An-Najah National University

Faculty of Graduate Studies

Phylogenetic Relationship among Closely Related Species of the Genera *Lens*, *Vicia*, *Lathyrus* and *Pisum* (Leguminosae) in Palestine

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Dedication

To my dear parents, sisters (Sana', Wafa') and brothers (Ashraf, Alaa') with love and respect.

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الاقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Phylogenetic Relationship among Closely Related Species of the Genera *Lens*, *Vicia*, *Lathyrus* and *Pisum* (Leguminosae) in Palestine

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Declaration

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

Student`s name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

Table of Contents

List of Tables

List of Plates and Figures

List of Abbreviations

DNA	Deoxyribonucleic acid
cpDNA	Chloroplast deoxyribonucleic acid
mtDNA	Mitochondrial deoxyribonucleic acid
rDNA	Ribosomal deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
ITS	Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
Taq	Thermus aquaticus
$MgCl_2$	Magnesium Chloride
NH_4Cl	Ammonium Chloride
HgCl	Mercuric Chloride
Mix	Mixture
Fl	Flowering
bp	Base pair
S	Svedberg unit
L	*Lens* or *Lathyrus;* according to the species.
P	*Pisum*
V	*Vicia*
S	*Salvia*
L.	Carl Linnaeus
Sm	James Edward Smith
Medik	Friedrich Kasimir Medikus
Boiss	Pierre Edmond Boissier
Mill	Philip Miller
MEGA5	Molecular Evolutionary Genetics Analysis version 5
UPGMA	Unweighted Pair Group Method
K2P	kimura 2- parameter model
NCBI	National Center for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
v	Version
Mt	Mountain
W	West, western
E	East, eastern
N	North, northern
C	Central
bs	Bootstrap
V. no.	Voucher number

Phylogenetic Relationship among Closely Related Species of the Genera *Lens, Vicia, Lathyrus* and *Pisum* (Leguminosae) in Palestine

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Abstract

Leguminosae or Fabaceae is the third largest flowering plant family, which is an important economically for food production and soil fertility. Four Palestinian genera of the Fabaceae family which are *Lens*, *Vicia*, *Lathyrus and Pisum* have overlapping morphological characteristics among them were the subject of this study. Different molecular techniques are used to determine the relationship and genetic diversity among Leguminosae species of interest including internal transcribed spacers (ITS) sequencing.

This study was performed to identify the phylogenetic relationship among 9 closely related species belong to 4 genera of Leguminosae family: *Lens culinaris, Vicia sativa, Vicia palaestina, Vicia peregrina, Vicia faba, Vicia narbonensis, Lathyrus aphaca, Pisum fulvum* and *Pisum sativum*.

Moreover, In spite of that *L. culinaris* and *V. Palaestina* are two species belong to different genera; their morphological features are similar to a high degree. Therefore, this study was performed to determine the taxonomic classification of *V. palaestina* and its phylogenetic relationship to the morphological closely related species *L. culinaris*.

Morphological classification was conducted for some closely related species of the Leguminosae family in Palestine. The studied plant species were collected from natural wild habitat. Then a representative plant specimen of each species was deposited at An-Najah National University Herbarium. Then molecular analysis was determined using 18S and 28S as universal primers for amplifying and sequencing the ITS region. Evolutionary divergence between sequences was estimated and phylogenetic tree was constructed using the Kimura 2-parameter model and Unweighted Pair Group method, respectively.

Each sample ITS sequence of the examined species as well as *Salvia dominica*, as an out group, were introduced into the GenBank. The phylogenetic analysis among studied species revealed that, *V. palaestina* (KJ864941) and *P. sativum* (KJ864946) have a high average of intraspecies genetic divergence (8.95%). While, low average of intraspecies genetic divergence (1.81%) between *V. faba* (KJ864957, KJ864958) and *V. peregrina* (KJ864954) was recorded. Moreover, *V. peregrina*, *V. faba*, *V. narbonensis*, *V. palaestina*, *L. culinaris*, and *V. sativa* were grouped into one clade (clade I). However, *V. sativa* occupied the farther position in clade I and separated from other species of *Vicia* genus. While, *P. sativum, P. fulvum* and *L. aphaca* were grouped into the same clade (clade II). On the other hand, *L. culinaris* formed a cluster within the core of *Vicia* near to *V. Palaestina*.

In this study, phylogenetic relationship among the closely related species under study of Leguminosae family was detected accurately using ITS sequencing. Also this technique revealed the close relationship between *L. culinaris* and *V. palaestina*, as the two species are related to the same genus instead of two different genera.

Chapter One

Introduction

1.1. General Background

Biological diversity is used to explain the variety of life forms; the genetic diversity that they include and the ecological role that they have, which is defined at three levels; genetic diversity, diversity of species and diversity of ecosystems (1). Genetic diversity is related to the diversity within a species according to the variations in genes and their recombination (2, 3). It determines the set of biochemical, physiological and morphological changes of an individual that are known as the phenotypes of the species. The main cause of genetic variability is the random mutations of DNA nucleotide sequence (1). Through the evolutionary process, the genetic diversity is very important for the survival of the species, because it assists the species to adapt to environmental changes through the natural selection process and decrease the risk of species extinction (4). The diversity of species expresses the species richness, the number of species present in a defined area, and the abundance of different species that locate in the same habitat (phylogenetic diversity) (4, 5). The stability of the ecosystem and the entire biosphere mainly depends on the species diversity (1). The large differences between the ecological process, types of ecosystems and habitat diversity of each type of ecosystem are known as ecosystem diversity (6). The concept of ecosystem diversity is closely related to the genetic diversity and species diversity (1).

Mediterranean climate regions have unique ecosystems due to its distinct climate; hot drought summer and cool moist winter (7). Although this climate mainly spreads on 5% of the land surface of the earth, 20% of the plant species were identified in these regions (8).

There is an obvious association between legumes distribution in nature and their morpho-physiological differences with the soil characteristics, related to the nature of parent rock, soil chemical properties and soil texture (9). Legumes in the Mediterranean semiarid areas play a main role as a pasture plants for their forage quality and assisting nitrogen fixation, which are increase soil fertility and giving the nutritional requirements for other plant species (10).

Palestine is situated at a meeting point among Asia, Africa and Europe in the southeastern of the Mediterranean Sea. This location gives Palestine floral diversity in a wide range (11, 12, 13), which contains a large number of species and a high density of endemic species, approximately 5% (120 endemics) of the total number of plants that grow in Palestinian Territory. The West Bank and Gaza Strip contain about 2,076 plant species, where 1,959 species in 115 families inhabit the West Bank and 1,290 species in 105 families inhabit the Gaza Strip (14). A great wealth of biodiversity (in terms of the number of species, ecosystems and landscape) was known in Palestine (15). It was recorded as an important center of genetic diversity to maintain the life of many crops like barley, onions, vines, wheat and pulses (14). Leguminosae, Compositae, Cruciferae, Liliaceae, Gramineae and Labiatae were identified as the most dominant families of Palestine (16, 17).

1.2. Leguminosae Family

Legumes are flowering plants belong to Leguminosae family (Fabaceae). They vary in size and inhabit from herbaceous to woody plants, which are widely distributed worldwide. However, legumes mainly grow in tropical and temperate temperature climate (1, 18, 19). Legumes are used as an important source of food for humans and animals; in addition to their synthesis of many secondary compounds for medical principles, colouring, etc. (1).

The leaves of this family are usually of pinnate compound form, and arranged alternately up the stem. Zygomorphic, hermaphrodite flowers with a five merous corolla are known in these family members. Their flowers are composed of one large petal, the standard, which folds over the rest petals for protection. In front of this petal, two horizontal petals are called wings, while, the last two petals are united by their margins that are known as the keel. Their fruit (pod) is often one celled, that usually dehisces along two opposite longitudinal splits which is known by legume fruit type. The pod of legumes contains one or few seeds with a large embryo and little endosperm for each seed. These plants can absorb nitrogen by the irregular nodules on their roots that are needed for plants growth (20).

Four genera of the Fabaceae family which are *Lens*, *Vicia*, *Lathyrus and Pisum*, have overlapping characteristics among them to a variable degree (19). Therefore, their phylogenetic relationship will be investigated in this study.

Lens is one of the oldest cultivated legumes, which grow in the Mediterranean, Europe, and Temperate West Asia (21, 22). According to its morphological characteristics, this genus was divided into five species:

L. culinaris, L. orientalis, L. ervoides, L. nigricans and *L. montbretti*; out of which the only cultivated one is *L. culinaris* as the others are wild types (23).

The temperate region of Asia, Europe and North and South America are the sites where *Vicia* genus is distributed, where, more than 166 species are recognized in the world. Nevertheless, its diversity site is in the Mediterranean region (24, 25). Different species of *Vicia* genus were identified in Palestine, for example, *V. ervilia, V. narbonensis, V. sativa, V. villosa, V. palaestina, V. peregrina* and others (20).

The third genus *Lathyrus* is the largest one, as represented by about 170 species, which are mainly located in the Mediterranean and Irano-Turanian region (26, 27, 28). Morphological characteristics of *Lathyrus* genus are more clear than *Vicia* genus although they have the same size (29). *Lathyrus sativus, L. cicera* and *L. orchrus* are the primary *Lathyrus* species that are used for human feeding (28), while other species are used as model organisms like: *L. odorus, L. niger* and *L. japonicas* (30, 31, 32, 33).

Pisum genus is one of the Fabaceae family, that is distributed throughout Southwest Asia and Northeast Africa (34). Five species of *Pisum* genus are used as food source, out of which three are wild, that are *P. humile, P. elatine* and *P. fulvum*. However, the other species are cultivated legumes: *P. abyssinicium* and *P. sativum* (35). Furthermore, the last one was used as a model organism in Mendel's low (36).

1.3. Relationship among the Four Fabaceae Genera

Each genus (*Lens, Vicia*, *Lathyrus* and *Pisum*) has at least one special character that makes it different from the others. However, many of the morphological continuum were observed in all genera, especially between *Lens* and *Vicia*, as the only difference between these two genera is the style. Hence, *Lens* is a *Vicia* with a *Lathyrus* style characters (19, 37). Moreover, overlapping characteristics were detected in *Lens culinaris*, *Vicia sativa* and *Lathyrus sativus* (38). Also *Vicia sativa platysperma* and *Vicia lunata* have an intermediate forms between *Vicia* and *Lens* (19).

Although the legumes classification depends on the morphological characters (1), 59 characters of some *Vicia* species and *L. culinaris* were studied. In that study, it was shown that similar characteristics between *Vicia faba* and its *Vicia* closest species were less than that of *L. culinaris* similarity to many *Vicia* species (39). From this point of view we can wonder why *V. faba* was classified in *Vicia* genus rather than other related genera.

On the other hand, chromosome numbers were reported for the studied species of the four genera. Slightly differences among them were observed ($2n = 10, 12, 14$) even among samples of the same species. For example, the different samples of *V. sativa* have variation in their chromosome numbers ($2n = 10, 12$) (20).

1.4. Molecular Phylogenetic Relationship

The chloroplast genome (cpDNA), the mitochondrial genome (mtDNA) and the nuclear genome (DNA) are sources of DNA in plant cells for studying the genetic diversity and phylogenetic relationship among plants (40). However, rDNA (ribosomal DNA) genes are specific genes of the nuclear genome that are used for genetic diversity (41). Internal transcribed spacers (ITS) have a high degree of variation, even between closely related species, so this portion of the genome is exploited in many biodiversity topic studies (42, 43, 44, 45).

In 1970, Davis and Plitmann, determined that many taxonomic problems in the genus *Vicia* exist, because it is very difficult to distinguish this genus from *Lathyrus* and *Lens*. Many species of *Vicia* and *Lathyrus* overlap in a variable degree both genetically and morphologically due to the environmental variations. For example the morphological characteristics of *Vicia koeiana* are very similar to many species of the genera *Lathyrus* and *Lens* with irrespective of style characteristics (46).

Later on, in 1982, Ladizinsky and saker, suggested that *Lens montebretti* should be moved from the genus *Lens* into the genus *Vicia* due to the morphological and cytological studies, and as a result, should be named as *Vicia montbretti* (47). Moreover, molecular analysis was used to differentiate *L. montbretti* from *V. montbretti*. Therefore, in 2002, Mayer and Bagga, placed *L. montbretti* in the genus *Vicia*, although it has lentoid calyx, style and flattened seed characteristics (48).

