Mandragora: Phylogenetic and Domestication

Thesis submitted in partial fulfillment
of the requirements for the degree of
"DOCTOR OF PHILOSOPHY"

by

Keren Fogel

Submitted to the Senate of Ben-Gurion University
of the Negev

June 2012

Beer-Sheva

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Approved by the Dean of the Kreitman School of Advanced Graduate Studies_____

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This work was carried out under the supervision of

Dr. Sergei Volis

and Dr. Khalil Kashkush

In the Life Science Department

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Research-Student's Affidavit when Submitting the Doctoral Thesis for Judgment

I_____, whose signature appears below, hereby declare that
(Please mark the appropriate statements):

___ I have written this Thesis by myself, except for the help and guidance offered by my Thesis Advisors.

___ The scientific materials included in this Thesis are products of my own research, culled from the period during which I was a research student.

___ This Thesis incorporates research materials produced in cooperation with others, excluding the technical help commonly received during experimental work. Therefore, I am attaching another affidavit stating the contributions made by myself and the other participants in this research, which has been approved by them and submitted with their approval.

Date: _____ Student's name: _____ Signature:_____

Thanks

I want to thank many people that helped me through my PhD:

Dr. Sergi Volis, for the opportunity to perform research on an interesting and unique plant.

Dr. Khalil Kashkush, for the help and support.

Dr. Michele Zaccai, for lending an attentive ear.

Dr.Yuval Sapir, for some of the pictures and helping in the beginning of my work.

Dr. Tu Tieyao, for the remote assistance.

Irena Shulgina, for helping with the lab work.

Asher Dvir, for helping in greenhouse and field and in every opportunity.

Yonghong Zhang for helping in AFLP analyzing.

Pavel Melnikov, for succeeding in germinating some stubborn mandrake seeds.

Zina Kraitshtein, Vadim Khasdan, Adva Levi and Beeri Yaakov, from Khalil's lab for helping me in AFLP and sequencing and for the friendship during my studies.

And thanks to my family:

My parents that have supported me all the way, it is thanks to them that I have reached so far.

My mother and father-in-law who helped whenever needed.

My dear partner-to-life, Shimon Fogel, who encouraged me from my first year of undergraduate studies, urged me through my M.Sc and PhD studies and is always there for me.

I received much of the strength to quest and learn from my belief in G-d, I am thankful for the power that He bestows to me. אֲבָרֵךְ אֶת יְהוָה אֲשֶׁר יְעָצָנִי (תהלים טז ז)

This work is dedicated to my little children:

Nitzan, Shaked and Shachar,

For the happiness they brought to my life.

I'm sure you'll get far!

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List of abbreviations

AFLP	Amplified Fragment Length Polymorphism
FACS	Florescence-Activated Cell Sorter
ITS	Internal Transcribed Spacer
cpDNA	Chloroplast DNA
NJ	Neighbor Joining
ML	Maximum Likelihood
MP	Maximum Parsimony

Samples name:

AM	Ammiad
ND	Nahal Dishon
RP	Rosh-Pina
BG	Beit Guvrin
Luz	Luzit Caves
Keram	Keramim
Lah	Lahav Forest
Meit	Meitar
MT	Turkmenistan

1. Abstract

The goal of this study was to resolve the phylogenetic relationships between species of the genus *Mandragora* based on eight regions in the chloroplast DNA, ITS region in nrDNA, AFLP, FACS and different morphometrics (flowers, fruits and seeds morphology). Additionally, characterization of the phenotypic variance and self and out crosses were done as the first step towards domesticating *Mandragora* fruits, and FACS was used to evaluate the ploidy level of *Mandragora* and associate it with its distribution range.

According to the morphometric data (fruits and seeds) and FACS analysis, *Mandragora* plants can be divided into three groups. One group includes *Mandragora* plants from Israel, Turkmenistan and Iran, which are characterized by globular and large fruits, with large seeds and higher DNA content. The second group includes *Mandragora* plants from Cyprus, Turkey, Italy, Spain and Morocco, which are characterized by ellipsoid and small fruits and medium seeds and lower DNA content. Tibetan plants are the third group with the smallest seed and different seed coat morphology.

The results of AFLP and sequencing of ITS and chloroplast DNA are very similar, showing a greater difference between European and Israeli *Mandragora* plants than between Israeli *Mandragora* plants and those from from Turkmenistan and Iran. Therefore, we conclude that *M. officinarum* that included European and Israeli plants is not a single species as was thought previously (Ungricht *et al.*, 1998 and Akhani and Ghorbani, 2003). Additionally, the recognition of *M. turcomanica* as a species of its own (Ungricht *et al.*, 1998 and Akhani and Ghorbani, 2003) is not supported by the data presented herein, as it seems to be very close to Israeli plants.

Thus it appears that the current taxonomy of the genus *Mandragora* L. (Solanaceae) including three species (M. *turcomanica* Mizg. from Central Asia/Iran, *M. officinarum* L. from the Mediterranean and *M. caulescens* C.B. Clark from Sino-Himalayan region) must be revised based on the results of this work. It follows from the new results, that *M. caulescens* is a highly separated clade from *M. officinarum* - *M. turcomanica*, while *M. turcomanica* is not a separate species but a relict population of *M. officinarum*. Moreover, *Mandragora* plants from Israel are very close to *M. turcomanica*, indicating a possible origin of *M. turcomanica* from the clade that is

present only in Israel. Additionally, this clade of Israel and Turkmenistan differs on DNA content from the clade widespread in Europe.

Key words

Mandragora; Taxonomy; Systematics; phylogeography; phylogeny; Domestication.

2. Introduction

2.1. Historic records and economic potential of Mandragora

Mandragora officinarum L. or mandrake is recognized as *Dudaim* in Hebrew (Fleisher and Fleisher, 1994), and is mentioned twice in the Bible. First in Genesis (30:14-16):

וַיֵּלֶךְ רְאוּבֵן בִּימֵי קְצִיר חִטִּים וַיִּמְצָא דוּדָאִים בַּשָּׂדֶה וַיָּבֵא אֹתָם אֶל לֵאָה אִמּוֹ. וַתֹּאמֶר רָחֵל אֶל לֵאָה: תְּנִי נָא לִי מִדּוּדָאֵי בְנֵךְ. וַתֹּאמֶר לָהּ: הַמְעַט קַחְתֵּךְ אֶת אִישִׁי וְלָקַחַת גַּם אֶת דּוּדָאֵי בְנִי? וַתֹּאמֶר רָחֵל: לָכֵן יִשְׁכַּב עִמָּךְ הַלַּיְלָה תַּחַת דּוּדָאֵי בְנֵךְ. וַיָּבֹא יַעֲקֹב מִן הַשָּׂדֶה בָּעֶרֶב וַתֵּצֵא לֵאָה לִקְרָאתוֹ וַתֹּאמֶר: אֵלַי תָּבוֹא כִּי שָׂכֹר שְׂכַרְתִּיךְ בְּדוּדָאֵי בְנִי. וַיִּשְׁכַּב עִמָּהּ בַּלַּיְלָה הוּא.

During the wheat harvest, Reuben, the eldest son of Jacob and Leah, found mandrake while walking in the field, and brought them to his mother. Rachel, Jacob's infertile second wife and Leah's sister, agreed to renounce a night with Jacob in order to get the mandrake, probably because she knew it was a cure for infertility. Thus this story shows that the *Mandragora* was an ancient remedy to help childless women conceive.

In another place in the Bible, Song of Solomon (7:14), the wonderful smell of *Mandragora* is praised: הַדּוּדָאִים נָתְנוּ רֵיחַ וְעַל פְּתָחֵינוּ כָּל מְגָדִים חֲדָשִׁים גַּם יְשָׁנִים דּוֹדִי צָפַנְתִּי לָךְ "The mandrakes give forth fragrance. At our doors are all manner of precious fruits, new and old, which I have laid up for you, my beloved…"

2.1.1. Mandragora use in the past

The roots of the *Mandragora* plant are massive and branched and therefore occasionally have a baby or human-like shape (Figure 1). This special shape of the root is the basis of many legends about the plant (Thompson, 1934; Moldenke and Moldenke, 1952; Berry and Jackson, 1976; Fleisher and Fleisher, 1994; Carter, 2003).

Mandragora was one of the most important medicinal plants in the ancient past. It was used as a pain killer, an anesthetic before surgery, a cure for infertility, a trance-inducing drug, a hallucinogenic, a substance to exile demons, etc (Randolph, 1905; Grover, 1965; Fleisher and Fleisher, 1994; Hanus *et al.*, 2005). The *Mandragora*'s name comes from the term 'man-dragon'. Other names of *Mandragora* are 'Satan's apple', *beid el-jinn*' ("jinn's eggs"), 'crazy apple' or *'al tuffah al majnoon'*

in Arabic, ‘‘*yavruchin* or *abu' l-ruch*’ ("master of the breath of life") and more of the same style (Fleisher and Fleisher, 1994).

Figure 1: Examples of different shapes of *Mandragora* roots.

Additionally, the term "Dudaim" in Hebrew means ‘love plant’ and it was considered to be the most powerful herb of love magic. It was supposed to have an aphrodisiac-like effect and witches used to prepare love potions from its root. The fruits of the plant, also-called love apples, were believed to increase fertility (Randolph, 1905; Thompson, 1934; Fleisher and Fleisher, 1994; Hanus *et al.*, 2005). In kibbutz Mishmar Ha'emeq, Micha Lin produces a mandrake liqueur, marketed under the name "*Mandragora*", which he recommends as a wedding present, to arouse passion.

2.1.2. The economic potential of *Mandragora* domestication

Mandragora appears to be a very promising plant, with great potential for domestication and developing into a crop due to the unique taste and aroma of its fruit. The plant has economic potential in the plant food industry, pharmaceuticals and agriculture and can grow in most soil types with modest irrigation. In addition, this is a new fruit that can easily enter the market, which is constantly searching for innovations.

2.2. Scientific background

2.2.1. Chemical properties of *Mandragora*

Mandragora belongs to the nightshades family (Solanaceae), and like other plants in this family (such as the tomato and potato) it is toxic when still green, because of its high Alkaloid content (Ungricht *et al.*, 1998). The major group of alkaloids found in this plant is the tropane alkaloids. Despite the toxicity of the tropanes, they are considered medicinally significant, when used correctly and in small amounts. It was found that *Mandragora*'s roots contain alkaloids such as atropine, scopolamine and hyoscyamine. These chemicals cause anesthetization, loss of senses and relax muscle tension which is probably the reason for the name 'crazy apple' and other names of the plant (Grover, 1965; Hanus *et al.*, 2005). These toxic chemicals are only found in the roots, seeds and unripe fruit, whereas there is no danger in the pulp of the mature fruit (Ungricht *et al.*, 1998). Recently, more than eighty substances were identified from all parts of this plant in different species of the genus *Mandragora* (Hanus *et al.*, 2005).

In addition, the ripe fruit contains many volatile chemicals that are responsible for its special smell. The fruit's odor is very special and smelled from afar. The chemical components of the fruit's aroma were examined and it was found that the ripe fruit contains many volatile chemicals, including various esters (also found in apples, guavas, mangos, papaya and passion fruit), γ-lactones (in small amounts but contribute significantly to the smell of *Mandragora*) and sulfur-containing chemicals (also found in onion, garlic and cabbage) (Grover, 1965; Fleisher and Fleisher, 1992; Fleisher and Fleisher, 1994; Hanus *et al.*, 2005; Hanus *et al.*, 2006).

2.2.2. The biology of *Mandragora*

In Israel, the *Mandragora*'s leaves dry up during the summer and the perennial root is dormant. Sprouting of young leaves (Figure 2a) starts from October, flowering (Figure 2b) lasts from December till February and the fruits (Figure 2c) begin to appear in March. In *Mandragora*, there is a preference for out-breeding through protogyny (a state in hermaphroditic flowers that is characterized by development of female organs or maturation of their products before the appearance of the corresponding male product, thus inhibiting self-fertilization), but it can also self fertilize. The fruit is tomato-like, (Figure 2d) two to four centimeters in diameter,

containing soft pulp and firm seeds. When the fruit ripens, it changes its color to yellow-orange and secretes a strong sweet smell, which is slightly reminiscent of melon. The smell attracts mammals that eat the fruit and disperse the seeds. The fruit's aroma and taste are very special and unique (Bernhardt and Dafni, 2000).

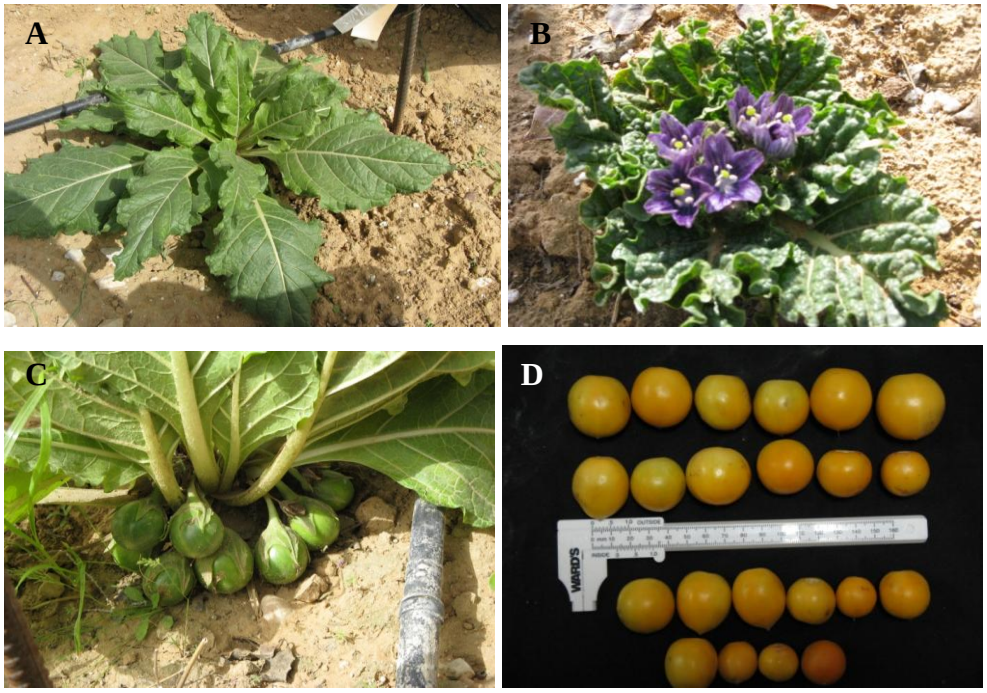


Figure 2: *Mandragora* plants from Israel: A- sprouting; B- flowering; C- unripe fruits on the plant; D- mature fruits.

Mandragora (Figure 3) is found at one location on the border between Turkmenistan and Iran, and is also distributed throughout Syria, Lebanon, Israel, Cyprus, Turkey (only on the shore line), Greece, Italy, Morocco and Spain. Other locations are in Tibet and China (Ungricht *et al.*, 1998). In Israel, *Mandragora* is found from Mount Hermon, Golan Heights, the upper Galilee, Samarian and Judean Mountains, through the coastal plain to the northern Negev, which is the southern limit of *Mandragora* in this area.

Figure 3: A map of *Mandragora* distribution (after Ungricht *et al.*, 1998)

2.2.3. The taxonomy of *Mandragora*

The long history of mythology and medicinal use of *Mandragora* and the variable morphology and phenology have created confused classification in *Mandragora*.

