising the whole work and for giving me so much attention and time. To her I shall be forever grateful.

I would like to extend my deepest appreciation to **Dr Mohamed**Hamdy Emam Ammar, Associate Professor, Director of Plant Gene
Bank, Alshekh zouaied, Sinai, Dissert Research Center, for his great efforts in conducting the molecular analysis. To him I shall be forever grateful.

Also, I would like to extend my deepest appreciation to Dr. Mahmoud Magdi Almossalamy, Lecturer, Department of Genetics, Faculty Agriculture, Ain Shams University, for his great efforts in conducting the molecular analysis. To him I shall be forever grateful.

Also, I would like to extend my deepest appreciation to Dr. Reda Rezk, Head Researcher, Center of Date Palm Institute, , for his great efforts in analysis of Tannin and sugar contents. To him I shall be forever grateful.

Also, I would like to extend my deepest appreciation to Mr. Mohamed Basheer Alheelo, for his great efforts in supplying us with some the date Palme genotypes. To him I shall be forever grateful.

#### **ABSTRACT**

In this investigation, some fruit characteristics of eight Libyan date palm genotypes (collected from three different regions in Libya) were measured as well as sugar and tannin contents were estimated to distinguish between these genotypes under investigation.

Moreover, finger printing of these eight Libyan date palm genotypes was performed on the molecular level using three different techniques, these were inter simple sequences repeats (ISSR), sequences related amplified polymorphism (SRAP) and Amplified fragments length polymorphism (AFLP). The results revealed a kind of biodiversity among the genotypes under investigation, either on the morphological or biochemical and molecular levels.

## Introduction

The botanical name of the date palm, *Phoenix dactylifera* L., is presumably derived from a Phoenician name "phoenix", which means date palm, and "dactylifera" derived from a Greek word "daktulos" meaning a finger, illustrating the fruit's form.

Another source refers this botanical name to the legendary Egyptian bird, "Phoenix", which lived to be 500 years old, and cast itself into a fire from which it rose with renewed growth, **Zaid and Wet (1983)**. This resemblance to the date palm, which can also re-grow after fire damage, makes the bird and the date palm share this name, while "dactylifera" originates from the Hebrew word "dachel" which describes the fruit's shape.

Belonging to the Angiosperms-Monocotyledons, *Palmaceae* is a family of about 200 genera and 1, 500 species, **Dowson** (**1982**). Phoenix (*Coryphoideae Phoeniceae*) is one of the genera which contain a dozen species, all native to the tropical or subtropical regions of Africa or Southern Asia, including *Phoenix dactylifera* L. According to **Dransfield and Uhl,** (**1986**) date palm is classified as follows: Group: Spadiciflora, Order: Palmea, Family: Palmaceae, Sub-family: Coryphyoideae, Genus: Phoenix, Species: *Dactylifera* L.

In 2001 the top five date producing countries were Egypt, Iran, Saudi Arabia, Pakistan and Iraq, accounting for about 69% of total production. If the next five most important countries are included, i.e. Algeria, United Arab Emirates, Libya, Oman and Morocco, then this percentage rises to 90%. This clearly indicates that most of the world's date production is concentrated in a few countries in the same region.

Date fruit has been identified as a highly nutritious food with many functional benefits to human health. Date fruits have been studied for their proximate, mineral, and phytochemical compositions, and several authors have reviewed the value of date fruits as an emerging "healthy" food (Al-Farsi and Lee 2008; Vayalil 2012). However, date seeds, the major by-product of commercial date fruit processing, have not been fully utilized. It is anticipated that date seeds could be an excellent source of some bioactive compounds with potential applications in the food and pharmaceutical industries. Date fruits and seeds are of high research interest due to their high nutritional value and high contents of functional ingredients.

This investigation aimed to study the variation among some date palm genotypes grown in different locations in Libya on the base of the fruits morphology, some of the fruit biochemical contents such as tannin and sugar contents and to study the biodiversity among these genotypes under investigation in the molecular level using different molecular techniques such as SRAP, ISSR and AFLP florescent dye.

#### LITERATURE REVIEW

## Morphological and biochemical study

Samarawira (1983) reported that the total sugars in the date palm fruit is between 70 and 80%. Approximately 60% of the dry weight at the Khalal and early Rutab stages of fruit development is sucrose. High-yielding varieties of date palm have fruit yield potentials of 12.0 tons/ha, equivalent to an estimated production of 7.2 tons/ha of sucrose if the fruit is harvested at the stage of maximum sucrose accumulation. The estimated sucrose production from the date palm compares very favourably with the world average of 6.6 tons/ha for sugarcane and the sugar beet average of 5.6 tons/ha for Europe. The relatively high sugar content of the fruit suggests that the date palm may have an important agro-industrial future as a potential source for refined sugar.

Bacha et al. (1987) studied some physical and chemical characteristics of the fruits of four different date palm cultivars during three stages of fruit development (kimri. khalal and tamar). The results showed that such characteristics varied greatly from one stage to another with some variations between cultivars and seasons. Fruit weight, size, length, diameter and seed weight increased from Kimri to Khalal stages followed by a slow decline in the tamar stage. Moisture, ash, protein and tannins contents decreased sharply especially in the tamar stage. Total soluble solids (T.S.S.) and total sugars increased progressively from Kimri, Khalal and tamar stages.

The date palm not only provided a concentrated energy food, it also yielded a variety of products for use in agricultural production and for domestic utensils, and practically all parts of the palm had a useful purpose. Examples of such uninhibited growth can still be found in some of the more remote areas of the Sahara, **FAO** (2002).