While, in 2003, Steel and Wojciechowski, showed that *Pisum sativum* is sister to monophyletic *Lathyrus* species based on morphological and molecular information. *Lens* species created a clade near to *Vicia* species, indicating that *Lens* is close genus to *Vicia* (49).

The genetic analysis of these four genera detect if *Lens, Vicia*, *Lathyrus* and *Pisum* have the same ancestor, due to the similar characteristics between them. Hence, comparative genomic studies were conducted to define patterns of chromosomal evolution and detect phylogenetic relationships among species. Moreover, comparative genomics can facilitate the use of genomic resources among different legumes species, and help to reduce cost and increase efficiency in genetic research as well as crop breeding. For example, the use of conserved genome structure to assist in transferring knowledge among related plant species is well established in grasses where synteny greatly assists in gene identification among related species (50).

1.5. Aim of this Study

- Phylogenetic relationship among four closely related genera will be constructed, which are represented by the species: *Lens culinaris, Vicia sativa, Vicia palaestina, Vicia peregrina, Vicia faba, Vicia narbonensis, Lathyrus aphaca, Pisum fulvum* and *Pisum sativum*.
- The taxonomic classification of *Vicia palaestina* and its phylogenetic relationship to the morphological closely related species *Lens culinaris* will be investigated in this study.

Chapter Two

Materials and Methods

2.1. Plant Material Collection

Two to five fresh plant specimens of species under study of *Lens, Vicia, lathyrus* and *Pisum* were collected from different localities of Palestine via intensive field trips during the period of study (December – April / 2014), and deposited under suitable conditions at An-Najah National University Herbarium, Department of Biology and Biotechnology, Faculty of Science, An-Najah National University. Coloured photographs, field observations, as well as some ecological notes were collected. Table 2.1 shows the scientific names, common names, wild or cultivated state and the different localities of the collected species.

When collecting herbarium material of Leguminosae, field notes on the following characters of taxonomic importance should be made: colour and shape of the flowers, which may change on drying; shape of the stipules, form and arrangement of the leaves and the hairy style distribution if present.

Measurements were taken for different parts of each specimen as presented in the morphological descriptions. These measurements were made on at least 15 specimens on average for each species provided that enough material was available.

The collected material of plant specimens were pressed until drying. Then poisoned chemically using a mixture of mercuric chloride and ammonium chloride (150 g of mercuric chloride (HgCl) and ammonium chloride (NH_4Cl) were dissolved in as little water as possible. After that 10 L of 96% ethanol were added to the previous mixture). The poisoned plant specimens were identified, labeled; provided with herbarium voucher number (V. no.) (Table 2.2) and mounted on herbarium sheets. Finally deposited at the herbarium, Department of Biology and Biotechnology, Faculty of Science, An-Najah National University.

Table 2.1. Scientific names of studied species with their: common names, wild or cultivated states and the locations they were collected from.

No. of species	**Scientific name**	**Common name**	**Wild vs. Cultivated**	**Place**
1	*L. aphaca* L.	Yellow pea, Yellow vetch	Wild	Tubas
			Wild	Tubas
2	*P. fulvum* Sm.	Tawny pea	Wild	Salfit
			Wild	Taluza
			Wild	Taluza
			Wild	Taluza
3	*P. sativum* L.	Garden pea	Cultivated	Aqqaba
			Cultivated	Yasid
			Cultivated	Qalqilia
			Cultivated	Maithaloun
			Cultivated	Salfit
4	*V. palaestina* Boiss.	Palestine vetch	Wild	Beit-Wazan
			Wild	Beit-Leed
			Wild	Beit-Leed
			Wild	Al-Ameryah
			Wild	Tubas
5	*V. peregrina* L.	Rambling vetch	Wild	Salfit
			Wild	Salfit
			Wild	Beit-Wazan

			Wild	Beit-Wazan
			Wild	Al-Ameryah
6	*V. sativa* L.	Common vetch	Cultivated	Yamun
			Cultivated	Yamun
			Cultivated	Tamun
			Cultivated	Tamun
			Wild	Beit-Wazan
7	*V. faba* L.	Broad bean, fava bean	Cultivated	Maithaloun
			Cultivated	Salfit
8	*V. narbonensis* L.	Narbon bean	Wild	Tubas
			Wild	Tubas
9	*L. culinaris* Medik.	Lentil	Cultivated	Yasid
			Cultivated	Yasid
			Cultivated	Qalqilia
			Cultivated	Qalqelia
			Cultivated	Maithaloun

Table 2.2. Voucher numbers, GenBank numbers of the studied species and their scientific names.

No. of species	Scientific name	Voucher numbers	GenBank no.
1	*L. aphaca* L.	1606a	KJ864924
		1606b	KJ864925
2	*P. fulvum* Sm.	1598	KJ864933
		1607a	KJ864934
		1607b	KJ864935
		1607d	KJ864936
3	*P. sativum* L.	1579	KJ864943
		1580	KJ864945
		1584	KJ864942
		1586	KJ864944
		1609	KJ864946
4	*V. palaestina* Boiss.	1603	KJ864940
		1610a	KJ864937
		1610b	KJ864941
		1611	KJ864938
		1570	KJ864939
5	*V. peregrina* L.	1600a	KJ864952
		1600b	KJ864955
		1613a	KJ864953
		1613b	KJ864956
		1614	KJ864954
6	*V. sativa* L.	1601a	KJ864947
		1601b	KJ864950
		1602a	KJ864948
		1602b	KJ864951
		1612	KJ864949
7	*V. faba* L.	1578	KJ864957
		1582	KJ864958
8	*V. narbonensis* L.	1571a	KJ864959
		1571b	KJ864960
9	*L. culinaris* Medik.	1581a	KJ864928
		1581b	KJ864929
		1585a	KJ864930
		1585b	KJ864931
		1599	KJ864932

2.2. Taxonomical Analysis and Identification of the Collected Plant Species

The collected legumes genera and species under study were classified and identified according to their morphological properties, based on botanical characters of different parts using Flora Palaestina (20); such as:

1. Leaves arrangement, shape and size.
2. Stipules presence, shape and size.
3. Flowers colour, texture and form.
4. Fruits style, texture and size.
5. Seeds colour, shape and number.

Moreover, a specific key for the studied species was constructed and provided.

2.3. Genomic DNA Extraction

Total genomic DNA extraction was performed from previously frozen leaf samples. *Salvia dominica* (labiatae) was included as an outgroup (Table 2.1). Samples were ground to a fine powder using mortar and pestle in the presence of liquid nitrogen. Then 0·1 g of leaf tissue was processed with PureLink^{TM} Plant Total DNA Purification Kit (Invitrogen, USA), following the manufacturer's protocol for isolation of total genomic DNA. That contains three major steps; plant lysate preparation, washing and elution of DNA.

2.4. PCR Amplification and Gel Electrophoresis

Ribosomal DNA (rDNA) codes for the RNA component of the ribosome. It is arranged in tandem arrays separated by the intergenic spacer and each unit codes for the large (28S) and small (18S) rRNA subunits. Separated from, and between the two subunits lie the gene 5.8S as well as two spacers, ITS-1 and ITS-2. The ITS region is a widely used marker since the development of universal primers.

The nuclear ribosomal DNA encompassing the ITS regions (ITS-1 spacer, 5.8S rDNA and ITS-2 spacer) were amplified using universal primers (51). Here the primer sequences were CCT TMT CAT YTA GAG GAA GGA G for 18S and CCG CTT ATT KAT ATG CTT AAA for 28S. The PCR reaction mix with a final volume of 25 μL, was performed with 12.5 μL of PCR premix with 1.5 mM MgCl2 (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma, USA), 0.4 μM of each primer and 2 μL of template DNA. The amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94ºC was followed by 35 cycles of denaturation at 94ºC for 45 s, annealing at 56ºC for 1 min and extension at 72ºC for 2 min, with a final extension step at 72ºC for 5 min. The PCR products were resolved by electrophoresis through 1.5% agarose gels to determine the size of amplified fragment after ethidium bromide staining (0.5 μg/ml).

2.5. DNA Cleaning and Sequencing

The PCR products were cleaned with ChargeSwitch®-Pro PCR Clean-Up Kit (Invitrogen, USA), following the manufacturer's protocol PCR product clean up. DNA PCR products were sequenced by dideoxynucleotide chain termination method using 3130 Genetic Analyzer (Applied Biosystems®, USA), Bethlehem University, Bethlehem, Palestine. This method depends on the principle that single-stranded DNA molecules that differ in length, due to the presence of dideoxynucleotides triphosphates as DNA chain terminators, can be separated from one another using polyacrylamide gel electrophoresis. The sequencing PCR reaction was performed with 18S and 28S primers used singly in forward and reverse reactions and BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems®, USA). Sequence information was further submitted for accession number in primary bioinformatics web servers.

2.6. Sequence Alignment and Phylogenetic Analysis

The comparison of the continuous sequences of ITS region of 9 Leguminosae species was made with previously available sequences in NCBI (National Center for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool) system. Multiple alignments were done using ClustalW of the computer program CLC Main Workbench software (version 5.6.1, 2009, CLC bio, Aarhus, Denmark). Phylogenetic analysis was based on alignments obtained from ClustalW of a 600 bp sequence. Phylogenetic tree was constructed using the program Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in the same software. The robustness of the groupings in the UPGMA analysis was assessed with 1000 bootstrap (bs) resamplings. Reference sequences were retrieved from GenBank used for phylogenetic analysis and *S. dominica* was used as an outgroup. Pairwise distances were computed using the Kimura 2-parameter method (52).

Chapter Three

Results

3.1. Morphological Characterization of Leguminosae Family

3.1.1. Classification of Leguminosae Family

Kingdom: Plantae
Super division: Spermatophyta
Division: Angiosperma
Class: Dicotyledoneae
Family: Leguminosae

3.1.2. Description of the Leguminosae Family

Herbaceous or woody plants, rarely creeping or climbing, sometimes spiny. Leaves alternate, rarely opposite, usually pinnate, 3-foliolate or digitate, sometimes terminating in or reduced to a tendril, rarely leaves simple; stipules usually present. Flowers hermaphrodite, in axillary or terminal racemes, panicles, heads or spikes, rarely solitary, zygomorphic. Calyx 5-, rarely 4-merous, with sepals more or less united at base, often 2-lipped. Corolla 5-merous, papilionaceous, consisting of a posterior, outermost petal (the standard), 2 lateral, often horizontal, petals (the wings) and 2 anterior and innermost ones usually united by their margins (the keel). Fruit usually a 1-celled, many seeded pod (legume), dehiscing by 2 valves along the ventral and dorsal sutures. Seeds usually fairly large, sometimes arillate.

3.1.3. Key to the Genera under study (Leguminosae)

1. All or part of leaves simple with tendrils, or compound leaves of 1 pair of leaflets; rarely leaves of more than 2 pairs but then the pod with 2-4 prominent longitudinal nerves or leaflets digitate; stipules and leaflets always entire, the former not dark-spotted. . . . . **1. *Lathyrus***

1. All or part of leaves compound, digitate, pinnate. Leaves of 2 to many pairs of leaflets, rarely only of 1 pair but then stipules dentate or dark-spotted or both.

2. Stipules usually longer than leaflets, 1 cm broad or more, dentate or lobed, nerve dark-spotted; leaflets 1-3 pairs, dentate or lobed. . . . . **2. *Pisum***

2. Stipules shorter than leaflets, less than 1 cm broad, entire, rarely dentate but then dark-spotted; leaflets mostly in several pairs, entire or only notched at apex, rarely dentate but then pod with ciliate-tuberculate margin.

3. Pod at least twice as long as broad. Style hairy all around or only on lower (anterior) side (that facing the keel). Seeds not lenticular. . . . . **3. *Vicia***

3. Pod shorter than above. Style hairy on upper (posterior) surface and glabrous beneath. Seeds compressed, lenticular.
. . . . **4. *Lens***

3.1.4. Description of the Genera and their Studied Species

3.1.4.1. *Lathyrus* L. P. Taubert, *Lathyrus* L., in: Engl. & Prantl, Nat. *P flznfam*. III, 3: 353-354, 1894. P. F. A. Ascherson & K. O. P. P. Graebner, *Lathyrus,* in: Aschers. & Graebn., *Syn. Mitteleur. Fl.* 6, 2: 1000-1063, 1909-1910. V. A. Fedchenko, *Lathyrus* L., in: *Flora URSS* 13: 479-520, 1948 (20); Tackholm, Fl. Egy. 278 (1974); Zohary, Fl. Pal. 2: 211 (1987).