Ancient cultures distinguished between two species of the Mediterranean plants, the female mandrake (autumn flowering) and male mandrake (spring flowering). Linnaeus (Linnaeus, 1753) combined these two variants as one species, called *M. officinarum*. After Linnaeus further subdivisions of the genus (Figure 4) were made, into a few vernal and autumnal species (Ungricht *et al.*, 1998). Since 1950, there were again only two groups- *M. officinarum* L. (vernal) and *M.* autumnalis Bertol. (autumnal) (Hawkes and Edmonds, 1972; Hawkes *et al.*, 1972; Hawkes, 1972; Jackson and Berry, 1979). However, two groups with different flowering periods should have seasonal isolation and could hardly interbreed. In fact, there are no clear-cut clusters in the flowering time of these groups, so in the latest classification only one species of Mediterranean *Mandragora* is recognized (Ungricht *et al.*, 1998).

Classification type	Vernal species	Autumnal species
1 VERNAL, 1 AUTUMNAL TAXON		
Sprengel (1825)	*M. vernalis* Bertol.	*M. autumnalis* Bertol.
1 VERNAL, 2 AUTUMNAL TAXA		
Bertoloni (1835), Dunal (1852)	*M. vernalis* Bertol.	*M. officinarum* L. *M. microcarpa* Bertol.
2 VERNAL, 2 AUTUMNAL TAXA		
Heldreich (1886)	*M. vernalis* Bertol. *M. haussknechtii* Heldr.	*M. autumnalis* Spreng. *M. microcarpa* Bertol.
3 VERNAL, 2 AUTUMNAL TAXA		
Vierhapper (1915)	*M. mas* Garsault *M. hispanica* Vierh. *M. haussknechtii* Heldr.	*M. autumnalis* Bertol. *M. foemina* Garsault

Figure 4: The increased taxonomic subdivision of the Mediterranean *Mandragora*, adapted from Ungricht et al. (1998).

The Sino-Himalayan plants (Central and East Asian), *M. caulescens* C.B. Clarke (Figure 5), were split into four subspecies, differentiated on the basis of corolla color, calyx and corolla lengths and overall plant size (Grierson and Long, 1978). Additionally, the subspecies *M. chinghaiensis* K.Z. Kuang and A.M. Lu from Qinghai and Xizang provinces in Tibet was described (Kuang and Lu, 1978) (Figure 6).

However, the Sino-Himalayan and Mediterranean plants vary in the size, shape and color of their flowers and leaves within local populations and even individuals, so the subdivision within these groups according to these morphometric parameters is not clear (Ungricht *et al.*, 1998).

The Turkemenian plant (from Turkmenistan and Iran), *M. turcomanica* Mizg. is a larger and geographically isolated plant (restricted to southwestern Kopet Dag on the Turkmenistan-Iranian border) and therefore was accepted as a distinct species (Mizgireva, 1978; Ungricht *et al.*, 1998).

The current taxonomy of the genus *Mandragora*, based on morphometric analysis of herbarium specimens, phenology, habitat and distribution (Table 1), includes only three recognized species: *M. turcomanica* Mizg. from central Asia/Iran, *M. officinarum* L. from the Mediterranean and *M. caulescens* C.B. Clarke from the Sino-Himalayan region (Ungricht *et al.*, 1998; Akhani and Ghorbani, 2003).

Mandragora species are described by Ungricht *et al.* (1998) as perennial herbs with very long stout tap-roots (the tap-root is the perennating organ) and pedicellate actinomorphic flowers born in leaf axils. The flower has five calyx lobes that enlarge in fruit, five corolla lobes and five stamens. The fruit is a fleshy berry, strongly aromatic, with many seeds.

1. *Mandragora officinarum* L. is stem-less and has elliptic leaves in a rosette (max. 45 cm long). The pedicels are variable (max. 15 cm long) and the campanulate corolla is 12-65 cm long, greenish white to pale blue or violet. The fruit is 5-40 mm in diameter, globular to ellipsoid, glossy yellow to orange when ripe. Flowering is from September to April, fruiting is from November to June and the plant is dormant during the summer until autumn rains begin. The distribution is circum Mediterranean.

2. *Mandragora turcomanica* Mizg. is stem-less and has broadly elliptical or ovate leaves in a rosette (90 × 60 cm). The pedicels are 2-3 cm long and the campanulate corolla is 20-25 cm long, violet or purple with three narrow white stripes at its base. The fruit is 40-60 mm in diameter, glossy yellow to orange when ripe. Flowering is from October to March, fruiting is until June and the plant is dormant during the summer until autumn rains begin. Distribution is in small populations in a restricted area in Turkmenistan and possibly in Iran as well.

3. *Mandragora caulescens* C.B. Clarke has a stem, max 60 cm long (Figure 5) with oblanceolate or spatulate leaves arranged in a cluster on the top stem (max. 30 cm). The pedicels are 5-10 cm long and the corolla is 5-30 cm long campanulate to cup-shaped and colored yellow to purple. The fruit is 10-25 mm in diameter, globular, pale greenish white to greenish yellow. Flowering is from April to September, fruiting is from August to October and the plant is dormant during the winter when the area may be covered by ice. Distribution is in the Sino-Himalayan region.

Table 1: Summary of the comparison between the three species of *Mandragora* (based on Ungricht *et al.*, 1998 and Akhani and Ghorbani, 2003).

Species / Character	*M. officinarum* L.	*M. turcomanica* Mizg.	*M. caulescens* C.B. Clarke
Stem	Stem-less	Stem-less	Present, max 60 cm
Leaves	Rosette (max. 45 cm)	Rosette (90 x 60 cm)	Cluster on stem top (max. 30 cm)
Flower Pedicel	Variable, max 15 cm	2-3 cm	5-10 cm
Corolla length	12-65 cm	20-25 cm	5-30 cm
Corolla color	Greenish white to pale blue or violet	Violet or purple with three narrow white stripes at base	Yellow to purple
Fruit diameter	5-40 mm	40-60 mm	10-25 mm
Flowering period	September to April	October to March	April to September
Fruiting period	November to June	Until June	August to October
Dormancy period	Summer	Summer	Winter
Distribution	Mediterranean	Irano -Turanian	Sino-Himalayan

Figure 5: *Mandragora caulescens* from Tibet (photos by Hang Sun (A) and Tu Tieyao (B)).

Figure 6: *Mandragora chinghaiensis* from Tibet (photo by Tu Tieyao).

2.2.4. Polyploidy in *Mandragora*

Polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plant evolution (Ramsey and Schemske, 1998). It is estimated that over 70% of flowering plants are polyploid (Hilu, 1993; Ma *et al.*, 2004; Mable, 2004). By having a different number of alleles at a locus, polyploid species often display new traits and genetic variability that differ from their diploid ancestors in overall gene expression levels (Ramsey and Schemske, 1998; Ojiewo *et al.*, 2007).

Most domesticated crops are polyploids, allowing the development of desirable agricultural traits and compatibility to plant domestication (Eckardt, 2004). Polyploidy leads to an increase in plant organ size, in most cases, and sometimes also in fruit size, and leads to the development of new features (Singh and Wafai, 1984; Eckardt, 2004). It can be explained by the fact that increase in nuclear ploidy has been associated with an increase in cell volume, and as a result the whole organ is larger (Hilu, 1993).

It is hard to determine the number of chromosomes and the ploidy level of the *Mandragora*, because its chromosomes are small and numerous (Figure 7). There are remarkable differences between the chromosome numbers and ploidy level in different studies, from 24 chromosomes for a ploidy level of 4x to 96 chromosomes for a ploidy level of 16x (Smith, 1927; Tu *et al.*, 2005) (Table 2). *Mandragora* was suggested to have x = 12 (2x, 4x, 7x and 8x) by previous studies (Olmstead and Palmer, 1992; Badr *et al.*, 1997; Olmstead *et al.*, 1999), but because odd-number polyploids are sexually unstable and cannot normally comprise entire natural populations, Tu and colleagues (Tu *et al.*, 2005) suggested the chromosome base number of x = 6 for these taxa, making *Mandragora* 4x, 8x, 14x and 16x (Table 2).

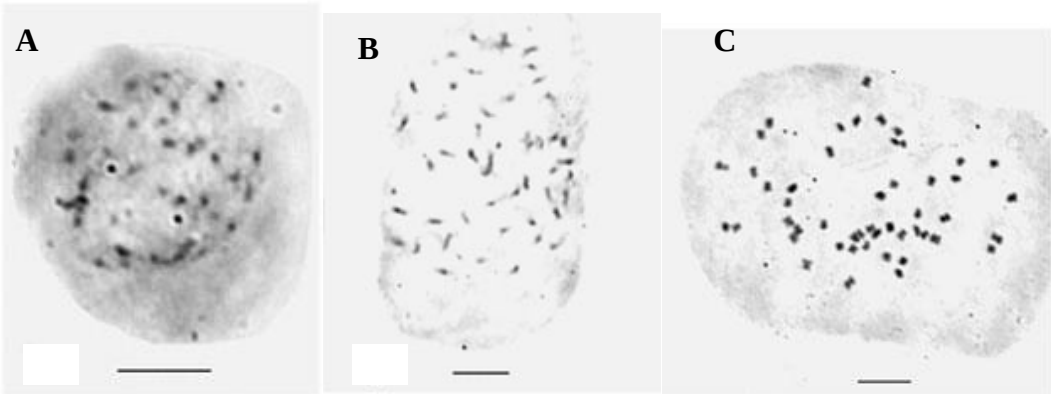


Figure 7: *Mandragora* cytology: A- Interphase nuclei of smooth-faced round prochromosomes of *M. caulescens*. B- Mitotic prophases of proximal type of *M. caulescens*. C- Mitotic metaphases of *M. caulescens*, 2n = 48. Scale bars = 5 µm (adapted from Tu *et al.*, 2005).

Table 2: Summary of the previously reported *Mandragora* chromosome number (adapted from Tu *et al.*, 2005).

Taxon	2n/ base no./ ploidy level	Karyotype formula (*n*)	Locality	Data source
M. caulescens	48/6/8x	?	Hengduan Mountains	Tu *et al.*, 2005
M. autumnalis	84/6/14x	?	Mediterranean region	Hawkes, 1972
	96/6/16x	?	Europe (Slovakia)	Murin, 1978
	24/6/4x	?	Europe (Italy)	Lentini *et al.*, 1988

2.3. Chloroplast DNA and Internal Transcribed Spacer (ITS)

Chloroplast DNA (cpDNA) is a haploid circular molecule (155 kb in *Nicotiana tabaccum*), which is highly conserved in size and structure, with low mutation and recombination rates, and thus makes a good tool to study genetic variation of closely related species (Grivet *et al.*, 2001; Shaw *et al.*, 2005; Heinze, 2007). It usually possesses two long inverted repeats (IR) which separate a large single copy region (LSC) from a small single copy region (SSC). As cpDNA is maternally inherited, the geographical distribution of cpDNA variants is largely dependent on the species migration.

However, despite these advantages of cpDNA, in order to reconstruct phylogeny and avoid potential bias due to maternal inheritance, there is a need to compare chloroplast DNA sequences to nuclear ribosomal DNA (nrDNA) sequences (Rieseberg and Soltis, 1991; Sang *et al.*, 1997; Xu *et al.*, 2010).

The internal transcribed spacer (ITS) region of 18S-26S nrDNA has proven to be very useful for phylogenetic studies in many angiosperm families. The need for phylogenetic markers from the nuclear genome, to complement the rapidly growing body of cpDNA data, makes the ITS region a particularly valuable resource for plant systematists (Baldwin, 1992; Baldwin *et al.*, 1995; Wen and Zimmer, 1996; Sang *et al.*, 1997; Wen and Shi, 1999; Alvarez and Wendel, 2003; Bohs, 2007), and thus it was employed in this study.

3. Research hypothesis and objectives

I hypothesized that *Mandragora* of different geographic origins have different ploidy level and possess sufficient variation in fruit size and weight for successful domestication. In addition, I hypothesized that the current genus taxonomy is out-dated and needs to be revised.

The three specific goals of the research were as follows:

1. Characterization of the phenotypic variance of *Mandragora* and different crosses trials as initial step towards *Mandragora* domestication.
2. Assessment of ploidy level and its association with distribution range.
3. Species delimitation and revision of the current taxonomy of genus *Mandragora*.

4. Materials and Methods

4.1. Plants

Sergei Volis' lab has a collection of *Mandragora* from Israel and abroad. All plants (Israeli and foreign accessions) were grown in the greenhouse, Bergman Campus, Ben-Gurion University of the Negev, Beer Sheva (Figure 8). The accession name, country, province and geographic coordinates of sampling locations are listed in Table 3 and Table 4.

Figure 8: *Mandragora* plants in Bergman Campus, Ben-Gurion University of the Negev, Beer Sheva grown in the greenhouse.

The source of most of the plants were from vegetative origin, one plant from each clone, except for Cyprus that had three plants from the same clone, and the Iranian plant that originated from seed.

Table 3: Accession name, region and geographic coordinates of samples from Israel, *M. officinarum* (according to Ungricht *et al.*, 1998). The last four columns detail the analyses conducted.

Accession name	Region	Geographic coordinates	Cross-breeding	AFLP	cpDNA	ITS
M-j-sh1	Golan	33°16′N 35°46′E	-	V	V	V
M-j-sh2	Golan	ditto	-	V	-	-
Mevo Hama2/07	Golan	32°44′N 35°39′E	V	V	-	-
Mevo Hama4/07	Golan	ditto	V	V	-	-
Mevo Hama5/07	Golan	ditto	V	-	-	-
Mevo Hama7/07	Golan	ditto	V	-	-	-
Mevo Hama8/07	Golan	ditto	V	V	-	-
Mevo Hama11/07	Golan	ditto	V	-	-	-
Am1/99	Galilee	32°55′N 35°31′E	V	V	-	-
Am1/01	Galilee	ditto	V	-	-	-
Am3/01	Galilee	ditto	V	V	-	-
Am4/01	Galilee	ditto	V	V	-	-
Avel	Galilee	33°15′N 35°34′E	-	V	-	-
Karmiel	Galilee	32°54′N 35°17′E	-	V	-	-
Yiftah1/08	Galilee	33°8′N 35°33′E	-	V	-	-
NDX 13/05	Galilee	32°2′N 35°25′E	V	V	-	-
NDX 26/05	Galilee	ditto	V	-	-	-
NDX 27/05	Galilee	ditto	V	V	-	-
NDX 28/05	Galilee	ditto	V	V	-	-
NDX 30/05	Galilee	ditto	V	-	-	-
R-P1/00	Galilee	32°57′N 35°32′E	V	-	-	-
R-P3/00	Galilee	ditto	V	-	-	-
R-P8/00	Galilee	ditto	V	-	-	-
R-P12/00	Galilee	ditto	V	-	-	-
R-P28/00	Galilee	ditto	V	-	-	-
R-P33/00	Galilee	ditto	V	V	-	-
R-P43/00	Galilee	ditto	V	-	-	-
R-P49/00	Galilee	ditto	V	V	-	-
R-P53/00	Galilee	ditto	V	V	-	-
R-P16/99	Galilee	ditto	V	-	-	-
Dalt1/06	Galilee	33°0′N 35°31′E	V	-	-	-
Dalt3/06	Galilee	ditto	V	V	-	-
Dalt4/06	Galilee	ditto	V	-	-	-
Dalt5/06	Galilee	ditto	V	V	-	-
Givat More2/08	Galilee	32°36′N 35°21′E	-	V	-	-
Yaqum2/08	Galilee	32°14′N 34°50′E	-	V	-	-
Lapidim1/06	Gilboa	32°31′N 35°23′E	-	V	-	-
Lapidim2/06	Gilboa	ditto	-	V	-	-
Bark1/06	Gilboa	32°30′N 35°23′E	V	V	-	-
Bark2/06	Gilboa	ditto	-	V	-	-
Gilboa1/05	Gilboa	32°26′N 35°24′E	V	V	-	-
Mazleg3	Judean Mountains	31°44′N 35°4′E	-	V	-	-
Mazleg3/06	Judean Mountains	ditto	V	V	-	-
Mazleg4/06	Judean Mountains	ditto	V	V	-	-
Hadasah4/06	Judean Mountains	31°43′N 35°5′E	V	V	-	-
Hadasah7/06	Judean Mountains	ditto	V	V	-	-
Bar Giora	Judean Mountains	31°43′N 35°4′E	V	V	-	-
BG2000	Shefela	31°35′N 34°53′E	V	V	-	-
Luz1/02	Shefela	31°40′N 34°53′E	V	V	-	-