Annual or perennial herbs. Stems green, mostly with leafy wings. Leaves pari- or imparipinnate, consisting of 1 or rarely of a few pairs of leaflets, or reduced to leaf-like petioles or to stipules, usually with a tendril or with a terminal awn. Racemes 1- to many-flowered, pedunculate. Flowers rather large. Calyx tube usually having a somewhat oblique rim; calyx teeth equal or the upper teeth somewhat shorter. Standard usually obovate to oblong; wings slightly adhering to keel. Pod dehiscing by 2 valves, mostly many-seeded, mostly compressed, linear or oblong-rhomboidal, rarely cylindrical. Seeds mostly globular, sometimes compressed or angular.

3.1.4.1.1. *Lathyrus aphaca* L., Sp. Pl. 729 (1753); Boiss., Fl. 2: 602 (1872). *L. aphaca* L. var. *biflorus* Post, Fl. Syr. Pal. Sin. 292 (1883-1896) (20); Tackholm, Fl. Egy. 279 (1974); Zohary, Fl. Pal. 2: 220 (1987). [Plate 1, A & B]

Annual, glaucescent, subglabrous, 10-75 cm. Stems usually erect or ascending, branched mainly at base, flexuous, angular. Leaves abortive, reduced to stipules and tendrils; stipules 0.5-4 cm., sessile, simple, leaf-like, ovate, sagitate-hastate or truncate at base, apiculate; tendrils 1-6 cm., simple, filiform. Peduncles as long as tendrils, longer than stipules, muticous or short-aristate. Racemes axillary, 1(-2)-flowered. Pedicles about as long as calyx tube, erect or slightly curved, often hairy. Flowers 1-1.5 cm. Calyx about 1 cm.; teeth much longer than tube, almost equal, lanceolate, acute. Corolla longer than calyx, yellow; standard longer than the wings and the incurved, whitish and pink-veined keel. Pod 3(-4-7)-seeded, 2-3 x 0.4-0.6 cm., erect, compressed, oblong-linear, sometimes falcate, slightly torulose, beaked, reticulately veined. Seeds 2-4 mm., subglobular, brown-black, smooth. Fl. February-April.

Habitat: Fields, field edges and roadsides. Acco Plain, Sharon Plain, Philistean Plain, Upper and Lower Galilee, Mt. Carmel, Esdraelon Plain, Mt. Gilboa, Samaria, Judean Mts., W. and N. Negev, Hula Plain, Upper Jordan Valley, Gilead, Moav, Edom. Very common (20).

Area: Mediterranean, Irano-Turanian and Euro-Siberian (20).

Distribution: Tubas, Taluza, Kafr Qud, Al-Ameryah. [Plate 5].

3.1.4.2. *Pisum* L. Tackholm, Fl. Egy. 280 (1974); Zohary, Fl. Pal. 2: 221 (1987).

Annual or perennial. Leaves of 1 or more pairs of leaflets and terminal, often branched, tendril; stipules leaf-like, often larger than leaflets. Racemes 1-3-flowered. Flowers conspicuous. Calyx campanulate; tube oblique; teeth unequal, the upper teeth shorter. Standard obovate to orbicular, 2-gibbous at base; wings adherent to keel. Pod dehiscent, 2-valved, somewhat compressed, oblong-linear, obliquely truncate, beaked. Seeds almost globular.

3.1.4.2.1. *Pisum fulvum* Sm. in Sibth. et Sm., Fl. Gr. Prodr. 2: 62 (1813) (20); Tackholm, Fl. Egy. 280 (1974); Zohary, Fl. Pal. 2: 222 (1987). [Plate 1, C & D]

Annual, glabrous, 15-70 cm. Stems ascending or procumbent, rarely erect, slender. Leaves 3-12 cm., spreading; stipules 1-4 cm., ovate, semicordate, dentate or incised all around or up to middle; leaflets 1 (-2)-paired, 1.5-2.5 x 1-1.5 cm., ovate, mostly dentate. Racemes 1 (-2-3)-flowered, with peduncles longer than stipules. Flowers about 1 cm. or less. Corolla rusty-yellow or riddish-brown, pale in subterranean flowers; standard broad, ovate to orbicular, retuse to 2-lobed. Pod 2.5-3 (-4) x 0.7-1 cm., short-beaked, net-veined. Seeds about 4 mm., black, velvety, punctulate. Fl. February-April.

Habitat: Mainly on scree and pebble heaps. Upper and Lower Galilee, Mt. Carmel, Samaria, Judean Mts. Fairly frequent (20).

Area: E. Mediterranean (20).

Distribution: Al-Ameryah, Yamun, Kifl-Harith, Taluza, Salfit. [Plate 5].

3.1.4.2.2. ***Pisum sativum*** Tackholm, Fl. Egy. 280 (1974). [Plate 1, E & F; Plate 2, A & B]

Annual, glabrous, 40-150 cm. Stems angular or roundish, hollow, covered with a waxy bloom. Leaves 6-15 cm., spreading; stipules about 8 cm., ovate, semisagittate; leaflets (0-) 1-2 (-3)-paired, 1-5 x 1-4 cm., broad, elliptic to oblong, entire to coarsely toothed. Racemes 1-4-flowered, with peduncles shorter than stipules. Flowers about 3 cm. Corolla white to pink or purple; standard broad, ovate to orbicular, retuse to 2-lobed. Pod 4-15 x 1.5-2.5 cm., short-beaked, net-veined. Seeds about 5 mm., whitish, gray, green or brownish, smooth or wrinkled punctulate. Fl. February-April.

Habitat: Mainly on scree and pebble heaps. Upper and Lower Galilee, Mt. Carmel, Samaria, Judean Mts. Fairly frequent.

Area: E. Mediterranean.

Distribution: Maithaloun, Aqaba, Yasid, Jaba', Tubas, Qalqilia, Salfit. [Plate 5].

3.1.4.3. ***Vicia*** L. P. F. A. Ascherson & K. O. P. P. Graebner, *Vicia*, in: Aschers. & Graebn., *Syn. Mitteleur*. Fl. 6, 2: 902-995, 1909. A. I.

Tupikova, Botanico-agronomical investigations of annual vetches, *Bull. Appl. Bot. Pl. Breed. Leningr*. 16: 151-246 (1926). I. N. Sveshnikova, Karyological studies on *Vicia, Bull. Appl. Bot. Pl.Breed. Leningr*. 17: 843-854 (1927). B. Schischkin & E. Bobrov, *Vicia* L., in: *Flora URSS* 13: 406-475, 1948. E. Guinea, *Estudio Botanico de las Vezas y Aruejas Espanolas*, 1-227, Madrid, 1953. F. J. Hermann, Vetches of the United States, *Agric. Handb*. 168: 1-84 (1960). D. Mettin & P. Hanelt, Cytosystematische Untersuchungen in der Artengruppe um *Vicia sativa* L., *Kulturpflanze* 12: 163-225 (1964). P. Ball, *Vicia*, in: T. G. Tutin & H. V. Heywood (ed.), *Fl. Europaea* (MS). U. Plitmann, *Biosystematical Studies in the Annual Species of Vicia and Lathyrus of the Middle-East*, Ph. D. Thesis, Jerusalem, 1966 (MS.) (20); Tackholm, Fl. Egy. 272 (1974); Zohary, Fl. Pal. 2: 194 (1987).

Annual or perennial herbs, rarely shrubs. Leaves paripinnate, with many, rarely 1-2 pairs of leaflets and branched, rarely simple tendrils; stipules mostly semisagittate. Racemes many- to few-flowered, often reduced to single axillary flowers. Bracts small, caducous. Flowers small to large. Calyx often with oblique tube and equal teeth or with upper teeth shorter than lower. Corolla blue, violet to purple or white to yellow; petals short-clawed; standard mostly obovate to oblong, notched; wings often adhering to keel. Pod dehiscent, mostly compressed, rarely cylindrical; valves leathery, rarely fleshy. Seeds mostly globular, sometimes angular.

3.1.4.3.1. *Vicia palaestina* Boiss., Diagn. Ser. 1, 9: 116 (1849) et Fl. 2: 592 (1872) (20); Zohary, Fl. Pal. 2: 197 (1987). [Plate 2, C & D]

Annual, sparingly appressed-hairy, 15-80 cm. or more. Stems climbing, simple to branched, slender. Leaves 2-7.5 cm., subglabrous to pubescent;

stipules 2-4 mm., semihastate, those of the uppermost leaves lanceolate to oblanceolate; tendrils often branched; leaflets (5-) 6-10-paired, 0.5-3 x 0.05-0.3 (-0.5) cm., subsessile, narrowly linear to narrowly oblanceolate, acute to obtuse, mucronulate. Peduncles long but shorter than subtending leaves, muticous. Racemes (2-) 3-8 (-9)-flowered, generally one-sided. Pedicels about as long as calyx, pubescent. Flowers (5-) 6-9 mm., deflexed. Calyx about 2 mm., somewhat hairy; rim of tube slightly oblique; teeth a little shorter than tube, the lower teeth longer, lanceolate-triangular. Corolla about 3 times as long as calyx; standard longer than wings, blue, slightly retuse at apex; wings white-blue or cream-blue; keel dark blue at apex. Style subcompressed, hairy at apex. Pod (1.3-) 2-2.5 x (0.4-) 0.5-0.8 cm., stipitate, 1-4-seeded, compressed, rhombic-elliptical to oblong, more or less torulose, short-beaked, glabrous, somewhat net-veined. Seeds 3-6 mm., globular to compressed-ovoid, brown to blackish-brown, smooth; hilum short, linear. F1. February-May.

Habitat: Maquis and batha. Acco Plain, Sharon Plain, Philistean Plain, Upper and Lower Galilee, Mt. Carmel, Esdraelon Plain, Samaria, Judean Mts., Judean Desert, Dan Valley, Hula Plain, Upper and Lower Jordan Valley, Gilead, Ammon, Moav. Fairly common (20).

Area: E. Mediterranean, with slight eastward extensions (20).

Distribution: Yasid, Al- Nasiriya, Kifl-Harith, Tubas, Taluza, Ramallah-road, Nablus, Azoon, Beit-Wazan, Beit-Leed, Al-Ameryah. [Plate 5].

3.1.4.3.2. ***Vicia peregrina*** L., Sp. P1. 737 (1753); Boiss., Fl. 2: 576 (1872) (20); Tackholm, Fl. Egy. 276 (1974); Zohary, Fl. Pal. 2: 202 (1987). [Plate 2, E & F]

Annual, appressed-puberulent or pubescent, 15-60 (-75) cm. Stems procumbent to erect, usually branched, angular. Leaves 1.5-6.5 cm.; stipules 2-4 mm., narrow, semihastate or semisagittate, free portion subulate or lanceolate, pilose; tendrils simple to branched; leaflets (2-) 3-7-paired, 1-3 x 0.1-0.6 cm., subsessile, narrowly linear to oblanceolate, tapering at base, retuse, rarely acuminate, mucronulate. Racemes axillary, mostly 1-flowered. Pedicels about as long as to a little longer than calyx, hairy. Floweres 1.1-2 cm. Calyx 6-7 mm., slightly gibbous, with an oblique limb; teeth almost as long as tube, the upper teeth shorter, connivent, lanceolate, acuminate. Corolla about twice as long as clayx, purple or blue-violet, paler at base, sometimes white; standard longer than wings, notched. Style hairy at apex. Pod 2-4 x 0.4-1.2 cm., short-stipitate, 3-7-seeded, deflexed, more or less compressed, oblong-linear, shorter-beaked, appressed-hairy to subglabrous, sometimes with violet-purple spots. Seeds about 4 mm., subglobular, sometimes subangular, mostely dark brown or mottled with black; hilum oblong, dark. Fl. February-May.

Habitat: batha and fallow fields. Coastal Galilee, Acoo Plain, Sharon Plain, Philistean Plain, Upper and Lower Galilee, Mt. Carmel, Esdraelon Plain, Samaria, Judean Mts., Judean Desert, W., N. and C. Negev, Dan Valley, Gilead, Ammon, Moav, Edom. Common (20).

Area: Mediterranean and Irano-Turanian (20).

Distribution: Yamun, Kifl-Harith, Salfit, Beit-Wazan, Al-Ameryah. [Plate 5].

3.1.4.3.3. *Vicia sativa* L., Sp. Pl. 736 (1753) (20); Tackholm, Fl. Egy. 275 (1974); Zohary, Fl. Pal. 2: 205 (1987). [Plate 3, A & B]

Annual, hairy to subglabrous, 20-80 cm. Stems erect to procumbent, branching from base. Leaves 3-11 cm.; stipules varying in length, semihastate, dentate, usually with a purple nectary spot beneath; tendrils usually branched; leaflets 4-10-paired, varying in size and shape, 1-3 x 0.4-1 cm., linear or lanceolate to oblong or obovate, sometimes elliptical, obcordate or cuneate, acutish or obtuse to truncate or retuse, mucronate, mostly entire. Racemes axillary, almost sessile, 1-3-flowered. Flowers 1-3 cm., short-pedicelled. Calyx 1-3 cm., campanulate, hairy, rim of calyx tube even (not oblique); calyx teeth 0.3-1 x 0.1-0.2 cm., subequal, linear-subulate or lanceolate, acute-mucronate. Corolla one and a half to two and a half times as long as calyx, sometimes 2-coloured; standard 0.7-1.3 cm., broad, obvate-orbicular, notched, whitish-pink to purplish-violet, claw about as long as limb; wings shorter than standard, bluish-pink to purplish-violet; keel shorter than wings, paler, usually darker at apex. Pod 3-6.5 x 0.4-1 cm., 2-10-seeded, pods compressed to turgid, linear, torulose or not, more or less pubescent, net-veined, yellowish to brown or black, rarely whitish. Seeds 3-6 mm., rarely larger, subglobular, sometimes compressed, plain or variegated, greenish-grey or brown-yellow or black; hilum short, linear. Fl. (February-) March-May (-June).

Habitat: Cultivated and escaped, but also spontaneous in fallow field and elsewhere. Coastal Galilee, Acco Plain, Sharon Plain, Philistean Plain, Upper and Lower Galilee, Esdraelon Plain, Mt. Gilboa, Judean Mts., Judean Desert, W. and N. Negev, Dan Valley, Hula Plain, Upper Jordan Valley. Fairly common (20).

Area: Mainly Mediterranean, with wide extensions into the Irano-Turanian and Euro-Siberian regions (20).

Distribution: Nablus, Kifl-Harith, Ramallah-road, al-Nasiriya, Yamun, Tamun, Beit-Wazan. [Plate 5].

3.1.4.3.4. *Vicia faba* L. [Plate 3, C & D]

Annual, glabrous, 30-160 cm. Stems erect, unbranched. Leaves 6-12 cm.; stipules 1-2 mm., sagittate, base toothed; tendrils 0 or rudimentary; leaflets 2-6-paired, 5-8.5 x 2.2-3.5 cm., ovate to elliptic, obtuse to acute. Racemes axillary, subsessile, mostly 2-6-flowered. Floweres 2-3 cm., dull white. Calyx 7-15 mm., campanulate, unequal toothed; calyx teeth 0.5 x 0.3 mm., Corolla about twice as long as clayx, white wings with dark blotches; standered 1.5 cm., broad, wings shorter than standard, keel shorter than wings. Pod 5-15 x 1-2 cm., 2-5-seeded, cylindrical or flattened, glabrous or pubescent. Seeds 10-30 mm., flat, green. Fl. February-May.

Habitat: Cultivated; fallow fields. Acco Plain, Sharon Plain, Philistean Plain, Upper and Lower Galilee, Mt. Carmel, Esdraelon Plain, Judean Mts., W. and N. Negev, Upper and Lower Jordan Valley, Gilead, Ammon, Moav. Fairly common.

Area: Mediterranean, with extensions into adjacent Euro-Siberian and Irano-Turanian territories.

Distribution: Maithaloun, Salfit, Nablus, Tubas, Jaba', Taluza, Yamun, Tamun. [Plate 5].

3.1.4.3.5. *Vicia narbonensis* L., Sp. Pl. 737 (1733) (20); Tackholm, Fl. Egy. 275 (1974); Zohary, Fl. Pal. 2: 208 (1987). [plate 3, E & F]

Annual, subglabrous to sparingly pubescent or hirsute, 15-50 cm. Stems ascending, procumbent to erect, branched, thick, angular. Leaves 1.5-9 cm.; stipules semihastate to semiorbicular, entire or dentate to incised, with a dark nectary spot beneath; tendrils simple or somewhat branched, lacking in the lower leaves; leaflets (1-) 2-3 (-4)-paired, 1-6x1-3 cm., subsessile, elliptical or oblong-lanceolate or ovate to obovate, obtuse or rarely acute, rounded or truncate to retuse, mucronulate, entire or dentate-serrate at margin or denticulate near apex, sparingly to densely hairy, especially along nerves, ciliate at margin. Peduncles very short and thick. Racemes (1-) 3-6-flowered. Flowers 1.8-3.2 cm., short-pedicelled, deflexed to erect. Calyx 0.7-1.3 cm., sparingly hairy; tube with oblique rim; teeth unequal, the lower teeth longer, as long as tube, mostly ciliate. Corolla 2-2.5 times as long as calyx, purple-violet; standard longer and keel shorter than wings. Style hairy at apex. Pod 3.5-6.5x0.8-1.5 cm., short-stipitate, flattened, linear to oblong-rhomboidal, curved and beaked, hairy or glabrescent, ciliate and tuberculate-denticulate at margin, often nerved. Seeds 4-6 mm., subglobular, brown-black, more or less smooth; hilum oblong-elliptical to oblong-ovate, whitish. Fl. February-June.

Habitat: Spontaneous and sometimes also cultivated; fallow fields. Acco Plain, Sharon Plain, Philistean Plain, Upper and Lower Galilee, Mt. Carmel, Esdraelon Plain, Judean Mts., W. and N. Negev, Upper and Lower Jordan Valley, Gilead, Ammon, Moav. Fairly common (20).

Area: Mediterranean, with extensions into adjacent Euro-Siberian and Irano-Turanian territories (20).

Distribution: Beit-Leed, Tubas, Al-Ameryah, Taluza, Yamun. [Plate 5].

3.1.4.4. *Lens* Mill. H. I. Barulina, Lentils of the Ud.S.S.R. and of other countries, *Bull. Appl. Bot. Pl. Breed. Leningr*. Suppl. 40 (1930; Russian with an English summary). D. V. Zaitschek, Studies in Palestinian lentils II, *Palest. Journ. Bot. Jerusalem* Ser. 1: 55-64 (1938) (20); Zohary, Fl. Pal. 2: 209 (1987).

Annual or rarely perennial, erect or climbing herbs. Leaves pari-, rarely imparipinnate; stipules semisagittate, petiole mostly terminating with a tendril or an awn. Racemes axillary, few- or 1-flowered. Flowers small, long-pedicelled. Calyx teeth long, nearly equal. Corolla mostly whitish to bluish; standard obovate; wings adherent to acute, slightly beaked keel. Pod short, 2-valved, 1-2-seeded, compressed. Seeds compressed, lenticular.

3.1.4.4.1. *Lens culinaris* Medik., Phil. Bot. 2: 361 (1791). *L. esculenta* Moench, Meth. 131 (1794). *Ervum lens* L., Sp. Pl. 738 (1753); Boiss., Fl. 2: 598 (1872) (20); Zohary, Fl. Pal. 2: 210 (1987). [Plate 4, A & B]

Annual, 16-20 cm., hairy. Stems few to many, erect, sparingly branching, angular. Leaves paripinnate, at least part of them terminating in a branched tendril; stipules small, lanceolate, entire; leaflets 3-7-paired, 0.8-1.5 x 0.4-0.6 cm., oblong-linear to linear. Peduncles shorter than leaves, ending in an awn up to 1 cm. Racemes 1 (-2)-flowered. Flowers 4-6 mm. Calyx short-campanulate; teeth much longer than tube, nearly as long as or longer than corolla, almost equal, filiform-subulate. Corolla white, rarely pink or violet. Staminal tube oblique. Pod 0.7-1.2 x 0.3-0.5 cm., deflexed, ovate-rhombic. Seeds 1-2, lenticular, rarely almost globular. Fl. April.

Habitat: Widely cultivated and often subspontaneous (20).

Area: Origin probably E. Mediterranean (20).

Distribution: Yasid, Qalqilia, Maithaloun. [Plate 5].

3.1.5. Continuum Morphological Properties between *V. palaestina* and *L. culinaris*

Vicia palaestina and *L. culinaris* are characterized by overlapping in their morphological characters; branched stems, branched tendrils and number of seeds. As well as the number of leaflets, number of raceme flowers and the size and shape of the stipules were observed. Style is hairy all around or only on lower side in *V. palaestina*. In contrast, to *L. culinaris* in which the style is hairy only on the upper side. In addition to that, *V. palaestina* and *L. culinaris* vary in their seeds as being round globular or lenticular, respectively.