Luz5/02	Shefela	ditto	V	-	-	-
Luz6/02	Shefela	ditto	V	-	-	-
Luz7/02	Shefela	ditto	V	-	-	-
Luz8/02	Shefela	ditto	V	-	-	-
Luz9/02	Shefela	ditto	V	-	-	-
Luz10/02	Shefela	ditto	V	-	-	-
Luz11s/02	Shefela	ditto	V	V	-	-
Zaf1/99	Shefela	31°37′N 34°55′E	-	V	-	-
Pura4/04	Northern Negev	31°29′N 34°46′E	V	V	-	-
Pura5/04	Northern Negev	ditto	V	V	-	-
Pura1/06	Northern Negev	ditto	V	V	V	V
Keram1/04	Northern Negev	31°20′N 34°54′E	V	V	-	-
Keram2/04	Northern Negev	ditto	V	V	-	-
Keram4/04	Northern Negev	ditto	V	-	-	-
Keram5/04	Northern Negev	ditto	V	-	-	-
Lah1/02	Northern Negev	31°21′N 34°50′E	V	-	-	-
Lah4/02	Northern Negev	ditto	V	-	-	-
Lah9/02	Northern Negev	ditto	V	-	-	-
Lah13/02	Northern Negev	ditto	V	V	-	-
Lah15/02	Northern Negev	ditto	V	V	-	-
Lah16/02	Northern Negev	ditto	V	V	-	-
Meit1/04	Northern Negev	31°19′N 34°55′E	V	V	-	-
Meit1/05	Northern Negev	ditto	-	V	-	-
Meit1/08	Northern Negev	ditto	V	-	-	-
Meit4/08	Northern Negev	ditto	V	-	-	-
Meit5/08	Northern Negev	ditto	V	-	-	-
Meit6/08	Northern Negev	ditto	V	-	-	-
Yatir1/04	Northern Negev	31°20′N 34°58′E	V	V	-	-
Yatir2/04	Northern Negev	ditto	V	-	-	-
Yatir3/04	Northern Negev	ditto	V	-	-	-
Yatir4/04	Northern Negev	ditto	V	-	-	-
Yatir 5/04	Northern Negev	ditto	V	-	-	-
Yatir 6/04	Northern Negev	ditto	V	-	-	-
Yatir 7/04	Northern Negev	ditto	V	-	-	-
Yatir9/04	Northern Negev	ditto	V	V	-	-
Yatir10/04	Northern Negev	ditto	V	-	-	-
Yatir 12/04	Northern Negev	ditto	V	-	-	-
Yatir2/05	Northern Negev	ditto	V	-	-	-
Yatir3/05	Northern Negev	ditto	V	-	-	-
Tane1/04	Northern Negev	31°21′N 34°57′E	V	V	-	-
Livne2/8	Northern Negev	31°21′N 35°4′E	V	V	-	-
Livne9/08	Northern Negev	ditto	V	V	-	-

Table 4: Current taxonomy according to Kuang and Lu (1978) and Ungricht *et al.* (1998), accession name, country and remarks (source of material, collection place, province or geographic coordinates) of samples of non-Israeli origin. The last four columns detail the analyses conducted.

Current taxonomy	Accession name	Country	Remarks	Cross-breeding	AFLP	cpDNA	ITS
M. turcomanica	Mt7-1	Turkmenistan		V	V	-	-
M. turcomanica	Mt10-2	Turkmenistan		V	V	V	V
M. turcomanica	Mt11-2	Turkmenistan		V	V	-	-
M. turcomanica	Mt12	Turkmenistan			V	-	-
M. turcomanica	Mt13-1	Turkmenistan			V	-	-
M. turcomanica	Mt14	Turkmenistan			V	-	-
M. turcomanica	Mt18-4	Turkmenistan			V	-	-
M. turcomanica	Mt20	Turkmenistan		V	V	-	-
M. turcomanica	Tur99	Turkmenistan		V	V	V	V
M. turcomanica	Iran	Iran		V	V	V	V
M. officinarum	Turkey1	Turkey	within 10 km from Antalia	V	V	V	V
M. officinarum	Turkey2	Turkey	Ditto	-	-	V	V
M. officinarum	Cyprus1	Cyprus		V	V	V	V
M. officinarum	Cyprus2	Cyprus		V	-	-	-
M. officinarum	Paros	Greece		-	V	V	V
M. officinarum	Crete	Greece		-	V	V	-
M. officinarum	Italy2008	Italy		-	V	V	V
M. officinarum	Sevillia1/08	Spain	Alcala de Guadaira 37º20'N 5º52'W	V	V	V	V
M. officinarum	Sevillia2/08	Spain	ditto	V	V	-	-
M. officinarum	Sevil1a2	Spain	ditto	-	V	-	-
M. officinarum	Sevil1a3	Spain	ditto	-	V	-	-
M. officinarum	Sevil1a4	Spain	ditto	-	V	-	-
M. officinarum	Sevil1a5	Spain	ditto	-	V	-	-
M. officinarum	Sevil1a6	Spain	ditto	-	V	-	-
M. officinarum	Morocco	Morocco		V	V	-	-
M. officinarum	Meknes	Morocco	between Boufakrane and Mrirt 33º39N 5º26´W	-	V	-	-
M. officinarum	Meknes65-1	Morocco	ditto	-	V	-	-
M. officinarum	Meknes65-3	Morocco	ditto	-	V	-	-
M. officinarum	Meknes65-4	Morocco	ditto	-	V	-	-
M. officinarum	Meknes66-1p1	Morocco	ditto	-	V	-	-
M. officinarum	Meknes66-2p2	Morocco	ditto	-	V	-	-
M. officinarum	Meknes66-3p1	Morocco	ditto	-	V	-	-
M. officinarum	Meknes1/08	Morocco	ditto	V	V	V	V
M. officinarum	Meknes2/08	Morocco	ditto	V	V	-	-
M. chinghaiensis	Tu153	Tibet	Qinghai province 34°22'N 100°27'E	-	-	V	-
M. chinghaiensis	Mc31534	Tibet	Xizang province 31°18'N 97°57'E	-	-	V	V
M. chinghaiensis	Tu521-1	Tibet	Qinghai province 34°27 'N 100°12' E	-	V	V	V

M. chinghaiensis	Tu521-2	Tibet	Qinghai province 34°27 'N 100°12' E		V	-	-
M. caulescens	YueMC1	Tibet	Yunnan province	-	V	-	-
M. caulescens	YueMC5	Tibet	Yunnan province	-	V	-	-
M. caulescens	Yue13	Tibet	Yunnan province	-	-	V	V
M. caulescens	MS6	Tibet	Xizang province	-	V	-	-
M. caulescens	Ms9	Tibet	Xizang province	-	-	V	V
M. caulescens	Ms10	Tibet	Xizang province	-	-	V	V
M. caulescens	MS11	Tibet	Xizang province	-	V	-	-
M. caulescens	MS13	Tibet	Xizang province	-	V	-	-
M. caulescens	MG202-1	Tibet	Yunnan province	-	-	V	V
M. caulescens	MG202-2	Tibet	Yunnan province	-	V	-	-
M. caulescens	MG202-3	Tibet	Yunnan province	-	V	-	-
M. caulescens	MG202-4	Tibet	Yunnan province	-	V	-	-
M. caulescens	MG47-1	Tibet	Yunnan province	-	V	-	-
M. caulescens	MG47-2	Tibet	Yunnan province	-	V	-	-
M. caulescens	MZ 7	Tibet	Yunnan province	-	V	-	-
M. caulescens	MZ8	Tibet	Yunnan province	-	-	V	V
M. caulescens	MZ11	Tibet	Yunnan province	-	V	-	-
M. caulescens	MZ14	Tibet	Yunnan province	-	V	-	-
M. caulescens	MJ5	Tibet	Sichuan province	-	-	V	V
M. caulescens	MJ15	Tibet	Sichuan province	-	V	-	-
M. caulescens	MD7	Tibet	Yunnan province	-	-	V	V
M. caulescens	MD15	Tibet	Yunnan province	-	V	-	-
M. caulescens	MD16	Tibet	Yunnan province	-	V	-	-
M. caulescens	ZhoaMC2	Tibet	Yunnan province	-	V	-	-
M. caulescens	ZhoaMC5	Tibet	Yunnan province	-	V	-	-

4.2. Methods

4.2.1. Self and cross-pollination experiment

Controlled pollinations were performed between accessions from different regions, the same region, the same population or by selfing to test for presence of reproductive isolation and self-incompatibility. Besides, a variety of crosses were needed to create a genetically diverse background, which will serve as the basis for selection in the future.

4.2.2. Morphological measurements

All reproducing plants were measured for flower, fruit and seed morphology. Fruit measurements included diameter, length and weight. Flowers were measured for corolla lobe width and length, sepal width and length, style and stigma length, filament and anther length and length of the flower stalk. Fruit weight was measured on a digital scale. Measurements of seed size and surface structure were conducted by SEM (Scanning Electron Microscope, JSM 5610 LV) and under a binocular.

4.2.3. Florescence-activated cell sorting (FACS)

Florescence-activated cell sorting (FACS) was used for estimation of DNA content per nucleus on the basis of relative fluorescence intensity. The method involves preparation of aqueous suspensions of intact nuclei whose DNA is stained using a DNA fluorochrome, propidium iodide (PI) (Dolezel and Bartos, 2005). The method allows measuring relative ploidy level by comparison of the DNA content of a studied plant individual using a reference standard plant, whose genome size is known. An ideal DNA reference standard should have a genome size close to the target species. Garden pea (*Pisum sativum*) appears to be the most suitable candidate. The current estimate of its 2C-value is 9.09 pg DNA (Dolezel *et al.*, 1992), which is in the middle of the known range of genome sizes in plants. The pea plants are easy to grow and multiply and a high quality nuclei suspension is easily prepared from their leaves. Therefore, I used *Pisum sativum* cv. Citrad as a standard (Dolezel *et al.*, 1998; Dolezel and Bartos, 2005) and also Tobacco (*Nicotiana tabacum* Samsson, 10.4pg DNA).

Every plant was tested at least twice in the Flow Cytometer (BD Biosciences, Franklin Lakes, NJ). Preparation of suspensions of intact nuclei for estimation of absolute DNA amounts was according to the method of Galbraith *et al.* (1983) with several modifications. In this procedure, the nuclei are released into a nuclei isolation buffer by mechanical homogenization of a small amount (about 100 mg) of fresh plant tissue. The composition of the isolation buffer is critical to facilitate the release of nuclei free of cytoplasm and in sufficient quantities, maintain the integrity of isolated nuclei, protect their DNA against endonucleases, and facilitate DNA staining (Dolezel *et al.*, 1998).

The nuclei isolation buffer that was used contained 0.2M Sucrose, 10mM MES, 2.5mM EDTA, 10mM NaCl, 10mM KCl, 0.15% Triton X-100, 0.1mM Spermine and 2.5mM DTT (all materials from Sigma-Aldrich, Rehovot Israel). The tubes with the buffer and the homogenized leaves were placed in ice and shaken for 30 to 50 minutes. Afterwards, samples were filtered through a nylon mesh of 150 microns and then centrifuged for 10 minutes at 550 g, at 4^0C. The upper supernatant was carefully decanted and the pellet was dissolved in 0.5 ml buffer and kept in ice till the next day. Before inserting the sample to the FACS machine, another filtration through a nylon mesh of 50 microns was performed and 10 μl of PI (2 mg/ml, Sigma-Aldrich) were

added. Each sample was tested twice, once alone and the second time with the standard inside. The results were obtained using a Becton Dickinson FACSVantage SE machine equipped with an air-cooled argon-ion laser tuned to 180mw and operating at 488 nm with a 585/42-nm band-pass filter (BD Biosciences, Franklin Lakes, NJ). The results were processed using BD CellQuestTM pro V. 5.1.1. software.

The sample 2C DNA content was calculated by sample G1 peak mean divided by standard G1 peak mean multiplied by standard 2C DNA content (pg DNA) (Dolezel and Bartos, 2005).

4.2.4. DNA extraction

DNA was extracted from leaf tissue using either the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) or the CTAB method (Doyle and Doyle, 1987). For the CTAB method, 150 mg of fresh or frozen leaf was homogenized with liquid nitrogen, then 500 µl of 2xCTAB buffers + β mercaptoethanol (1:1:0.2) was added and samples were incubated at 60°C for 45 to 90 minutes. 500 µl 5M potassium acetate (KAcO, 5M, pH 4.8) was added, samples were mixed gently for 10 min and put in ice for 20 minutes. Afterwards the tubes were centrifuged for 20 minutes at 18,000 g. The supernatant was transferred to a new eppendorf tube and 1.5 volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added, samples were mixed gently for 5 min and centrifuged again for 5 minutes at 18,000 g. The aqueous phase was transferred to a fresh tube with cold (-20°C) isopropanol, Mixed gently to precipitate DNA, incubated at -20°C for 1 hour and then centrifuged again at 18,000 g for 10 minutes. The supernatant was decanted and the pellet (DNA) was washed twice with 400 µl ice cold ethanol (70% and 96%) and centrifuged at 18,000 g for 2 minutes. The pellet was dried, dissolved in 100µl water and then incubated for 0.5-1 hour at 37°C and stored at -20°C. The quality and quantity of genomic DNA was accurately measured by NanoDrop spectrophotometer-1000 (Thermo Fisher Scientific, Wilmington, DE).

4.2.5. Fluorescent Amplified Fragment Length Polymorphism (AFLP)

fAFLP (Fluorescent Amplified Fragment Length Polymorphism) is a mapping technique used to visualize polymorphisms in genomic DNA. The AFLP system combines the restriction fragment length polymorphism (RFLP) technique and the

polymerase chain reaction (PCR) to generate a large number of amplified restriction fragments from genomic DNA. When separated by electrophoresis, the samples yield unique band or peak patterns that, when visualized by fluorescence-based fragment analysis, can be used for polymorphism detection. In this research, AFLP for 106 samples was used for analyzing genetic differences between collected accessions for taxonomic inferences.

The original protocol of (Vos *et al.*, 1995) was followed with some modifications. After each step, the PCR product was checked on 2% agarose gel. The adaptors and primers that were used are listed in Table 5.