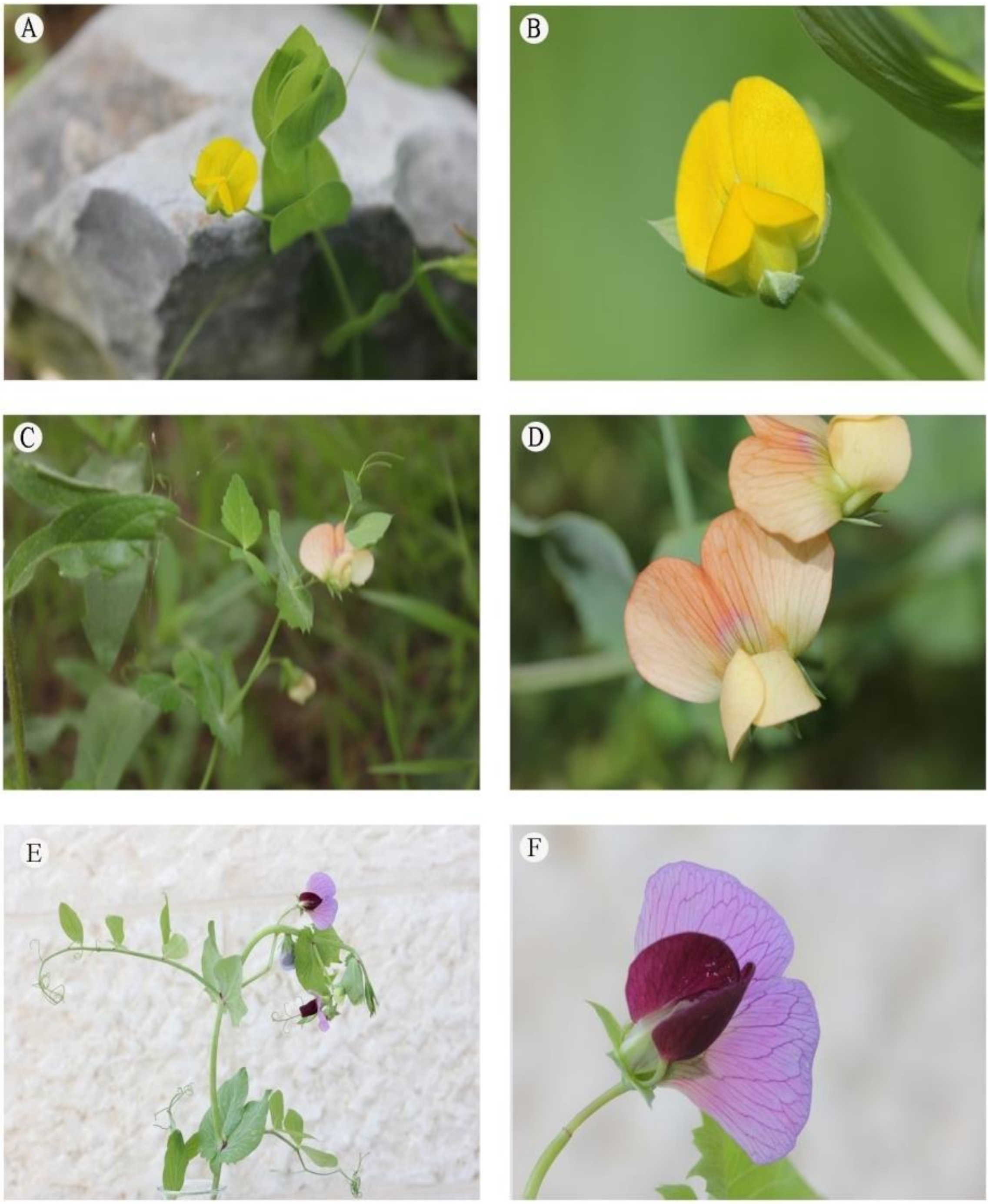

Plate 1: Coloured photographs of studied species

A. ***Lathyrus aphaca*** **B.** ***Lathyrus aphaca***

C. ***Pisum fulvum*** **D.** ***Pisum fulvum***

E. ***Pisum sativum*** **F.** ***Pisum sativum***

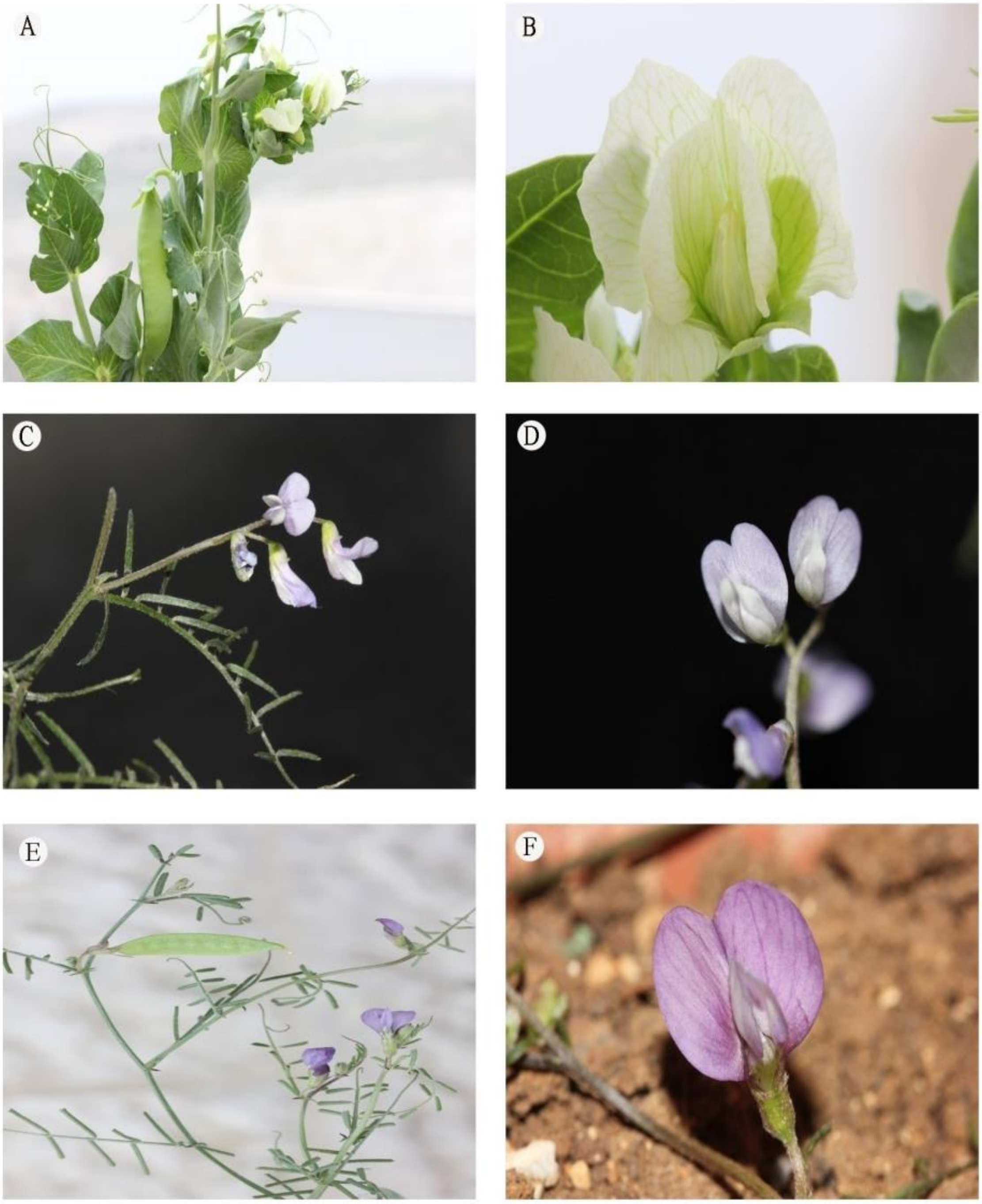

Plate 2: Coloured photographs of studied species

A. ***Pisum sativum*** **B.** ***Pisum sativum***

C. ***Vicia palaestina*** **D.** ***Vicia palaestina***

E. ***Vicia peregrina*** **F.** ***Vicia peregrina***

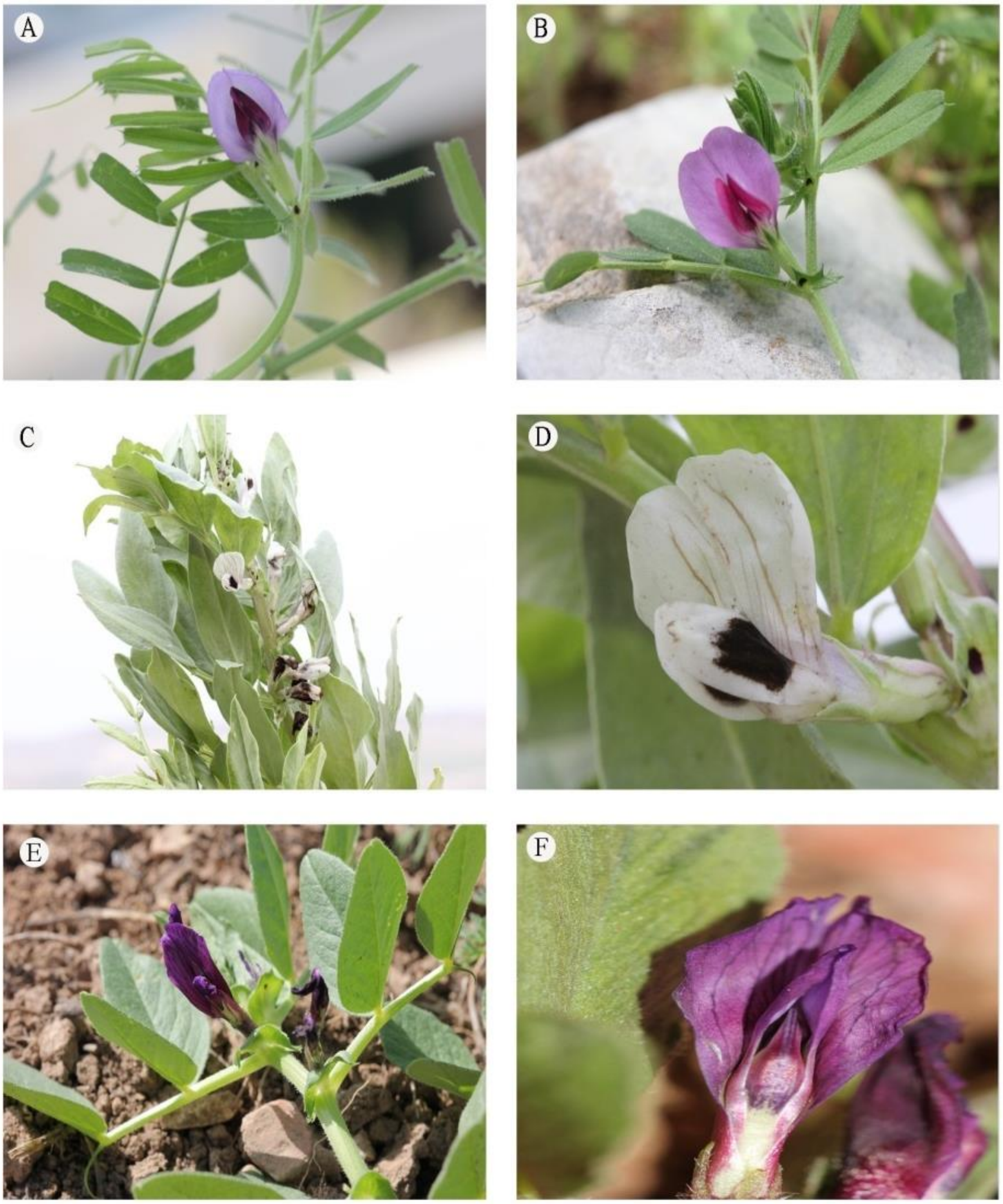

Plate 3: Coloured photographs of studied species

A. ***Vicia sativa*** **B.** ***Vicia sativa***

C. ***Vicia faba*** **D.** ***Vicia faba***

E. ***Vicia narbonensis*** **F.** ***Vicia narbonensis***

Plate 4: Coloured photographs of studied species

A. ***Lens culinaris*** **B.** ***Lens culinaris***

C. ***Salvia dominica*** **D.** ***Salvia dominica***

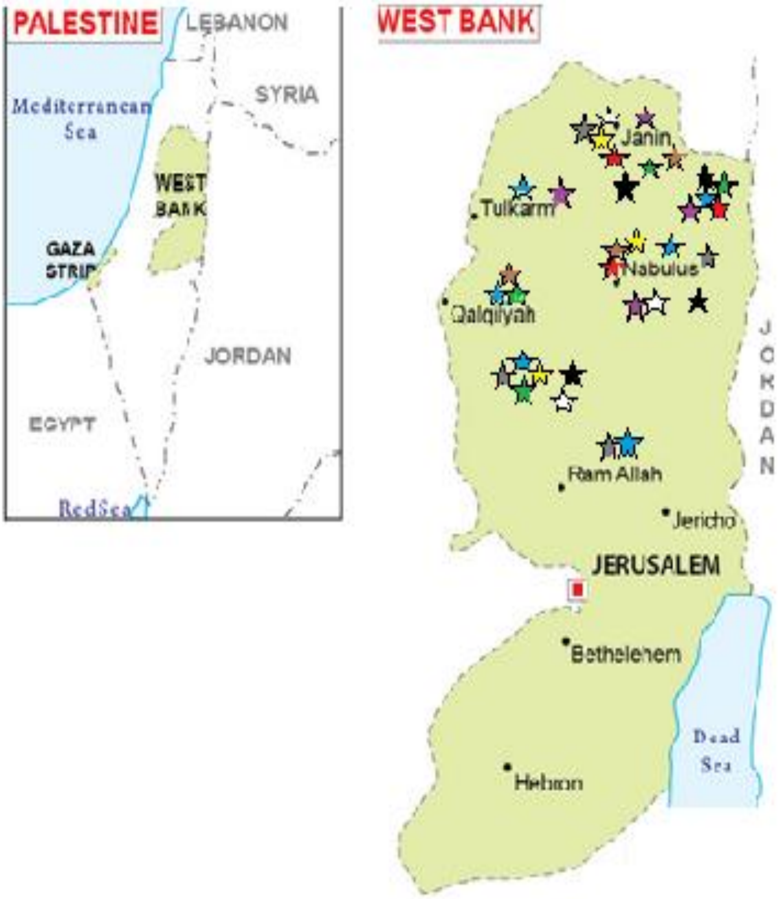

Plate 5: Map of Palestine showing the distribution of studied species

★ **Distribution of *L. aphaca***

★ **Distribution of *P. fulvum***

★ **Distribution of *P. sativum***

★ **Distribution of *V. palaestina***

☆ **Distribution of *V. peregrina***

★ **Distribution of *V. sativa***

★ **Distribution of *V. faba***

★ **Distribution of *V. narbonensis***

★ **Distribution of *L. culinaris***

3.2. Molecular Characterization of The Studied Species

3.2.1. Amplified ITS Sequence Analysis on Agarose Gel Electrophoresis

Specific sites of DNA; ITS-1 spacer, 5.8S rDNA and ITS-2 spacer in 4 genera of Leguminosae family as well as the outgroup, *S. dominica* (labiatae), were amplifed using universal primers 18S and 28S. On an agarose gel, the PCR products obtained from genomic DNA, yielded a single band of approximately 720 bp for all the tested species including *S. dominica* (Figure 3.1).