1. **Restriction/Ligation reaction** (R/L) – For each sample reaction: 1.0µl 10 ^{X} T4 DNA Ligase buffer, 1.0µl 0.5M NaCl, 1.0µl 10 mg/ml BSA, 1.0µl *Mse*I adaptor pair, 1.0µl *EcoR*I adaptor pair, 0.12µl *Mse*I (10,000 u/ml) , 0.5µl *EcoR*I (20,000 u/ml), 0.3µl T4 DNA Ligase (400,000 u/ml) (all from New England Biolabs, Ipswich, MA), 1.08µl DDW and 3µl genomic DNA (concentration of DNA- till 300 ng/µl), at a total volume of 10µl. Samples were mixed well, microcentrifuged for several seconds, and incubated at 37^{0}C for 2.5h.
2. **Preselective amplification** –Each sample reaction contained: 2.0µl 10 ^{X} TAQ DNA polymerase buffer, 2.0µl 20 mM $MgSO_4$, 1.6µl 2.5mM dNTPs, 0.2µl TAQ DNA polymerase (5 U/µl), 8.2µl DDW, 1.0µl MseI 50 pmol/µl Preselective primer, 1.0µl EcoRI 10 pmol/µl Preselective primer and 4.0µl of diluted (10 fold) R/L product. Samples were mixed well and microcentrifuged for a few seconds. The PCR conditions were as follows: 20 cycles of 30 s denaturation at 94°C, 1 min annealing at 56°C and 1 min extension at 72°C.
3. **Selective amplification –** Each reaction sample (*2 colors) contained: 2.0µl 10 ^{X} TAQ DNA polymerase buffer, 2.0µl 20 mM $MgSO_4$, 1.6µl 2.5mM DNTP's, 0.2µl TAQ DNA polymerase (5 u/µl), 1.0µl MseI selective primer (5 mM) (1 out of 8 primers), 1.0µl EcoRI (1 mM) (1 out of 2 fluorescently labeled selective primers FAM / VIC), 9.2µl DDW and 3.0µl of diluted (10 fold) preselective amplification reaction product. Samples were mixed well and microcentrifuged for a few seconds. The PCR conditions were as follows: One cycle at 94°C for 2 min, 65°C for 30 s and 72°C for 2 min. One cycle at 94°C for 30 s; 64°C (annealing) for 30 s, and 72°C for 2 min. Then, the annealing temperature was lowered each cycle by

1°C during 9 cycles (touch down), followed by 27 cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 2 min.

4. **ROX –** To prepare samples for running gene scan on 3130xl GENETIC ANALYSER, for each sample reaction 0.3µl GS-500 ROX-labeled size standard, 7.5 µl formamide and 2.2µl (2*1.1 for each colour) selective PCR product (Ma *et al.*, 2004) were mixed.

After tracking using the GeneScan analysis software (PE Applied Biosystems, Foster City, California), fAFLP lanes were saved as individual sample files. For each extracted sample lane, fragment sizing was performed by generating a sizing curve based on the predefined electrophoretic fragment distribution of the internal size standard GS-500 and scored 0 or 1. For numerical analysis, data intervals were delineated between the 50- and 500-bp bands of the internal size standard (Huys and Swings, 1999).

Table 5: List of the adaptors and primers used for fAFLP.

Primer name	**Sequence**
*Eco*RI adapters	CTC GTA GAC TGC GTA CC
	AAT TGG TAC GCA GTC TAC
*Mse*I adapters	GAC GAT GAG TCC TGA G
	TAC TCA GGA CTC AT
EcoRI	GAC TGC GTA CCA ATT C NNN
EcoRI pre selective primer	NNN=A
EcoRI selective primers (florescent labeled)	NNN= E_ACT (FAM), E_AGC (VIC).
MseI	GAT GAG TCC TGA GTA A NNN
MseI pre selective primer	NNN=C
MseI selective primers	NNN= (SAP2)M_CTT, (SAP3)M_CAG, (SAP7)M_CAA, (SAP8)M_CAC

4.2.6. AFLP data analysis

AFLP analysis was performed using two programs: *STRUCTURE* and *BAPS*. Every vertical colored bar corresponds to an individual and different colors are different clusters. *K* is the number of clusters. For each value of *K*, there is an estimation for each individual of the most likely cluster assignment. The admixture image shows for each individual the proportion of genome estimated to have ancestry in a particular cluster. The proportions are shown as colored segments of a vertical bar where the color determines the origin of a segment and the proportion of a particular color in the vertical bar corresponds to the proportion of the genome estimated to be represented by that source.

The *STRUCTURE* software implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. Individuals in the sample are assigned to *K* populations (where *K* may be unknown) and distinct genetic populations are identified (Evanno et al., 2005). The basic algorithm of *STRUCTURE* was described by Pritchard et al. (2000) and extensions to the method were published by Falush et al. (2003), Falush et al. (2007) and by Hubisz et al. (2009). The Admixture Model was used with 106 individuals and 646 loci. The length of Burnin Period (how long to run the simulation before collecting data to minimize the effect of the starting configuration) was 100000 and the number of Markov Chain Monte Carlo (MCMC) Reps after Burnin (how long to run the simulation after burnin to get accurate parameter estimates) was 500000. The range of tested *K* values ranged from 2 to the number of regions (10).

BAPS 5 (Bayesian Analysis of Population Structure) is a program for Bayesian inference of the genetic structure in a population (Corander and Marttinen, 2006). *BAPS 5* treats both the allele frequencies of the molecular markers and the number of genetically diverged groups in population as random variables. Admixture analysis was done based on mixture clustering of individuals with maximum number of *K* between 2 to 10, with 100 iterations for each *K*.

The data were also analyzed via Principal Coordinates Analysis (PCA) performed with *GenAlEx* 6.3 software (Peakall and Smouse, 2006) and cluster analysis via *Primer6* software (Clarke, 1993). Hierarchical agglomerative clustering used simple matching similarity index and Single linkage (nearest-neighbor) clustering algorithm. The similarity profile (SIMPROF) test was used on each node to assess the statistical

significance of the dendrogram. SIMPROF calculates a mean profile by randomizing each variable's values and re-calculating the profile. The pi statistic is calculated as the deviation of the actual resemblance profile of the resemblance matrix with the mean profile. This is compared with the deviation of further randomly-generated profiles to test for significance. In the dendrogram, significant branches ($p \leq 0.05$) were drawn in black and insignificant branches were drawn in red.

4.2.7. Chloroplast DNA fragment sequencing

Chloroplast DNA (cpDNA) sequences are routinely used in plant molecular systematic, phylogeographic and population genetic studies. Eight plastid markers were used for phylogenetic inferences, the atpB gene (encoding CF1 ATPase beta-subunit gene), the ndhF gene (encoding a subunit of the chloroplast NADH dehydrogenase), the rps16-trnK intergenic spacer, the rbcL gene (encoding the large subunit of the photosynthesis enzyme rubisco), the trnC-psbM region, the (trnF-trnV) region, the trnL-trnF intergenic spacer and the trnH-psbA intergenic spacer. The sequences of chloroplast genes or spacers showing nucleotide sequence polymorphism, have been widely used for inferring phylogeny and phylogeography in plants (Gielly and Taberlet, 1994; Bohs and Olmstead, 1997; Chiang *et al.*, 1998; Grivet *et al.*, 2001; Shaw and Small, 2004; Shaw *et al.*, 2005; Petit and Vendramin, 2007; Shaw *et al.*, 2007; Melotto-Passarin *et al.*, 2008; Tu *et al.*, 2008). Usually the phylogenetic information provided by one chloroplast genetic marker does not have enough power to separate close taxa, especially at low taxonomic levels. For this reason, in order to improve the resolution of phylogenetic analysis, we need to increase the number of markers (Melotto-Passarin *et al.*, 2008).

The analysis included 22 samples covering the whole known genus range, including Mediterranean, Central Asian and Sino-Himalayan locations. All new sequences obtained in this study were submitted to GenBank (Table 8).

Primers for ndhF, trnH-psbA, rps16-trnK, trnC-psbM, atpB, trnL-trnF and trnF-trnV (FV) followed Dumolin-Lapegue *et al.* (1997), Shaw and Small (2005) and Shaw *et al.* (2007). Internal primers were designed using the software Primer3 version 0.4.0 (http://frodo.wi.mit.edu/primer3/input.htm). The primer sequences are given in Table 6.

Table 6: List of the primers, their length and sequence that were used for cpDNA and ITS sequencing.

Region name (length in bp)	**Primer name**	**Primer sequence**
FV (3358)	trnF	CTC GTG TCA CCA GTT CAA AT
	FV_966R	GAG AAT AGA GGA TGG TGT GGA T
	FV_870F	CCA AAC CCA GAA AAC AGA CG
	FV_1902R	CCA ACA GCC CAA AAT TCA GT
	FV_1549F	GTG GGC AAC CTG GCA AAT A
	FV_2470R	CTA AAT AGT TAT GGA TGA CTT GAC
	FV_2100F	AAT TAC GGA TAC ACC CAA TAC G
	FV_3245R	CAA ACC AAC CTT TCG TCA TT
	FV_2915F	GTG TTG TGC TTC GCT AGG TC
	trnV	CCG AGA AGG TCT ACG GTT CG
trnC-psbM (1850)	trnC	CCA GTT CAA ATC CGG GTG TC
	TRNC_1011R	GTA AGA GGC CGT TGA TTG GA
	TRNC_938F	AGG AAG AGG ATC CCA GGA AA
	2039R	TTT TCT ACT TAT CAT TTA CG
rbcL-accD (1392)	26F	GTG CAC CAC AAA CAG AGA CTA AAG C
	RBCL_892R	TTG CTA ATA CCC GGA AGT GG
	RBCL_626F	AAG CAC AGG CTG AAA CAG GT
	60123R	TGA GTT CTT TCT CCT TTA TCC TTC
rpS16-trnK (668)	rps16x2F2	AAA GTG GGT TTT TAT GAT CC
	trnKx1	TTA AAA GCC GAG TAC TCT ACC
atpB (1091)	56292F	TCA GTA CAC TAA GAT TTA AGG TCA T
	57472R	TGC TCG GAG AAC CTG TTG ATA A
psbA (503)	trnH(GUG)	CGC GCA TGG TGG ATT CAC AAT CC
	psbA	GTT ATG CAT GAA CGT AAT GCT C
trnL-trnF (950)	trnL5(UAA)F	CGA AAT CGG TAG ACG CTA CG
	trnF(GAA)	ATT TGA ACT GGT GAC ACG AG

ndhF (1999)	NDHF_1F	CCT ATG TTA ATAGGAGCGGGACT
	NDHF_970R	CGC TTC GAT AAG ACC CCA TA
	NDHF_780F	TCG GCT TCT TCC TCT TTT CA
	NDHF_2110R	CCC CCT ACT ATA TTT GAT ACC TTC TCC
ITS (692)	ITS4	TCC TCC GCT TAT TGA TAT GC
	ITS5	GGA AGT AAA AGT CGT AAC AAG G

PCR was performed using either Eppendorf or MJ Research thermal cyclers in 50 µl volumes. For each sample reaction: 5.0µl 10 x TAQ DNA polymerase buffer, 5.0µl 20 mM MgSO4 (except for trnC-psbM- 40mM), 4µl 2.5mM dNTP's, 2.5µl primer 1 (forward) 10mM, 2.5µl primer 2 (reverse) 10mM, 0.5µl TAQ DNA polymerase (5 u/µl), 28µl sterilized distilled water and 2.5µl DNA (~50 ng/µl) were mixed (Tu *et al.*, 2008).

For the regions rps16-trnK, atpB, rbcL-accD, ndhF and trnC-psbM the PCR conditions followed Shaw *et al.*, 2007 and Dillon *et al.*, 2009: template DNA denaturation at 95^0c for 3 min, 35 cycles of denaturation at 94^0c for 30 sec, primer annealing at 50^0c for 1 min and primer extension at 72^0c for 2 min, followed by a final extension step of 72^0c for 10 min.

For psbA region the reaction conditions followed Shaw *et al.*, 2005: template DNA denaturation at 80^0c for 5 min, 30 cycles of denaturation at 94^0c for 30 sec, primer annealing at 50^0c for 30 sec and primer extension at 72^0c for 1 min, followed by a final extension step of 72^0c for 5 min.

For trnL-trnF region the reaction conditions followed Shaw and Small, 2004: template DNA denaturation at 80^0c for 5 min, 35 cycles of denaturation at 94^0c for 1 min, primer annealing at 50^0c for 1 min and primer extension at 72^0c for 2 min, followed by a final extension step of 72^0c for 5 min.

For FV region the reaction conditions followed Demesure *et al.*, 1995 and Dumolin-Lapegue *et al.*, 1997: template DNA denaturation at 94^0c for 4 min, 30 cycles of denaturation at 94 ^{0}c for 45 sec, primer annealing at 57.5^0c for 45 sec and primer extension at 72 ^{0}c for 4 min, followed by a final extension step of 72^0c for 10 min.

PCR products were visualized on 1% agarose gel with 100bp ladder before being purified with either the MSB® Spin PCRapace Kit (Invitek) or illustra™ ExoStar kit

(USB). The cycle sequencing reactions were conducted in 10 µl volumes which contained 0.25 µl BigDye (Rhenium) 3.1, 0.5 µl primers, 2.0 µl purified PCR products (60-70 ng) and 1.75 µl sequencing buffer (Rhenium). The reaction conditions were 95^0c for 2 min, 30 cycles of 96 ^{0}c for 30 sec, 50^0c for 15 sec and 60 ^{0}c for 4 min, ending with 4^0c. The sequencing reactions were run on an ABI 3730 automated sequencer (Applied Biosystems, Ben-Gurion University of the Negev Beer Sheva Israel).

4.2.8. Internal Transcribed Spacer (ITS) sequencing

The ITS primer sequences are given in Table 6.

6 and the materials for the PCR reaction are as described above for cpDNA sequencing. For ITS region the reaction conditions followed White *et al.* (1990): template DNA denaturation at 94^0c for 4 min, 33 cycles of denaturation at 94^0c for 1 min, primer annealing at 57^0c for 1 min and primer extension at 72^0c for 1 min, followed by a final extension step of 72^0c for 10 min.

4.2.9. Phylogenetic analysis based on cpDNA and ITS sequences

Sequencher 4.10.1 (Gene Codes Corporation, 2005) was used to evaluate chromatograms for base confirmation and to edit contiguous sequences. Sequences were aligned with ClustalX. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura *et al.*, 2011). The phylogeny reconstruction based on the combined sequence matrix was performed using Maximum likelihood (ML), Neighbor Joining (NJ) and Maximum Parsimony (MP). The bootstrap percentages (Felsenstein, 1985) for confidences of the internal nodes were obtained with 1000 replicates. The analysis involved 22 nucleotide sequences representing the whole known genus range, including Mediterranean, Central Asian and Sino-Himalayan locations. All positions containing gaps and missing data were eliminated. The bootstrap values lower than 50% are not shown.

Maximum-likelihood (ML) is a method of estimating the parameters of a statistical model for the inference of phylogeny. It evaluates a hypothesis about evolutionary history in terms of the probability that the proposed model would give rise to the observed data set. The method searches for the tree with the highest probability or likelihood. In MEGA, the test for best model of maximum likelihood was: Tamura 3 parameter model (T92) for the atpB, FV, psbA, trnC-psbM, trnL-trnF

and ITS regions; Hasegawa-Kishino-Yano model (HKY) for ndhF region; Jukes-Cantor model (JC) for rbcL-accD region; and Hasegawa-Kishino-Yano model (HKY) for rpS16-trnK region. The trees were condensed with 50% cut-off value. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

Neighbor joining (NJ) is a clustering method for creation of phenetic trees (those that classify organisms based on overall similarity, regardless of their phylogeny or evolutionary relation). The algorithm requires knowledge of the distance between each pair of sequences to form the tree. The evolutionary distances were computed using the p-distance method and are in units of the number of base differences per site.

Maximum Parsimony (MP) is a non-parametric statistical method for estimating phylogenies. Under parsimony, the preferred phylogenetic tree is the tree that requires the least evolutionary change to explain the observed data. The MP tree has the most favorable score and therefore is taken as the best estimate of the phylogenetic relationships of the tested taxa. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

5. Results

5.1. Self pollination and cross-breeding

Mandragora plants from the living collection maintained at the Bergman Campus were self-pollinated (Figure 9) or cross-pollinated with pollen of other *Mandragora* plants of different country origin or with pollen of *Mandragora* plants from different regions within the country (Table 7). As can be seen in Figure 9, the following percentages of self pollinations were successful: 49% of plants from Israel, 25% of plants from Turkmenistan, 50% of plants from Iran, 41% of plants from Morocco, 75% of plants from Spain, 29% of plants from Cyprus and 38% of self-pollinations of plants from Turkey.

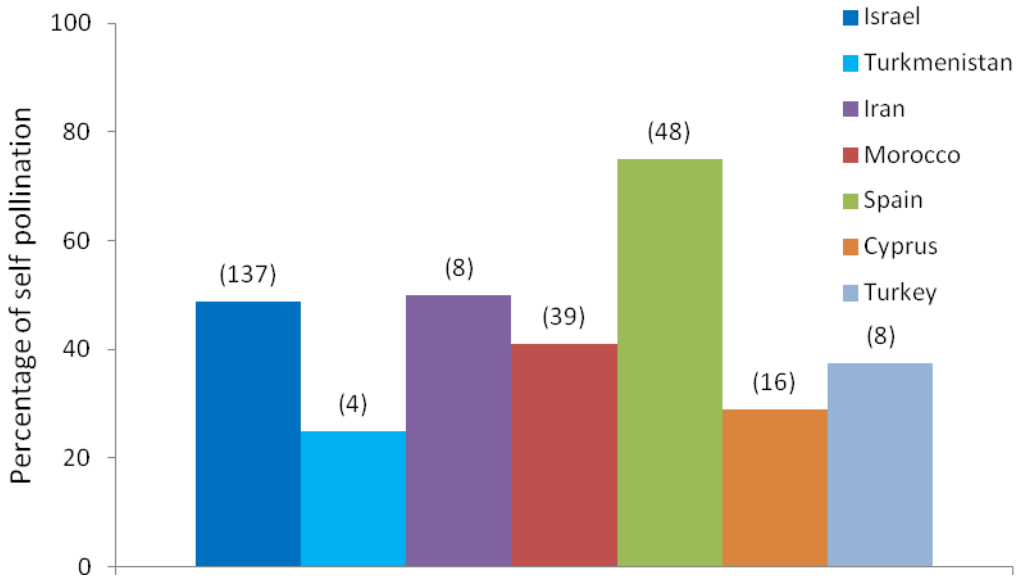


Figure 9: Percentage of successful self-pollination in *Mandragora* plants of different country origin. Number of pollinations is in parentheses.

Crosses between plants from Morocco to plants from Spain and vice versa resulted in the highest percentages of cross-pollination: 97% and 63%, respectively. Crosses between plants from different regions within Israel, from different regions within Morocco or from different regions within Spain succeeded in 27%, 79% and 80% of cases, respectively. We did not have different regions within plants of other country origin so crosses of different regions within them were not performed. There was only a short overlap in flowering time between plants from Israel and from Spain (Figure 12) so only few crosses between them were performed, and none succeeded.

There was no overlap in flowering time at all between plants from Morocco and Spain to plants from Turkmenistan and Iran so crosses between them were not performed (Figure 12). *Mandragora* plant from Iran flowered for the first time in January 2011, so only few crosses were performed with it.

Table 7: Percentage of successful cross-pollination in *Mandragora* plants of different country origin or from different regions within the country. The number of pollinations is in parentheses. An asterisk denotes crosses that were not performed.

♀ \ ♂	Israel	Turkmenistan	Iran	Morocco	Spain	Cyprus	Turkey
Israel	27% (476)	18% (22)	*	33% (18)	0% (4)	0% (46)	4% (26)
Turkmenistan	43% (7)	*	40% (5)	*	*	0% (1)	0% (3)
Iran	*	*	*	*	*	33 (3)	*
Morocco	67% (12)	*	*	79% (28)	97% (29)	*	*
Spain	0% (1)	*	*	63% (16)	80% (15)	*	*
Cyprus	19% (36)	100% (1)	*	*	*	*	33% (3)
Turkey	67% (3)	0% (2)	*	*	*	67% (3)	*

5.2. Morphology and phenology

5.2.1. Flower

Nine parameters of flower morphology were measured (Figure 10): corolla lobe width and length, sepal width and length, style and stigma length, filament and anther length and flower stalk length (Figure 11). The flower stalk length was very variable within and among plants of different origin. Flowers from Cyprus and Turkey were similar in all traits and smaller than flowers from other countries. Flowers from Morocco and Spain were similar in all traits except for style length. The corolla lobe (width and length) of flowers from Morocco and Spain was the largest, and from Cyprus and Turkey was the smallest. For corolla lobe width, flowers from Israel, Turkmenistan and Iran were similar and differed significantly from flowers of other origin – larger than flowers from Cyprus and Turkey and smaller than flowers from Morocco and Spain. Other flower parameters did not differ among different country origin.

5.2.2. Flowering time

For each flowering plant, the onset of flowering was recorded during the years 2007-2012 and distribution of flowering events per country origin is presented in Figure 12. The plants from Morocco and Spain usually flowered around October, while all other *Mandragora* plants – Israel, Turkmenistan, Iran, Cyprus and Turkey – flowered later, from December to February.

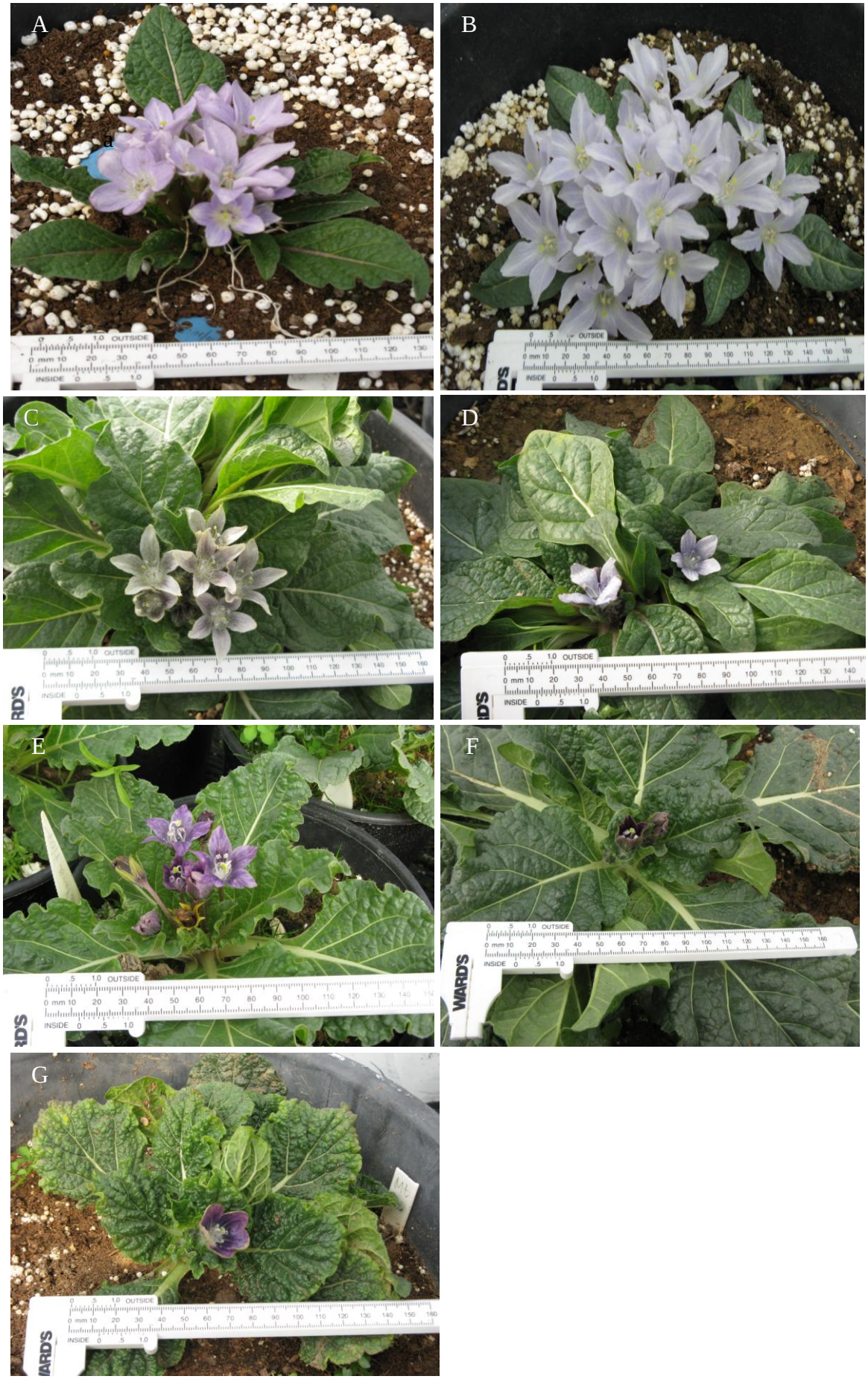


Figure 10: *Mandragora* flowers of different country origin: A- Morocco, B- Spain, C- Turkey, D- Cyprus, E- Israel, F- Iran and G- Turkmenistan.

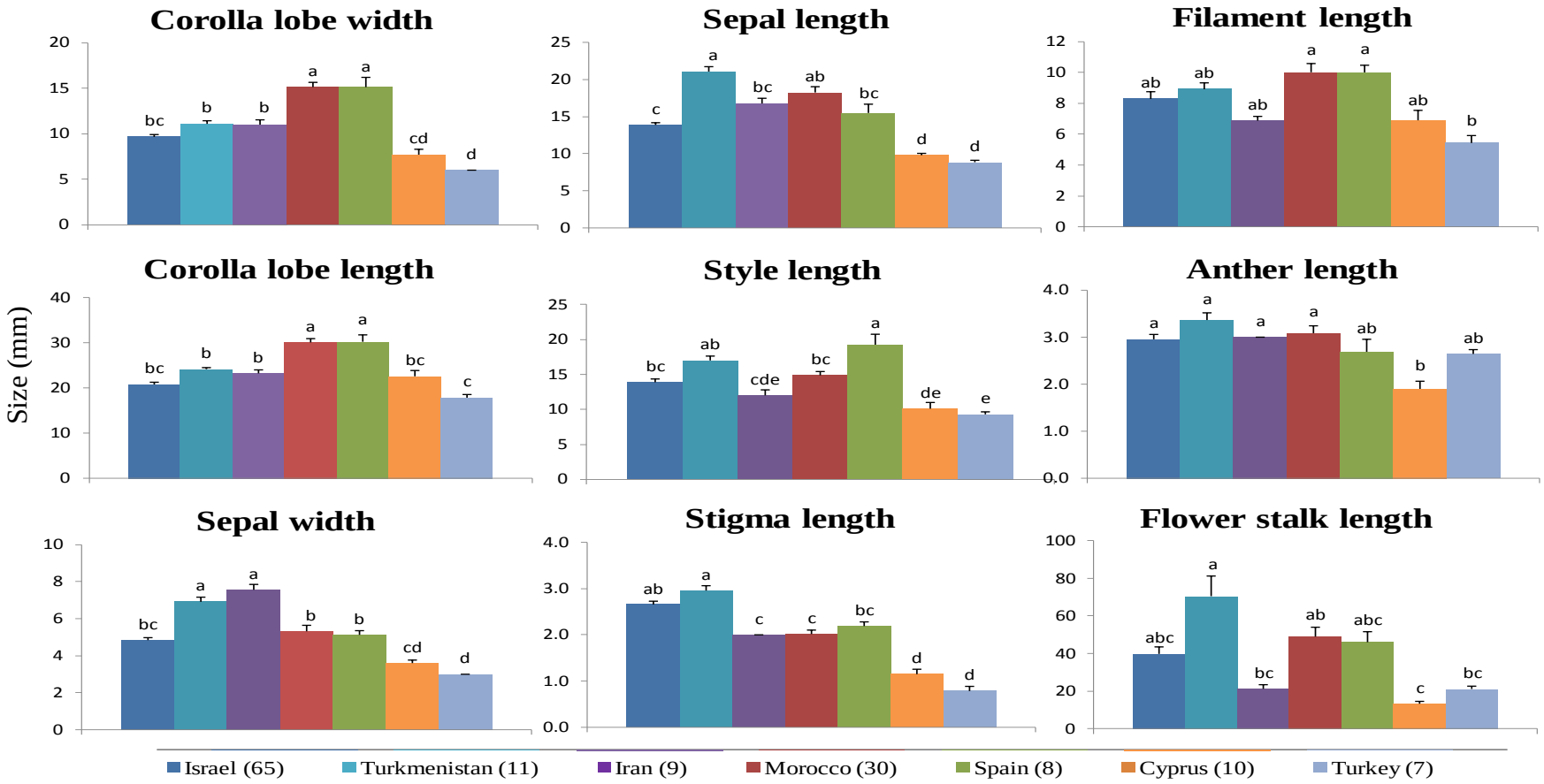


Figure 11: Different parameters of *Mandragora* flower morphology in mm ±SE. Letters denote the results of Tukey-Kramer test. The number of flowers measured is in parentheses.

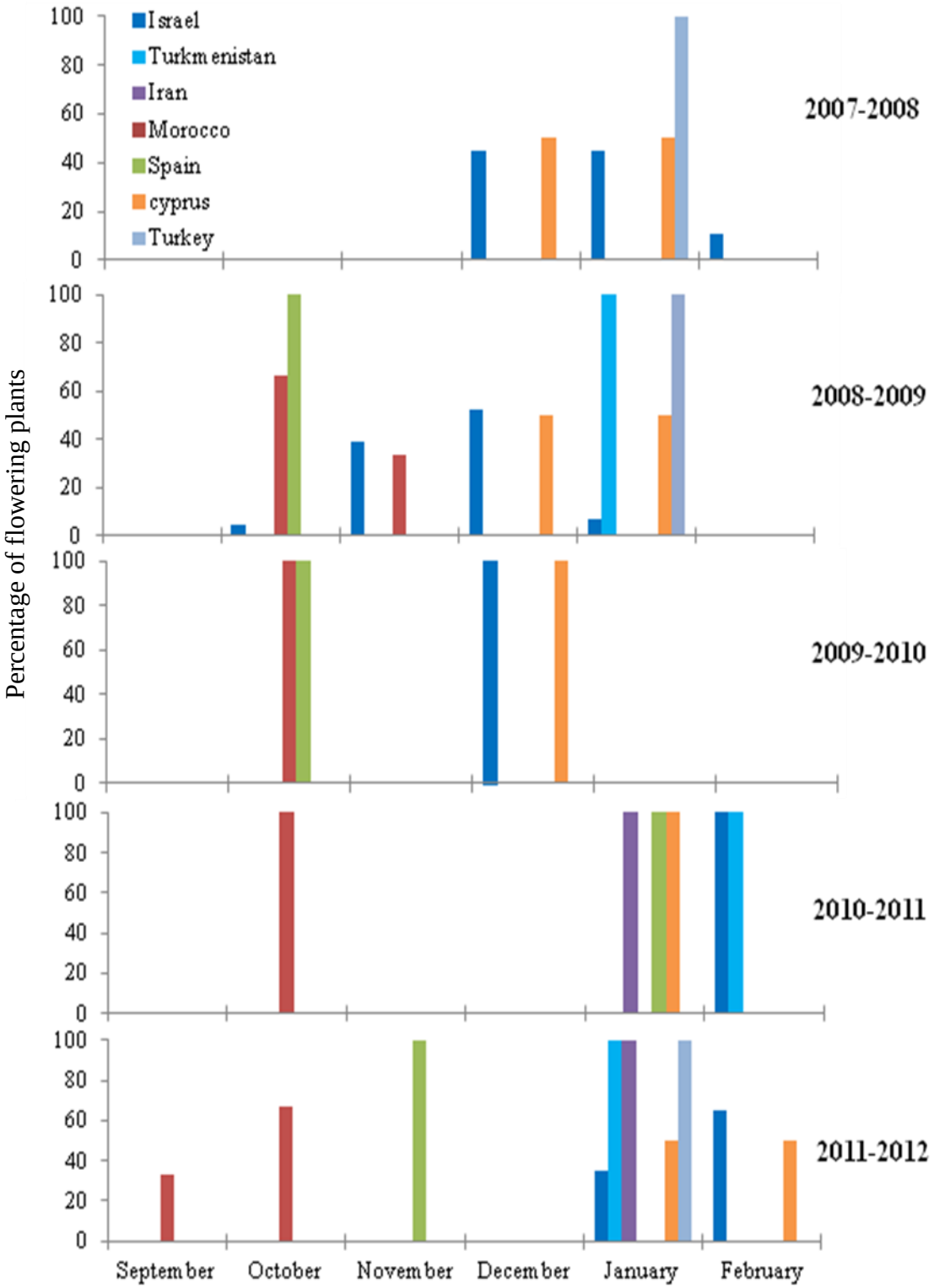


Figure 12: Flowering time frequency distribution for plants of different origin during five years of observation.