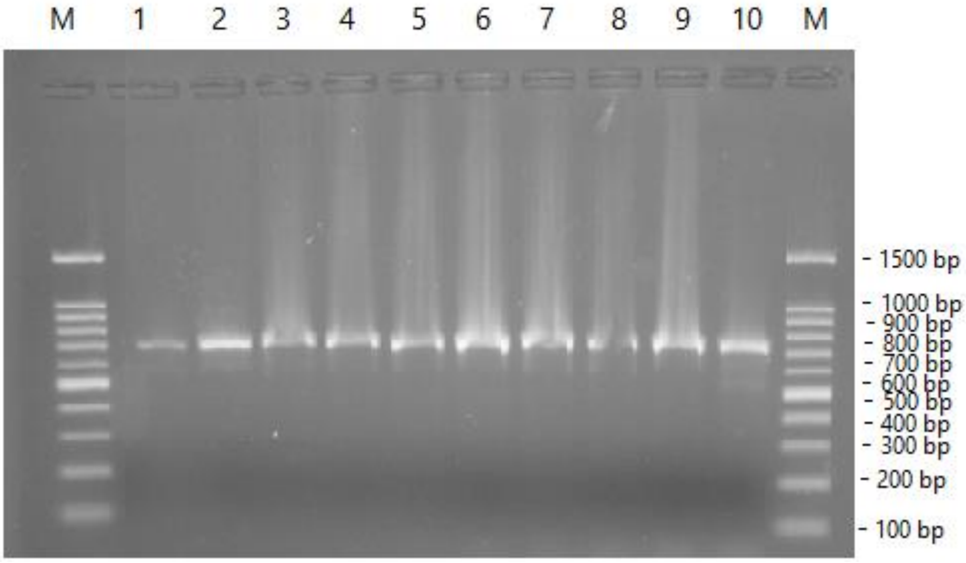

Figure 3.1. Agarose gel electrophoresis analysis showing detection of amplified ITS region of different species of Leguminosae family as well as *S. dominica* as an out group. Lanes: M, 50 bp DNA marker; 1, *L. aphaca*; 2, *P. fulvum*; 3, *P. sativum*; 4, *V. palaestina*; 5, *V. peregrina*; 6, *V. sativa*; 7, *V. faba*; 8, *V. narbonensis*; 9; *L. culinaris* and 10, *S. dominica*.

3.2.2. Genetic Relationship among Closely Related Species of Leguminosae Family

Thirty five classified samples of Leguminosae species which belong to *Lathyrus*, *Pisum*, *Vicia* and *Lens* genera were sequenced. Then the phylogenetic tree was constructed according to the similarity among the resulted sequences of Leguminosae species of the four genera; where the bootstrap consensus tree was inferred from 1000 replicates. The sequence information was between 663–683 bp. Sequences were further registered at the GenBank database under the accession numbers (KJ864924 - KJ864925 and KJ864928- KJ864961). Where, Table 2.2 shows the V. no. of the studied species (represented on the phylogenetic tree), GenBank numbers and their scientific names.

Distance matrix and phylogenetic tree of the amplified ITS region were established among the nine Leguminosae species under study (Table 3.1 and Figure 3.2, respectively).

Phylogenetic analysis in the current work revealed that ITS sequences of Leguminosae species of interest have the highest average of intraspecies genetic divergence (8.95%) was recorded between *V. palaestina* (KJ864941) and *P. sativum* (KJ864946). While, low average of intraspecies genetic divergence (1.81%) was between *V. faba* (KJ864957, KJ864958) and *V. peregrina* (KJ864954) (Table 3.1).

Table 3.1. Genetic differences between ITS region sequences derived from studied Palestinian Leguminosae species and fr

Leguminosae family were retrieved from GenBank (denoted by asterisk). DNA distances were created by K2P model using M

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	JX506052.1_L._aphaca *																																				
2	KJ864924 L. aphaca	0.0018																																			
3	KJ864925 L. aphaca	0.0018	0.0036																																		
4	AJ441060.1_L._culinaris_orientalis *	0.0578	0.0597	0.0597																																	
5	KJ864928 L. culinaris	0.0578	0.0597	0.0597	0.0000																																
6	KJ864929 L. culinaris	0.0578	0.0597	0.0597	0.0000	0.0000																															
7	KJ864930 L. culinaris	0.0578	0.0597	0.0597	0.0000	0.0000	0.0000																														
8	KJ864931 L. culinaris	0.0616	0.0635	0.0635	0.0036	0.0036	0.0036	0.0036																													
9	KJ864932 L. culinaris	0.0578	0.0597	0.0597	0.0000	0.0000	0.0000	0.0000	0.0036																												
10	AB546787.1_P.__fulvum *	0.0482	0.0501	0.0501	0.0618	0.0618	0.0618	0.0618	0.0657	0.0618																											
11	KJ864933 P. fulvum	0.0500	0.0519	0.0519	0.0677	0.0677	0.0677	0.0677	0.0716	0.0677	0.0163																										
12	KJ864934 P. fulvum	0.0500	0.0519	0.0519	0.0677	0.0677	0.0677	0.0677	0.0716	0.0677	0.0163	0.0000																									
13	KJ864935 P. fulvum	0.0500	0.0519	0.0519	0.0677	0.0677	0.0677	0.0677	0.0716	0.0677	0.0163	0.0000	0.0000																								
14	KJ864936 P. fulvum	0.0519	0.0538	0.0538	0.0696	0.0696	0.0696	0.0696	0.0735	0.0696	0.0181	0.0018	0.0018	0.0018																							
15	JX506267.1_V._palaestina *	0.0578	0.0597	0.0597	0.0366	0.0366	0.0366	0.0366	0.0404	0.0366	0.0656	0.0735	0.0735	0.0735	0.0734																						
16	KJ864937 V. palaestina	0.0597	0.0617	0.0617	0.0385	0.0385	0.0385	0.0385	0.0423	0.0385	0.0676	0.0755	0.0755	0.0755	0.0754	0.0018																					
17	KJ864938 V. palaestina	0.0578	0.0597	0.0597	0.0366	0.0366	0.0366	0.0366	0.0404	0.0366	0.0656	0.0735	0.0735	0.0735	0.0734	0.0000	0.0018																				
18	KJ864939 V. palaestina	0.0578	0.0597	0.0597	0.0366	0.0366	0.0366	0.0366	0.0404	0.0366	0.0656	0.0735	0.0735	0.0735	0.0734	0.0000	0.0018	0.0000																			
19	KJ864940 V. palaestina	0.0578	0.0597	0.0597	0.0366	0.0366	0.0366	0.0366	0.0404	0.0366	0.0656	0.0735	0.0735	0.0735	0.0734	0.0000	0.0018	0.0000	0.0000																		
20	KJ864941 V. palaestina	0.0733	0.0753	0.0753	0.0518	0.0518	0.0518	0.0518	0.0556	0.0518	0.0813	0.0893	0.0893	0.0893	0.0893	0.0144	0.0163	0.0144	0.0144	0.0144																	
21	AY143485.1_P._sativum *	0.0521	0.0540	0.0540	0.0658	0.0658	0.0658	0.0658	0.0697	0.0658	0.0036	0.0200	0.0200	0.0200	0.0218	0.0696	0.0716	0.0696	0.0696	0.0696	0.0854																
22	AY143473.1_P._sativum *	0.0540	0.0559	0.0559	0.0678	0.0678	0.0678	0.0678	0.0717	0.0678	0.0054	0.0218	0.0218	0.0218	0.0237	0.0716	0.0736	0.0716	0.0716	0.0716	0.0874	0.0018															
23	AY143462.1_P._sativum *	0.0560	0.0579	0.0579	0.0699	0.0699	0.0699	0.0699	0.0737	0.0699	0.0072	0.0237	0.0237	0.0237	0.0255	0.0737	0.0756	0.0737	0.0737	0.0737	0.0895	0.0036	0.0018														
24	KJ864942 P. sativum	0.0540	0.0559	0.0559	0.0678	0.0678	0.0678	0.0678	0.0717	0.0678	0.0054	0.0218	0.0218	0.0218	0.0237	0.0716	0.0736	0.0716	0.0716	0.0716	0.0874	0.0018	0.0000	0.0018													
25	KJ864943 P. sativum	0.0540	0.0559	0.0559	0.0678	0.0678	0.0678	0.0678	0.0717	0.0678	0.0054	0.0218	0.0218	0.0218	0.0237	0.0716	0.0736	0.0716	0.0716	0.0716	0.0874	0.0018	0.0000	0.0018	0.0000												
26	KJ864944 P. sativum	0.0559	0.0578	0.0578	0.0678	0.0678	0.0678	0.0678	0.0716	0.0678	0.0072	0.0237	0.0237	0.0237	0.0255	0.0736	0.0755	0.0736	0.0736	0.0736	0.0894	0.0036	0.0018	0.0036	0.0018	0.0018											
27	KJ864945 P. sativum	0.0540	0.0559	0.0559	0.0678	0.0678	0.0678	0.0678	0.0717	0.0678	0.0054	0.0218	0.0218	0.0218	0.0237	0.0716	0.0736	0.0716	0.0716	0.0716	0.0874	0.0018	0.0000	0.0018	0.0000	0.0000	0.0018										
28	KJ864946 P. sativum	0.0560	0.0579	0.0579	0.0699	0.0699	0.0699	0.0699	0.0737	0.0699	0.0072	0.0237	0.0237	0.0237	0.0255	0.0737	0.0756	0.0737	0.0737	0.0737	0.0895	0.0036	0.0018	0.0036	0.0018	0.0018	0.0036	0.0018									
29	KJ864947 V. sativa	0.0538	0.0557	0.0557	0.0404	0.0404	0.0404	0.0404	0.0442	0.0404	0.0636	0.0635	0.0635	0.0635	0.0655	0.0423	0.0442	0.0423	0.0423	0.0423	0.0575	0.0676	0.0676	0.0696	0.0676	0.0676	0.0696	0.0676	0.0696								
30	KJ864948 V. sativa	0.0557	0.0576	0.0576	0.0423	0.0423	0.0423	0.0423	0.0461	0.0423	0.0656	0.0655	0.0655	0.0655	0.0674	0.0442	0.0461	0.0442	0.0442	0.0442	0.0556	0.0696	0.0696	0.0716	0.0696	0.0696	0.0715	0.0696	0.0716	0.0018							
31	KJ864949 V. sativa	0.0538	0.0557	0.0557	0.0404	0.0404	0.0404	0.0404	0.0442	0.0404	0.0636	0.0635	0.0635	0.0635	0.0655	0.0423	0.0442	0.0423	0.0423	0.0423	0.0575	0.0676	0.0676	0.0696	0.0676	0.0676	0.0696	0.0676	0.0696	0.0000	0.0018						
32	KJ864950 V. sativa	0.0538	0.0557	0.0557	0.0404	0.0404	0.0404	0.0404	0.0442	0.0404	0.0636	0.0635	0.0635	0.0635	0.0655	0.0423	0.0442	0.0423	0.0423	0.0423	0.0575	0.0676	0.0676	0.0696	0.0676	0.0676	0.0696	0.0676	0.0696	0.0000	0.0018	0.0000					
33	KJ864951 V. sativa	0.0538	0.0557	0.0557	0.0404	0.0404	0.0404	0.0404	0.0442	0.0404	0.0636	0.0635	0.0635	0.0635	0.0655	0.0423	0.0442	0.0423	0.0423	0.0423	0.0575	0.0676	0.0676	0.0696	0.0676	0.0676	0.0696	0.0676	0.0696	0.0000	0.0018	0.0000	0.0000				
34	HM470633.1_V._americana *	0.0558	0.0577	0.0577	0.0423	0.0423	0.0423	0.0423	0.0461	0.0423	0.0656	0.0655	0.0655	0.0655	0.0674	0.0442	0.0461	0.0442	0.0442	0.0442	0.0595	0.0696	0.0696	0.0716	0.0696	0.0696	0.0716	0.0696	0.0716	0.0018	0.0036	0.0018	0.0018	0.0018			
35	HM470602.1_V._sativa *	0.0558	0.0577	0.0577	0.0423	0.0423	0.0423	0.0423	0.0461	0.0423	0.0656	0.0655	0.0655	0.0655	0.0674	0.0442	0.0461	0.0442	0.0442	0.0442	0.0595	0.0696	0.0696	0.0716	0.0696	0.0696	0.0716	0.0696	0.0716	0.0018	0.0036	0.0018	0.0018	0.0018	0.0036		
36	HM470605.1_V._incisa *	0.0577	0.0596	0.0596	0.0443	0.0443	0.0443	0.0443	0.0480	0.0443	0.0636	0.0635	0.0635	0.0635	0.0655	0.0461	0.0480	0.0461	0.0461	0.0461	0.0614	0.0676	0.0676	0.0696	0.0676	0.0676	0.0696	0.0676	0.0696	0.0036	0.0054	0.0036	0.0036	0.0036	0.0054	0.0054	
37	HM470607.1_V._nigra *	0.0577	0.0596	0.0596	0.0443	0.0443	0.0443	0.0443	0.0480	0.0443	0.0636	0.0635	0.0635	0.0635	0.0655	0.0461	0.0480	0.0461	0.0461	0.0461	0.0614	0.0676	0.0676	0.0696	0.0676	0.0676	0.0696	0.0676	0.0696	0.0036	0.0054	0.0036	0.0036	0.0036	0.0054	0.0054	0.0000
38	KJ864952 V. peregrina	0.0521	0.0540	0.0540	0.0330	0.0330	0.0330	0.0330	0.0367	0.0330	0.0560	0.0618	0.0618	0.0618	0.0637	0.0292	0.0310	0.0292	0.0292	0.0292	0.0442	0.0599	0.0619	0.0639	0.0619	0.0619	0.0638	0.0619	0.0639	0.0274	0.0292	0.0274	0.0274	0.0274	0.0292	0.0292	0.0311

Based on the phylogenetic tree; two main clades were revealed. Clade I included five species of *Vicia* genus; *V. peregrina*, *V. faba, V. narbonensis, V. palaestina* and *V. sativa*, in order in the phylogenetic tree respectively, as well as *L. culinaris*. While, clade II was composed of *P. sativum*, *P. fulvum* and *L. aphaca*. Furthermore, clade I was subdivided into two subclades: a and b. Subclade Ia had five species, four of them belong to the genus *Vicia* and the fifth one was *L. culinaris*. However, *V. sativa* occupied a basal position in the clade I. This may confirms that *V. sativa* represents a taxon distantly related to all other species of *Vicia*. *Lens culinaris* belonged to a subclade Ia. It was near to the species of *Vicia* and appeared basally sister to many *Vicia* species especially to *V. palaestina.*

Results of this research showed that all studied cultivated (KJ864951, KJ864950, KJ864947 and KJ864948) and wild type (KJ864949) samples of *V. sativa* were clustered close to each other in the same group (100% bs). The annual *V. faba* was classified near to *V. peregrina* (68% bs), then these two species were grouped into the same cluster with other species of *Vicia*; *V. narbonensis* and *V. palaestina*. Therefore, all of these species could be considered as sisters to each other according to their botanical and molecular properties.

However, clade II was subdivided into two subclades: c and d, which were represented by the genera *Pisum* and *Lathyrus*, respectively (68.9% bs). High molecular similarity between them was observed, which was more than their resemblance to other genera.

Pisum sativum and *P. fulvum* are two species in the same genus according to the morphological classification. Figure 3.2 showed that *P. sativum* and *P. fulvum* were clustered in one group and placed in the phylogenetic tree near to each other. So results confirm the strong relationship between them and endorse the classification of these two species in the same genus. Moreover, the positions of *P. sativum* and *P. fulvum* in the same group may indicate that *P. fulvum* is the wild origin of *P. sativum* (100% bs).

Finally, current work results confirm that the species of *Pisum* genus formed a monophyletic group because all species of *Pisum* genus were clustered into the same group and had the same ancestor. On the other hand, *V. sativa* clade splits from the other closely related species in the same genus, so the genus *Vicia* was considered as paraphyletic. However, *S. dominica* was found to be quite divergent and did not fall in any of the major clusters as illustrated in Figure 3.2.

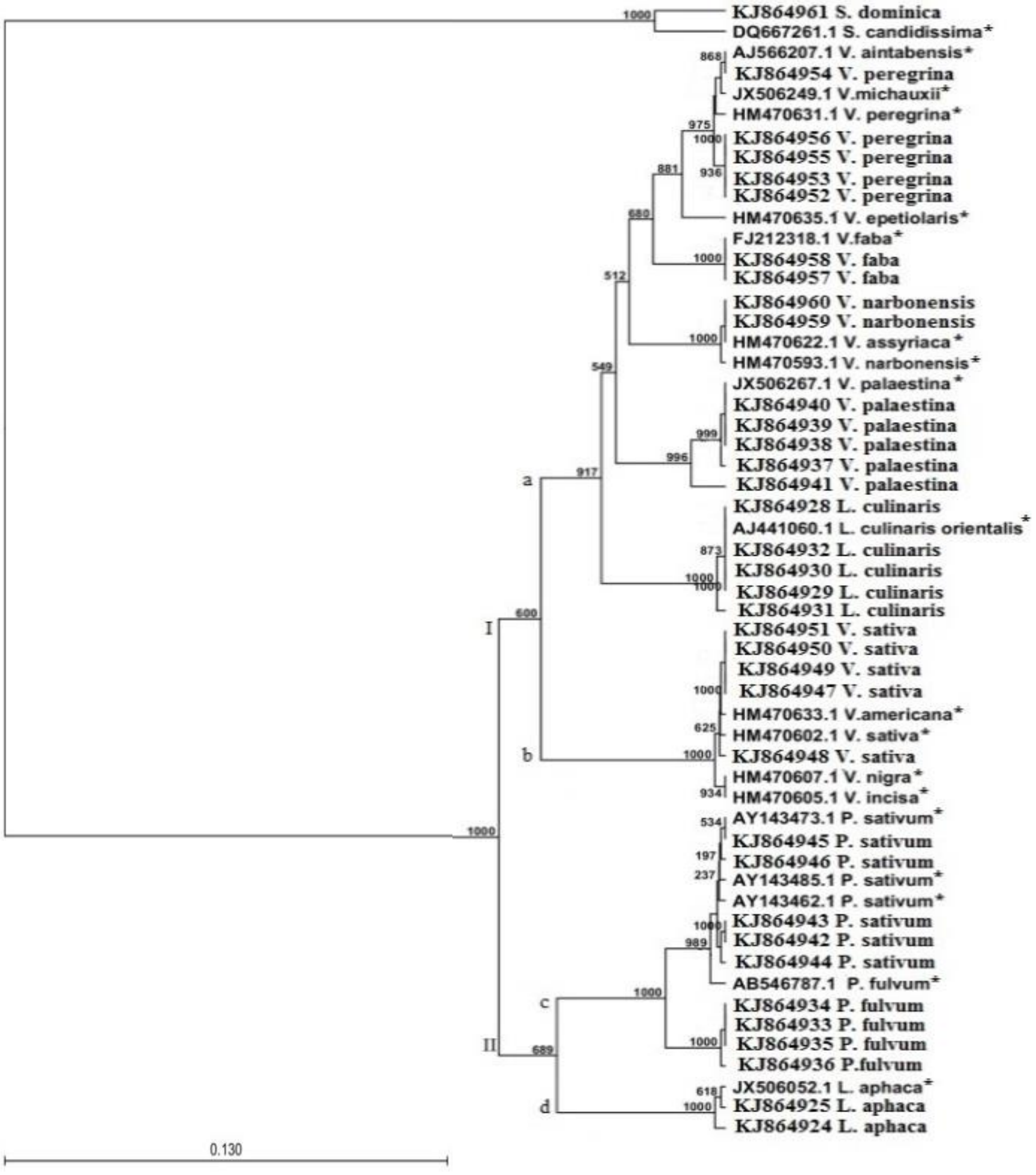

Figure 3.2. Phylogenetic analysis by UPGMA method based on ITS site. Sequence of some Palestinian Leguminosae species (*L. aphaca, P. fulvum, P. sativum, V. palaestina, V. peregrina, V. sativa, V. faba, V. narbonensis* and *L. culinaris*) as well as *S. dominica* as an outgroup were used for phylogenetic analysis. Reference sequences belong to species of Leguminosae family (denoted by asterisk) were retrieved from GenBank. The bootstrap consensus tree was inferred from 1000 replicates. The latin numbers (I, II) represent the clades, where the following letters (a, b, c and d) represent the subclades of the phylogenetic tree.

Chapter Four

Discussion

All Leguminosae species that were included in this study had simple or alternate compound leaves, with large or small stipules and five lobed corollas. Their fruit, usually a pod, has one or dozens of seeds. All of these morphological properties are similar to the Leguminosae description that was reported by Adelanwa (2008) (53).

Annual *L. aphaca* is characterized by erect stem, abortive leaves that are reduced into tendrils and stipules, 1-2 yellow flowers and straight pod with 2-4 mm seeds. This description agrees with the *L. aphaca* description by Çildir (2011) (54).

Pisum fulvum is an annual legume and it was known by different physical characters; ascending or procumbent stem, spreading leaves, dentate stipules, rusty-yellow or riddish-brown flowers and net-veined pod with black seeds. The similar description has been found in the Flora Palaestina (1987) (20).

Morphological examination results of *P. sativum* by having angular stem, ovate leaves, large stipules, white, purple or pink flowers and short-beaked pod with a green or brown seeds were also detected by Pavek (2012) (55).

In 1987, Zohary described *V. palaestina* in a similar way to the description in this work. *Vicia palaestina* has a climbing stem, subglabrous to pubescent leaves, semihastate stipules, deflexed flowers with white-blue wings and dark blue keel and oblong pod with 1-4 seeds (20).

Vicia peregrina was described in Flora of Egypt (1974) by many morphological characters; hairy plant, narrow and retuse leaflets, purple flowers with unequal calyx-teeth and calyx half as long as the corolla. This is the same description, which was recorded in this study (56).

Vicia sativa characteristics in this study are similar to that of Adam *et al.*, (1999). It has erect to procumbent stem, alternate leaves, small stipules with purple nectary spot, pink or purple flowers and linear pod; which is more than 3 cm long and vary in seeds number (57).

Vicia faba is a cultivated species of *Vicia* genus characterized by: erect stem, compound leaves, toothed base stipules, dull white flowers with dark spot and its pod contains 2-5 seeds. This is the same description of *V. faba* that was stated by Sattell *et al.* (1998) (58).

However, minor differences were noticed between the description of *V. narbonensis* in this study and the Tackholm one (1974). Lower leaves without tendrils, elliptical leaflets, semi-orbicular stipules and purple-violet flowers are the morphological properties of *V. narbonensis* in both descriptions. Glabrous plant with racemes 1-2 flowered were detected in his description, while subglabrous to sparingly pubescent or hirsute with Racemes (1-) 3-6-flowered were detected in the current description of this species (56).

A branched stem, alternate leaves, small stipules, white flowers and ovate pod with one or two seeds are the morphological properties of *L. culinaris* in this study as well as in the Yadav *et al.* (2007) and Muehlbauer *et al.* (1996) studies. Except the color of flowers; that is pale yellow, and the shape of the pod which is oblong legume in their studies (59, 60).

DNA sequences play an essential role in the reconstruction of the evolutionary relationships among different organisms. The rapid accumulation of DNA sequence data in GenBank has led to new genetic classifications that often conflicts with traditional taxonomy. The nuclear genes coding for the 18S and 25S rRNA components of the cytoplasmic ribosomes have been used extensively at family and higher taxa levels, for evaluating molecular phylogenetic relationships among plants. Internal transcribed spacer (ITS) regions have been shown to be relatively conservative in length and have exciting potential as a source of nuclear DNA characters to study phylogeny in plants among closely related genera or species. The coding regions showed little sequence divergence among closely related species, whereas the spacer regions exhibit higher rates of variability. Therefore, nuclear ribosomal ITS sequence data have a great potential to resolve plant phylogenies at various intrafamiliar levels in angiosperms. Sequence variability within these regions varies depending on the taxa, and most studies have been performed on species, population or genus level. Few studies are known about sequence divergence among taxa of entire families including different tribes and subtribes (61).

Many studies have been conducted using this molecular technique to study phylogeny in plants among closely related genera or different species. One of these studies has been carried out by Gurushidze *et al*. (2007). They studied the phylogenetic relationships of wild and cultivated species of *Allium* section *Cepa* inferred by nuclear rDNA ITS sequence analysis (62). Other studies were performed on the Leguminosae species, some of which are common to those under this study (49), while others considered different species (48). For example, Sonnante *et al*. (2003), studied the ITS sequence analysis and phylogenetic inference in the genus *Lens*. This study included *L. culinaris* and other species (63). Nevertheless, until now there is no phylogenetic analysis for Leguminosae species has been reported in Palestine using molecular techniques such as ITS region analysis.