5.2.3. Fruit

Fruits of plants from Israel, Turkmenistan and Iran were much larger and heavier than those from Morocco, Spain, Cyprus and Turkey (Figure 13 and Figure 14, ±SE). Fruits of *Mandragora* plants from Israel, Turkmenistan and Iran were in average 27.5±0.5, 29±0 and 26.4±2.4 mm long and weighed 11.7±0.4, 12.6±0 and 7.8±2.5 gram, respectively, while fruits of plants from Morocco, Spain, Cyprus and Turkey were in average 19.8±0.6, 15.9±0.6, 16.2±1.8 and 11.2±0.2 mm long and weighed 2.5±0.2, 2.1±0.2, 1.7±0.4 and 0.7±0 gram, respectively.

The range of minimum to maximum values of fruits of plants from Israel, Turkmenistan and Iran were 6 to 46, 29 to 29 and 23 to 33.5 mm long and 1 to 39, 12.6 to 12.6 and 3.5 to 15.1 gram weight, respectively. On the other hand, the range of minimum to maximum values of fruits of plants from Morocco, Spain, Cyprus and Turkey were 11 to 31, 8 to 25, 11 to 20 and 11 to 11.5 mm long and 0.2 to 5, 0.1 to 6, 0.6 to 2.6 and 0.65 to 0.8 gram weight, respectively.

Furthermore, fruits of plants from Israel, Turkmenistan and Iran were rather globular while those from Morocco, Spain, Cyprus and Turkey were ellipsoid (the length of the fruit was larger than its width) (Figure 15 and Figure 16). One fruit of Turkmenistan origin (Figure 16 D) was very large (55 mm in diameter), but it was collected from a plant grown in field in Sde Boqer and therefore cannot be compared directly to the other fruits.

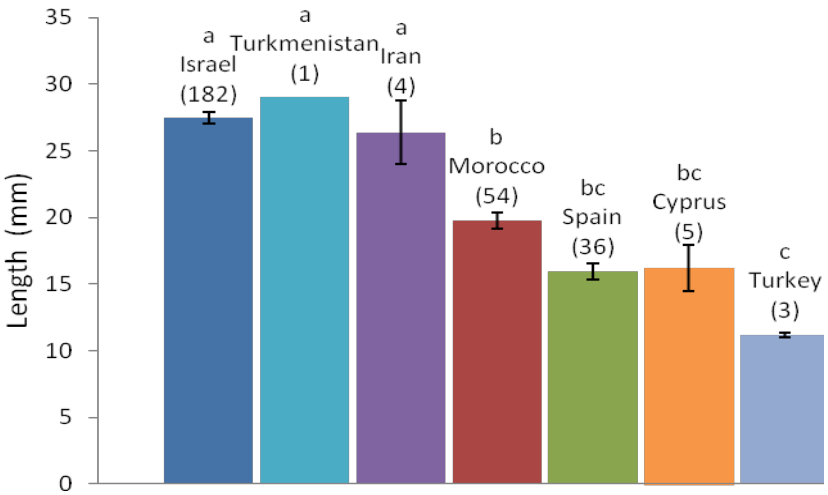


Figure 13: Fruit size (length in mm ±SE) of *Mandragora* plants of different country origin. Letters denote the results of Tukey-Kramer test. The number of fruits measured is in parentheses.

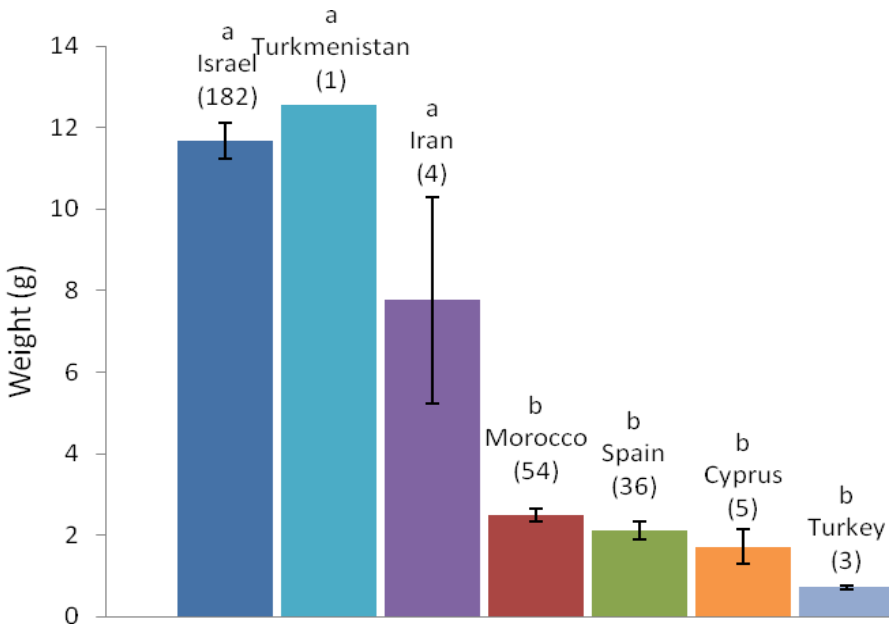


Figure 14: Fruit weight (in grams ±SE) of *Mandragora* plants of different country origin. Letters denote the results of Tukey-Kramer test. The number of fruits weighed is in parentheses.

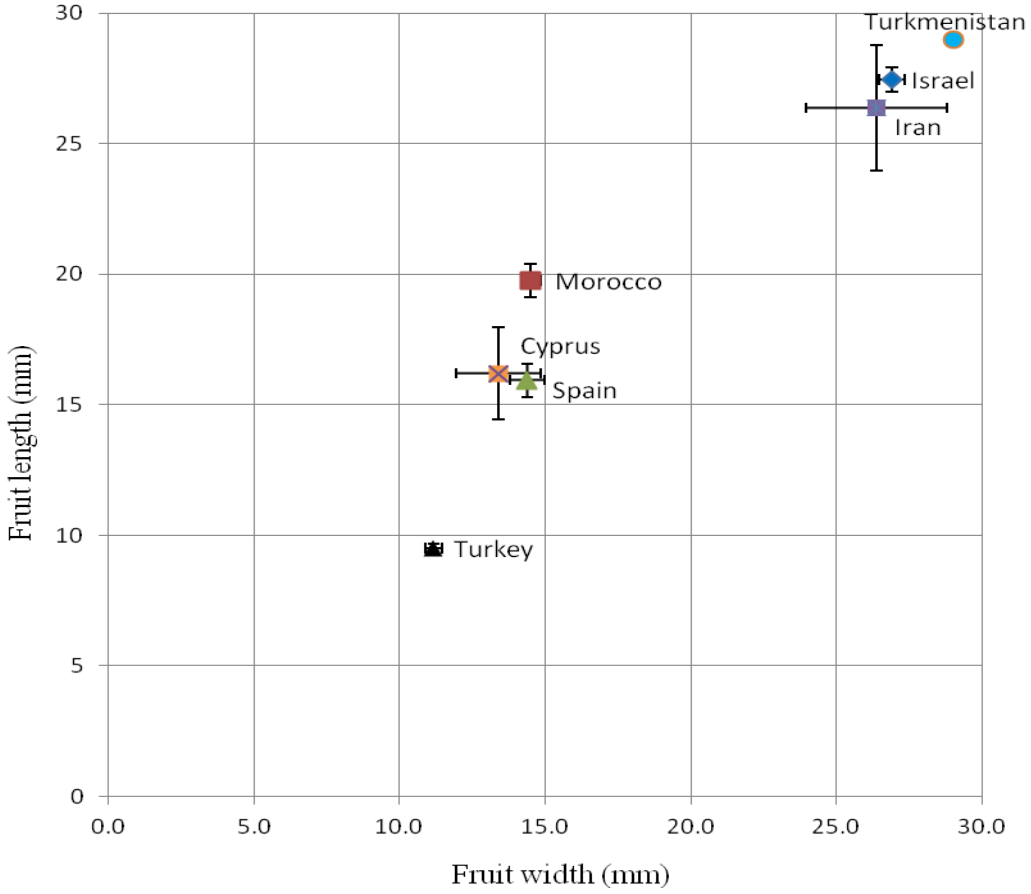


Figure 15: Fruit shape (fruit width versus length in mm ±SE) of *Mandragora* plants of different country origin.

Figure 16: Fruits of *Mandragora* plants of different country origin: A- Morocco, B- Spain, C- Israel and D- Turkmenistan.

5.2.4. Seeds

Seeds of *Mandragora* of different country origin were examined under the binocular (Figure 17 and Figure 18). The seeds from Turkmenistan, Israel and Iran are 5-6 mm long and distinctly larger than seeds from Morocco, Spain, Greece, Cyprus and Turkey that are 3-3.5 mm long, and much larger than seeds from Tibet (2 mm long) (Figure 17 and Figure 18). Additionally, three seeds of plants from Israel, Cyprus and Tibet were examined by SEM (Figure 19). The seed of Tibetan origin differed in its external surface morphology from the seeds of plants from Israel and Cyprus.

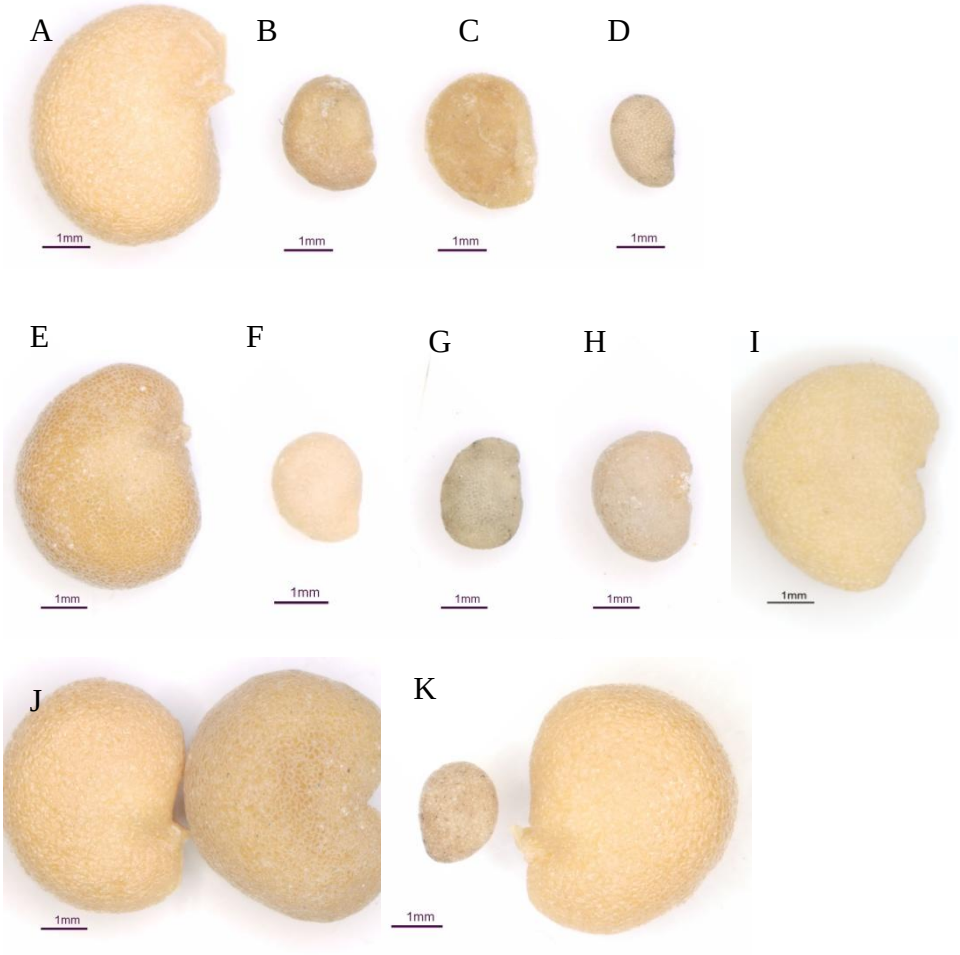


Figure 17: *Mandragora* seeds of different country origin examined under the binocular: A- Turkmenistan, B- Greece, C- Turkey, D-Tibet, E- Israel, F- Cyprus, G- Morocco, H- Spain, I- Iran, J- Israel and Turkmenistan, K- Tibet and Israel.

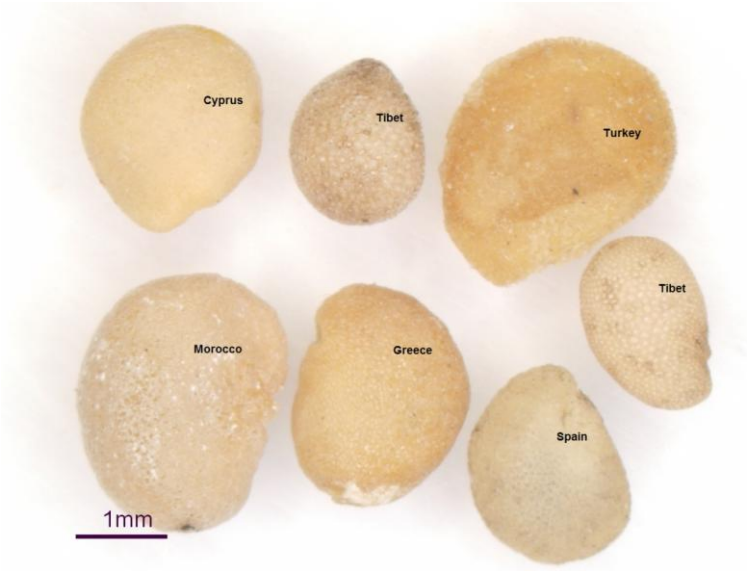


Figure 18: *Mandragora* seeds of different country origin.

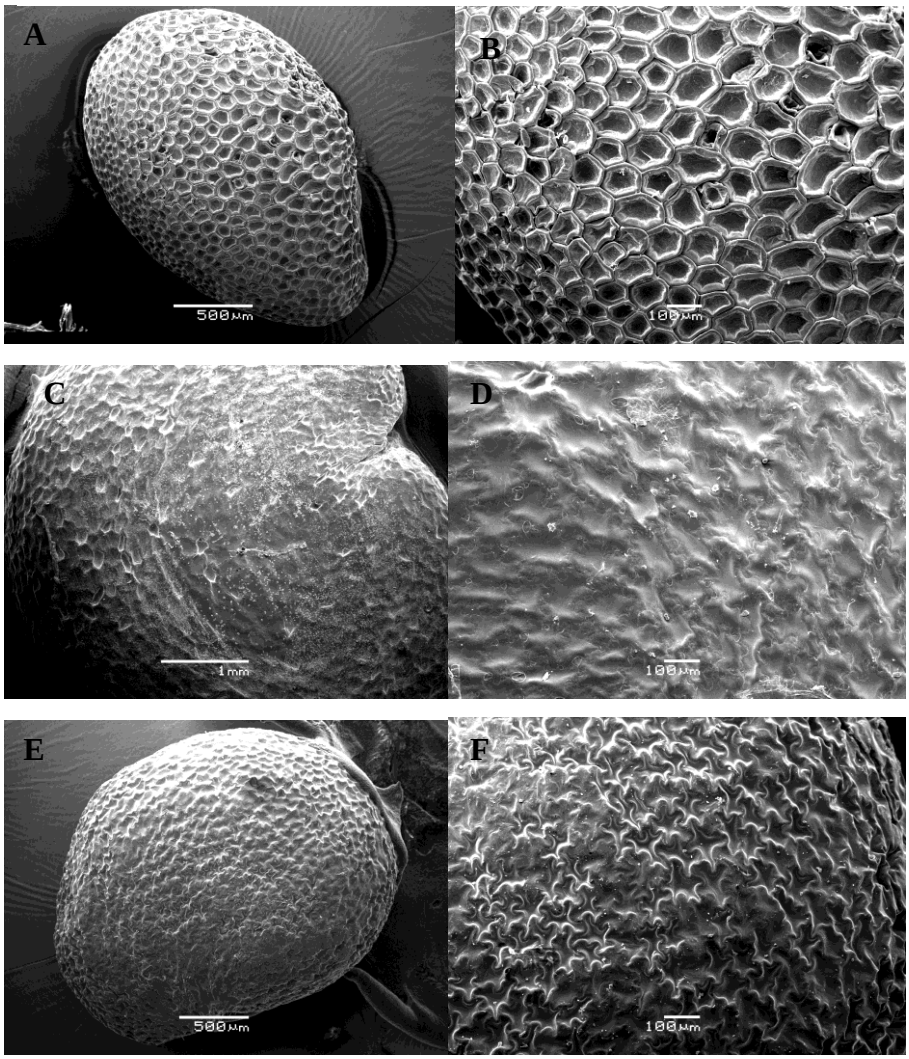


Figure 19: *Mandragora* seeds of different country origin examined in the SEM: A-B Tibet; C-D Israel; E-F Cyprus. Magnifications: A- 45x, B- 100x; C- 25x, D- 100x; E- 40x, F- 100x.

5.3. Florescence-Activated Cell Sorting (FACS)

The DNA content of plants collected in different regions of Israel or Turkmenistan was about 6.5 pg. The DNA content of plants from Morocco, Spain, Cyprus, Turkey and Italy was significantly lower (4.6-4.8 pg) (Figure 20). The Iranian plant had a DNA content of 5.7 pg that did not differ significantly from these two groups. However, the latter results were obtained from a single plant and should be treated with caution.

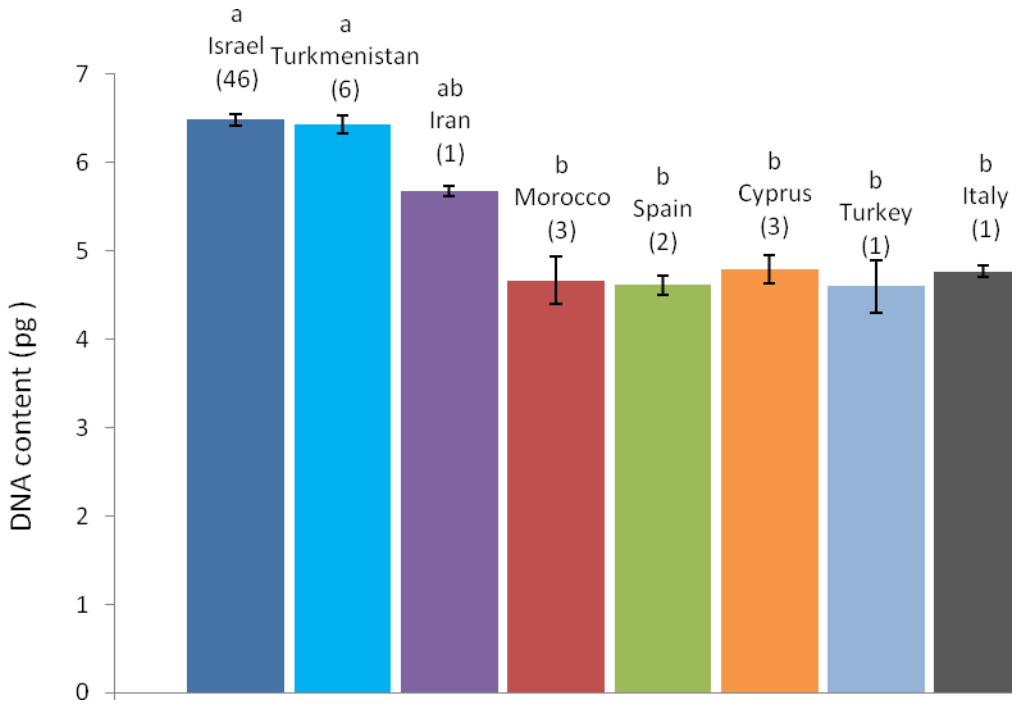
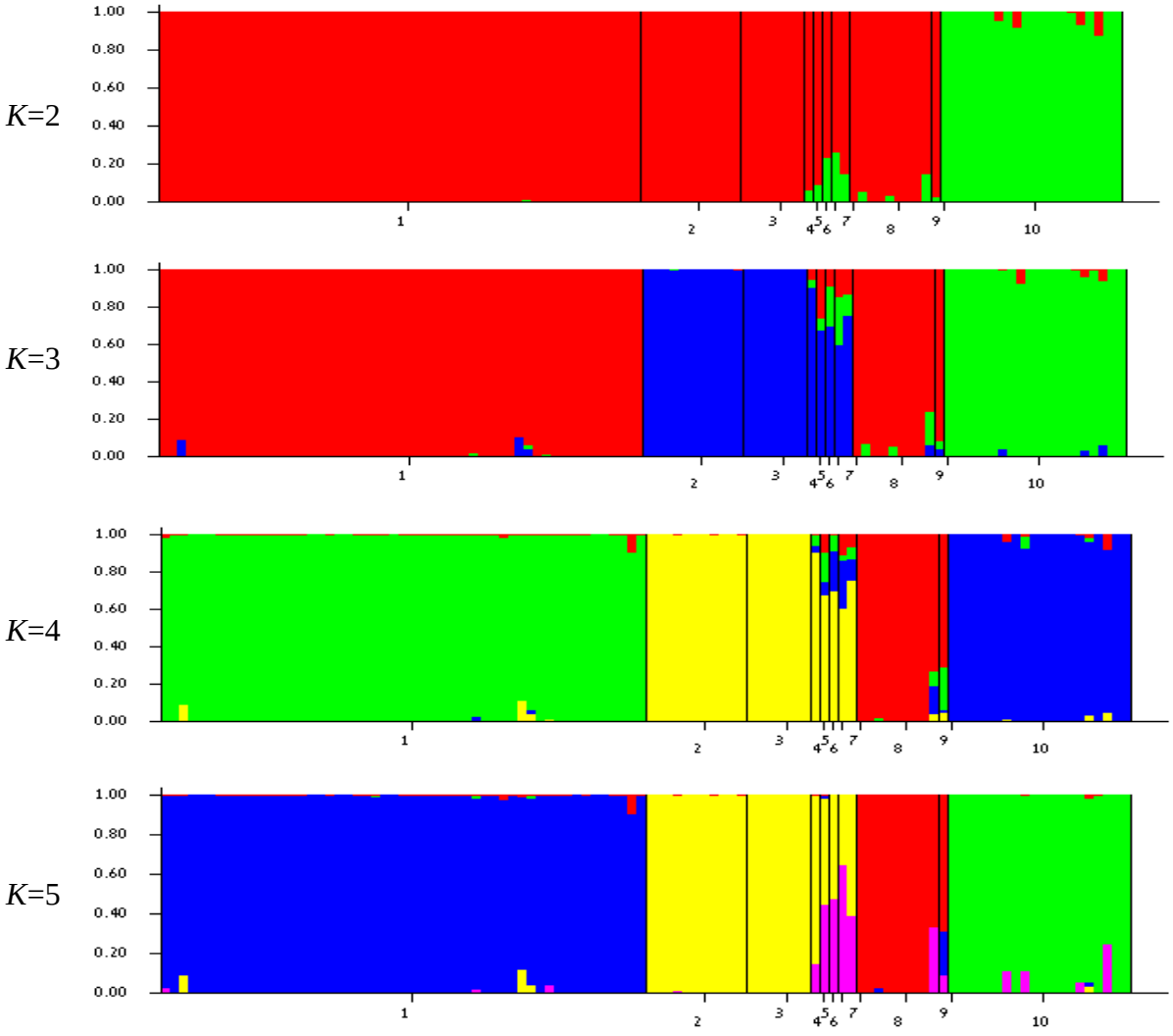


Figure 20: FACS analysis of *Mandragora* of different geographic origins (in pg DNA ±SE). Letters denote the results of Tukey-Kramer test. The number of plants tested is in parentheses.

5.4. Amplified Fragment Length Polymorphism (AFLP)

According to *STRUCTURE* software (Figure 21), when the number of clusters (K) = 2, plants from Israel, Turkmenistan, Iran and Europe formed one cluster and Tibetan plants formed the other one. When *K*= 3, plants from Israel, Turkmenistan and Iran comprised one cluster, all European plants comprised the second cluster and Tibetan plants the third cluster. When *K*= 4, Israeli plants were clustered separately from plants from Turkmenistan and Iran. When *K*= 5, there was internal division within Europe such that plants from Morocco, Spain and Italy formed one group and plants from Cyprus, Turkey and Greece formed another group. When *K*= 6, Iranian *Mandragora* was clustered with few groups: Turkmenistan, Israel and also to one of the accessions from Turkmenistan that form a different cluster. Further increase in the number of clusters determined had not changed the observed clustering, only another subdivision within Israeli, Tibetan and European plants.



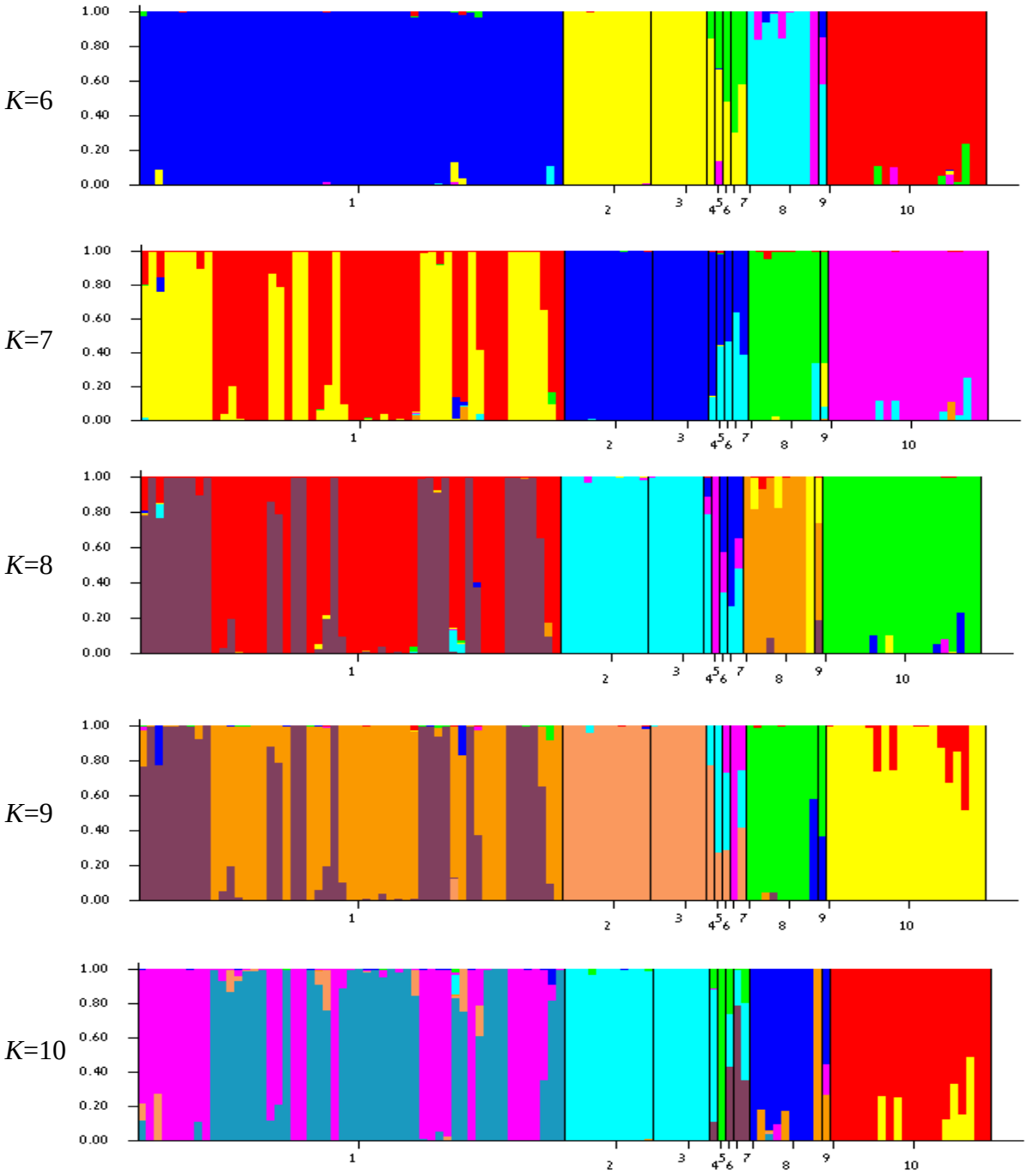


Figure 21: Estimated genetic structure of *Mandragora* in the *STRUCTURE* software. Each individual is represented by a thin vertical line, which is partitioned into *K* colored segments that represent the individual's estimated membership fractions in *K* clusters. Black lines separate individuals of different country of origin. Countries are labeled below the figure: 1=Israel, 2= Morocco, 3=Spain, 4=Italy, 5=Cyprus, 6=Turkey, 7= Greece, 8=Turkmenistan, 9= Iran, 10= Tibet.

According to *BAPS* software (Figure 22), when *K*= 2, Israeli plants were clustered with plants from Turkmenistan and Iran, and European samples were clustered with Tibetan plants. When *K*= 3, Israeli plants were clustered with plants from Turkmenistan and Iran, while European and Tibetan plants were separated. When *K*= 4, plants from Turkmenistan and Iran were separated from Israeli plants. When *K*= 5 there were five groups: the first group included Israeli plants; the second group included plants from Morocco, Spain and Italy; the third group included plants from Cyprus, Turkey and Greece (Paros and Crete); the forth group included plants from Turkmenistan and Iran; and the last group included Tibetan plants. Iranian plant was clustered with plants from Turkmenistan and also with Israeli plants. The difference between *BAPS* and *STRUCTURE* was in *K*= 6 and above, in which *BAPS* determined the true number of clusters to be 6, with internal division in Israeli population.