PCR results of this research showed that all studied Leguminosae species, which were amplified by universal primers produced single band of amplified ITS region of the same size (~720 bp). This result was consistent with Sonnante *et al*. (2003) observation as they concluded that the length of ITS sites in *Lens* species was the same to the other species of legumes (63). *Salvia dominica* which is a species used in this study as an out group revealed a ITS region band size similar to the ITS of the studied Leguminosae species (~720 bp). However, this is not the same for all different plant species. As, Gurushidze *et al*. (2007), showed that the length of ITS regions of the species of the *Allium* section *Cepa* were in the range between 626 and 644 bp (62). These results may indicate that the length of ITS is not the same for all plant species.

Constructed phylogenetic tree in this study showed that some reference species retrieved from GenBank database clustered together such as *V. assyriaca* and *V. narbonensis*; *V. michauxii*, *V. aintabensis* and *V. peregrina* or *V. American, V. sativa, V. nigra* and *V. incisa*. This may indicate that these species which clustered together have a close phylogenetic relationship. More studies are needed and necessary to reclassify the species belonged to family Leguminosae.

The species *L. culinaris* formed a cluster within *Vicia* genus, where this species was closest to *V. palaestina* more than any other species of *Vicia* genus (91.7% bs). Moreover, according to their morphological properties; these two species are similar to each other, except in the hairy style and the shape of the seed. Style is hairy all around or only on lower side and a round seeds, in contrast of style being hairy on upper side and compressed seeds for *V. palaestina* and *L. culinaris*, respectively. The morphological and molecular data strongly support the observation that *L. culinaris* and *V. palaestina* are two species related to the same genus.

This out finding coincides with a recent report (2003) in which it was shown that *Lens* is a close genus to *Vicia* as *Lens* species formed a clade near to the *Vicia* species based on ITS sequences (49). Moreover, Foladi *et al*. (2013) considered that *Lens* is a close genus to *Vicia* in a phylogenetic study of tribe *Vicieae* based on ITS analysis (64).

Radzhi (1970) and Tzvelev (1980, 1987) attached *V. narbonensis* and *V. faba* to section *faba* due to the high similarity between these two species (65, 66, 67). another study (2009) showed that *V. faba* and *V. narbonensis* are sisters to each other, and the obtained bootstrap supports the replacement of *V. faba* into the *V. narbonensis* group (68). The listed previous studies results support the ones obtained in this work. As *V. peregrina*, *V. faba* and *V. narbonensis* were clustered with each other in the phylogenetic tree and closely are related to each other. On the other hand, results showed that *V. sativa* was rather similar to the other *Vicia* species, since it was separated from other species of *Vicia* genus.

Furthermore, *V. sativa* and *L. culinaris* were located in different subclades in the resulted phylogenetic tree in this study. This agrees with their different morphological properties. Nevertheless, Erskine *et al.* (1994), proved the continuum morphological characters between *L. culinaris* and *V. sativa* (38).

Results showed that the cultivated and wild samples of *V. sativa* were clustered close to each other in the same group. This means that the wild and cultivated species have the same genetic content. Also the wild species can be considered as the origin of the cultivated one. This can be explained by the absence of hybridization occurrence or human Intervention.

The subclade which was represented by *L. aphaca* is sister to the one comprised of *P. sativum* and *P. fulvum*. Moreover, a monophyletic group was formed by them in the phylogenetic tree. This molecular analysis confirms the fact that *Pisum* and *Lathyrus* share the large stipule character, which was not found in *Vicia* and *Lens*. These results are in agreement with that were published previously (49).

Two species of *Pisum* genus; *P. sativum* and *P. fulvum* formed one subclade, which indicates the close relationship between them. In addition to that, the origin of the cultivated *P. sativum* can be concluded to be *P. fulvum*. However, more studies on other different species of *Pisum* genus are needed to prove that conclusion. Results of this research are in agreement with a previous report (69), which showed that *P. sativum* is sister to *P. fulvum*. However, in contrast, other studies showed that *P. fulvum* was the most divergent species from other *Pisum* genus species (70, 71, 72, 73, 74).

Four of the five samples of the *P. sativum* have white flower (KJ864945, KJ864946, KJ864943 and KJ864942). This character was considered as a recessive trait. While the fifth one (KJ864944) has purple flower, which is a character was considered as a dominant trait in Mendel's low [Plate 1, E & F; Plate 2, A & B]. In spite of that, all of the ITS sequences of the five samples were closely related to each other. Nevertheless, the purple flowered sample was farther from the other white flowered samples. As a result, different phenotypes, for example different flower colours can't be considered as different genotypes (20). This can be confirmed by that the genetic mechanisms controlling floral number and shape are apparently unstable, resulting in fluctuating asymmetry (75). In conclusion, it is not easy to establish a direct relation between the phenetic variation and the genetic variation (76). Accordingly, further studies which are related to that aspect could be conducted to provide a more elaborate clear view.

The genus *Vicia* did not form a monophyletic group; instead it formed a paraphyletic one with *Lens*. This result is consistent with a previous report (2003), which showed that genus *Vicia* formed a paraphyletic group with *Len*s, *Pisum*, and *Lathyrus* (49). This result was confirmed by a conclusion that a monophyletic *Vicia* could be obtained by transferring *Lens* and *Lathyrus saxatilis* to *Vicia* (69).

Conclusions

Internal transcribed spacer (ITS) sequencing is one of the molecular techniques, which can be used for studying the phylogenetic relationship among different species as well as closely related ones.

Phylogenetic relationship among plant species using ITS sequencing is an effective method for identifying the specimens that don’t have one or more essential parts like flowers, fruits, etc. which are important for plant morphological identification. Small amounts of leaves are enough to determine the specific name of any plant sample by this method accurately by referring to the GenBank data.

Furthermore, this technique can be used for classifying the new species according to the resulted clusters in the phylogenetic tree at family, genus and species levels.

Morphological and molecular properties of Palestinian *L. culinaris* and *V. palaestina* revealed the close relationship between these two species. Therefore, the shape of seed and style hairy characters are not enough to separate these two species into two different genera.

This study was the first study on the phylogenetic relationship among some Leguminosae species in Palestine, and paves the way for further in-depth researches on the same topic.

Recommendations

From the out findings in this study, it is recommended to transfer *L. culinaris* into *Vicia* genus based on the morphological and molecular characterization of this species. Therefore, the new classification of *Lens culinaris* is recommended to be *Vicia culinaris*.

Moreover, further studies on other different species of Leguminosae family, especially on the species that are related to the *Lens* and *Vicia* genera are needed and recommended. This may reveal more clarification of the relationship among Leguminosae closely related species.

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Appendixes

Appendix A

ITS Sequences of the Species of Leguminosae Family

Sequence KJ864924

CATTGTCGATGCCTTACATGCAGTCCAACACGTGAATTAGTTTGAACACATACGGTGGGCCT

GAGGTGTTCCACACCCAGGCTTACCACTGGTTTTGGAGGCGGACGACGAAGTGCGTTCTCCT

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CATCCGCATGGTACCGGAGACGGTTTCTGTGTGTGTTGTGTTTTGACACATTATATAGAATGA

CTCTCGGCAACGGATATCTAGGCTCTTGCATCGATGAAGAACGTAGCGAAATGCGATACTTG

GTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGATGCCAT

TAGGTTGAGGGCACGTCTGACTGGGTGTCACATCTCGAAGCCTCCTACCAATTTCCTTTTGAC

AGGAATTGTGCAGGGTGGATGTTGGCCTCCCGTGAGCTATTTCGTCTCATGGTTGGTTGAAA

ATTGAGACCTTGGTAGGGTGTGCCATGATAAATGGTGGTTGTGTGACCCACGAGACCAATCG

TGTGCCGCTCTATTGAATTTGGCCTCTTTTACCCATATGCGTTTCTAAACGCTCGTGATGAGA

CCCCAGTTCAGGCGGG

Sequence KJ864925

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Sequence KJ864928

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Sequence KJ864929

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Sequence KJ864931

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GAATGCGTCAAGGAAATTAAATTTTGCTCTGAGCGCACCTGCATGGCACCGGAGACGGTTTC

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GTCAAGGAATTAAAATTTTGCTCTGAGCGCACCTGCATGGCACCGGAGACGGTTTTCGTGCG

GGTTGTGTTTTGACACATGATATAGAATGACTCTCGGCAACGGATATCTAGGCTCTTGCATC

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G

Appendix B

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كلية الدراسات العليا

دراسة العلاقة الوراثية بين الانواع المتقاربة من الأجناس: *العدس، البيقة، الجلبان والبازيلاء* (العائلة البقولية) في فلسطين

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اشراف

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

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الملخص

تعد البقوليات او القرنيات ثالث اكبر عائلة في النباتات الزهرية، كما و لها اهمية اقتصادية حيث تعتبر مصدر للغذاء و خصوبة التربة. أربعة أجناس فلسطينية من هذه العائلة: العدس، البيقة، الجلبان و البازيلاء، تحتوي صفات شكلية متداخلة فيما بينها. تستخدم العديد من التقنيات الجزيئية لتحديد العلاقة الوراثية والتنوع الجيني بين انواع العائلة القرنية بما في ذلك تحديد تسلسل Internal transcribed spacer (ITS).

أجريت هذه الدراسة لتحديد العلاقة الوراثية التطورية بين تسعة أنواع متقاربة تنتمي لأربعة أجناس من العائلة البقولية: *Lens culinaris*، *Vicia sativa*، *Vicia palaestina*، *Vicia peregrina*، *Vicia faba*، *Vicia narbonensis*، *Lathyrus aphaca*، *Pisum fulvum* و *Pisum sativum*.

اضافة الى ذلك، *L. culinaris* و *V. palaestina* هما نوعان ينتميان الى جنسين مختلفين، الا أن خصائصهما الشكلية متشابهة لدرجة كبيرة. ولذلك، تهدف هذه الدراسة الى تحديد التصنيف الاسمي والعلاقة الجينية للنوعين *V. palaestina* و *L. culinaris* المتقاربين جدا من الناحية الشكلية.

تم تصنيف انواع الفصيلة القرنية المدروسة لعينات تم جمعها من بيئتها، وتم حفظ عينة مماثلة لكل نوع من الأنواع المدروسة في معشبة جامعة النجاح الوطنية. و تمت دراستها بناء على المواصفات الشكلية. ثم حددت الخصائص الجينية باستخدام البادئات 18S و28S لتكثير و معرفة تسلسل

منطقة ITS. قدّر الاختلاف بين تسلسل الجينات و رسمت شجرة النشوء والتطور باستخدام نماذج Kimura 2-parameter و Unweighted Pair Group Method، على التوالي.

أدخلت كل عينة من التسعة أنواع بالاضافة الى *Salvia dominica* كمجموعة خارجية الى بنك الجينات. *P. sativum* (KJ864946) و *V. palaestina* (KJ864941) امتلكت اعلى معدل اختلاف جيني بين الأنواع، بينما أقل معدل اختلاف جيني بين النوعين *V. faba* KJ864958, (KJ864957) و *V. peregrina* (KJ864954) تم الكشف عنه من تحليل جينات النشوء والتطور. *V. peregrina*، *V. faba*، *V. narbonensis*، *V. palaestina*، *V.*، *L. culinaris* و *V. sativa* تجمعت في فرع واحد (مجموعة 1). *V. sativa* احتلت أبعد موقع في المجموعة الاولى وانفصلت عن الانواع الأخرى من جنس البيقة. بينما *P. sativum* ، *P. fulvum* و *L. aphaca* تجمعت في نفس المجموعة (مجموعة 2). من ناحية أخرى، اتخذ *L. culinaris* موقعا ضمن تجمع البيقة بالقرب من *V. palaestina*.

في هذه الدراسة، تم الكشف عن العلاقة الوراثية التطورية بدقة بين الأنواع وثيقة الصلة من عائلة البقوليات باستخدام تسلسل ITS . كما وبينت هذه الدراسة العلاقة الجينية الوثيقة بين *L. culinaris* و *V. palaestina* كنوعين ينتميان الى جنس واحد بدلا من جنسين مختلفين.