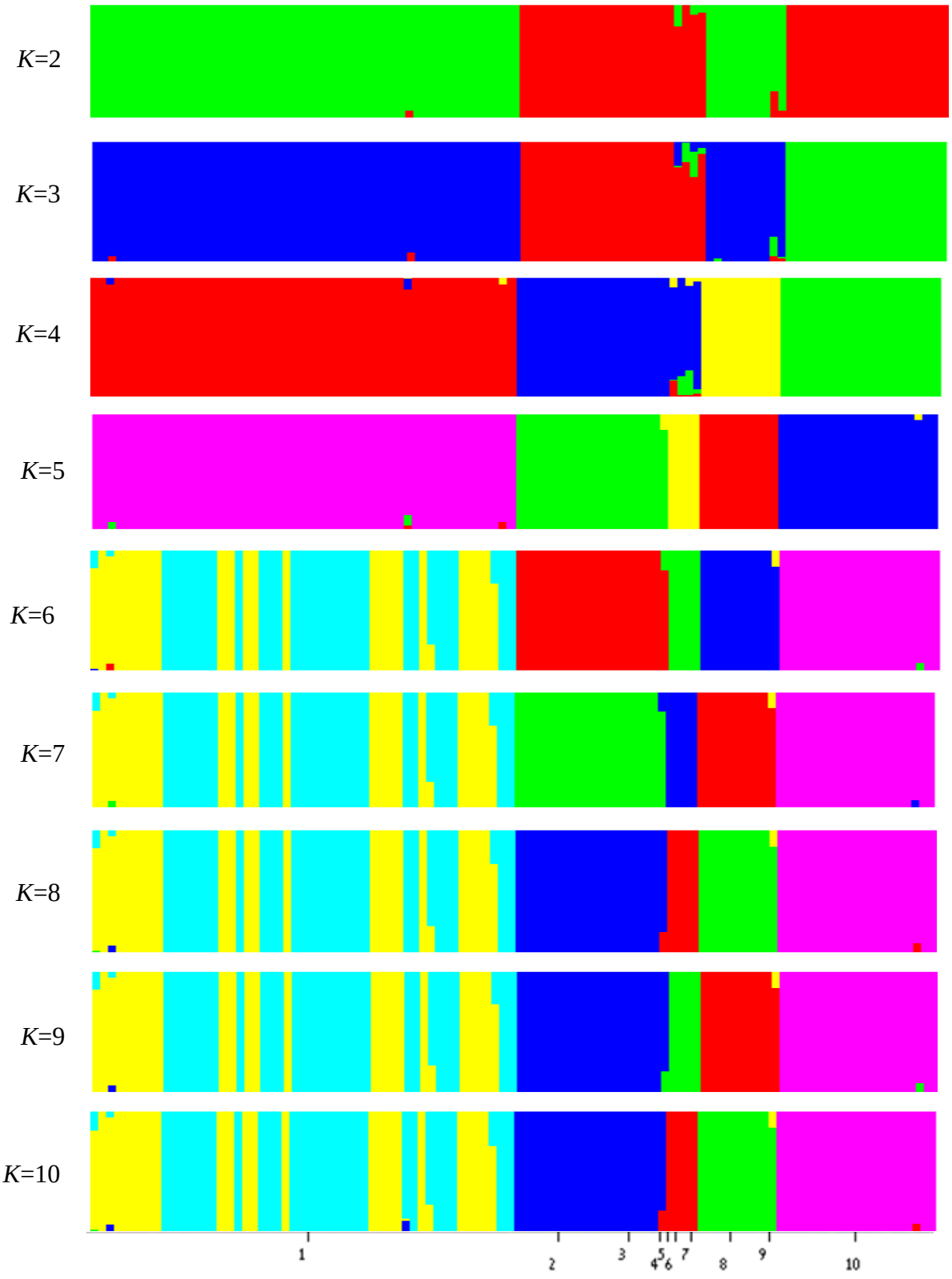


Figure 22: Estimated genetic structure of *Mandragora* in BAPS software. Each individual is represented by a thin vertical line, which is partitioned into *K* colored segments that represent the individual's estimated membership fractions in *K* clusters. Countries are labeled below the figure: 1=Israel, 2= Morocco, 3=Spain, 4=Italy, 5=Cyprus, 6=Turkey, 7= Greece, 8=Turkmenistan, 9= Iran, 10= Tibet.

Principal Coordinates Analysis (PCA) produced similar results (Figure 23). Plants from Israel, Turkmenistan and Iran were clustered together, all European populations were clustered together and Tibetan plants were separated from the others.

Cluster analysis (Figure 24) revealed three major clades. The clade including Israel, Turkmenistan and Iran was a sister to the European clade, and Tibetan plants were clustered alone from all other plants.

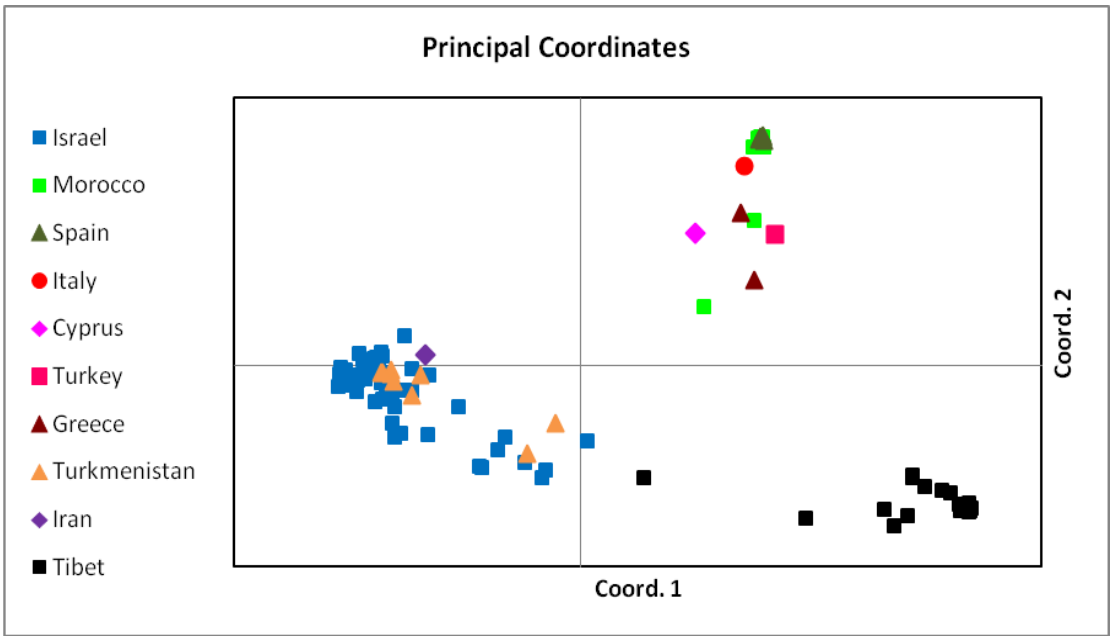


Figure 23: Principal Coordinates Analysis of *Mandragora* in *GenAlEx* software.

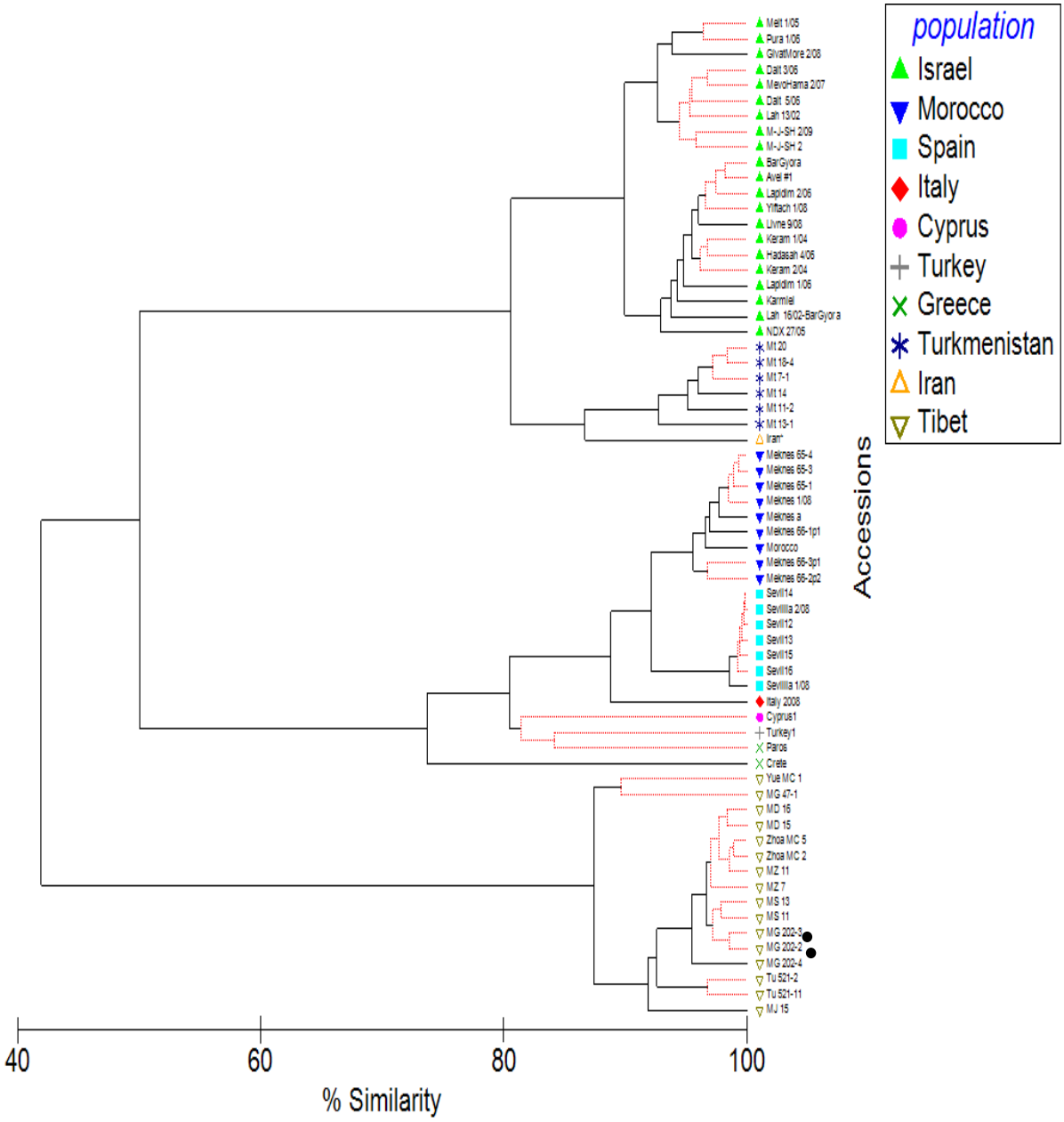


Figure 24: Hierarchical clustering of *Mandragora* of different country origin in *PRIMER6*. Significant branches ($p \leq 0.05$) in the phylogenetic tree were drawn in black and insignificant branches were drawn in red. Circles denote *M. chinghaiensis*.

5.5. Internal Transcribed Spacer (ITS) sequencing

I continued sequencing of *Mandragora* samples that had been started by Tu Tieyao. All new sequences obtained in this study were submitted to GenBank (Table 8). The nucleotide polymorphism in the ITS sequence is shown in Table 10 (in the Appendix) and summarized in Table 9. There were 50 nucleotide changes in the ITS sequence, 47 of them were informative, with no indels in the sequence. Neighbor Joining and Maximum Likelihood produced trees of identical topology (Figure 25). Plants from Israel, Turkmenistan and Iran were clustered together as a subclade within a clade including all European populations; and Tibetan plants were a very distinct separate clade. The European clade was subdivided into three sub-clades: the first group included plants from Morocco, Spain and Italy, the second group plants from Turkey and the third group plants from Cyprus and Paros. Plants from Turkey, Cyprus and Paros were closer to the clade of plants from Israel, Turkmenistan and Iran. However, bootstrap support for this clade subdivision, in contrast to 100% support of major clade division, was low (Figure 25). The Tibetan clade too was subdivided into two groups corresponding to two recognized species – *M. chinghaiensis* and *M. caulescens*.

Table 8: List of GeneBank accession numbers of ITS and cpDNA sequences. Sequences submitted by Tu Tieyao are in bold.

Sample' origin	ITS	atpB	trnL-trnF	rbcL-accD	trnH-psbA	ndhF	trnC-psbM	FV	rpS16-trnK
Israel (Golan)	JX067493	**HQ215968**	JX067472	**HQ216129**	**HQ216169**	**HQ216096**	**HQ216053**	JX067512	**HQ216008**
Israel (Negev)	JX067494	JX067445	JX067473	JX067456	JX067403	JX067432	JX067421	JX067513	JX090171
Turkmenistan	JX067495	**HQ215978**	JX067471	**HQ216139**	**HQ216177**	**HQ216104**	**HQ216063**	JX067514	**HQ216018**
Iran	JX067490	JX067444	JX067470	JX067455	JX067400	JX067431	JX067420	JX067511	JX067415
Turkey1	JX067483	**HQ215977**	JX067466	**HQ216138**	**HQ216176**	**HQ216103**	**HQ216062**	JX067503	**HQ216017.**
Turkey2	JX067484	**HQ215974**	x	**HQ216135**	**HQ216173**	**HQ216100**	**HQ216059**	JX067504	**HQ216014**
Cyprus	JX067485	JX067443	JX067469	JX067454	JX067411	JX067430	JX067419	JX067505	JX090170
Paros	JX067486	JX067441	JX067464	JX067452	JX067412	JX067428	JX067417	JX067506	JX067414
Crete	x	JX067440	x	JX067451	JX067401	JX067427	JX067416	JX067507	JX067413
Italy	JX067487	**HQ215975**	JX067468	**HQ216136**	**HQ216174**	**HQ216101**	**HQ216060**	JX067508	**HQ216015**
Spain	JX067488	JX067442	JX067465	JX067453	JX067402	JX067429	JX067418	JX067509	JX090169
Morocco	JX067489	**HQ215976**	JX067467	**HQ216137**	**HQ216175**	**HQ216102**	**HQ216061**	JX067510	**HQ216016**
M. ching. Qinghai 1	x	**HQ215973**	x	**HQ216134**	**HQ216172**	**HQ216099**	**HQ216058**	x	**HQ216013**
M. ching. Qinghai 2	JX067491	JX067446	JX067474	JX067462	JX067404	JX067438	JX067422	JX067515	JX090172
M. ching. Xizang MC	JX067492	**HQ215972**	JX067475	**HQ216133**	JX067405	JX067439	**HQ216057**	JX067516	**HQ216012**
M. caul. Xizang MS1	JX067496	**HQ215969**	JX067476	**HQ216130**	**HQ216170**	**HQ216097**	**HQ216054**	JX067517	**HQ216009**
M. caul. Xizang MS2	JX067497	**HQ215970**	JX067477	**HQ216131**	JX067409	JX067436	**HQ216055**	JX067518	**HQ216010**
M. caul. Yunnan Yue	JX067499	**HQ215971**	JX067479	**HQ216132**	**HQ216171**	**HQ216098**	**HQ216056**	JX067520	**HQ216011**
M. caul. Yunnan MG	JX067498	JX067450	JX067478	JX067461	JX067410	JX067437	JX067426	JX067519	JX090173
M. caul. Yunnan MZ	JX067500	JX067448	JX067480	JX067458	JX067407	JX067434	JX067424	JX067521	JX090174
M. caul. Yunnan MD	JX067502	JX067449	JX067482	JX067459	JX067408	JX067435	JX067425	JX067523	JX090176
M. caul. Sichuan MJ	JX067501	JX067447	JX067481	JX067457	JX067406	JX067433	JX067423	JX067522	JX090175

X - samples that failed to sequence

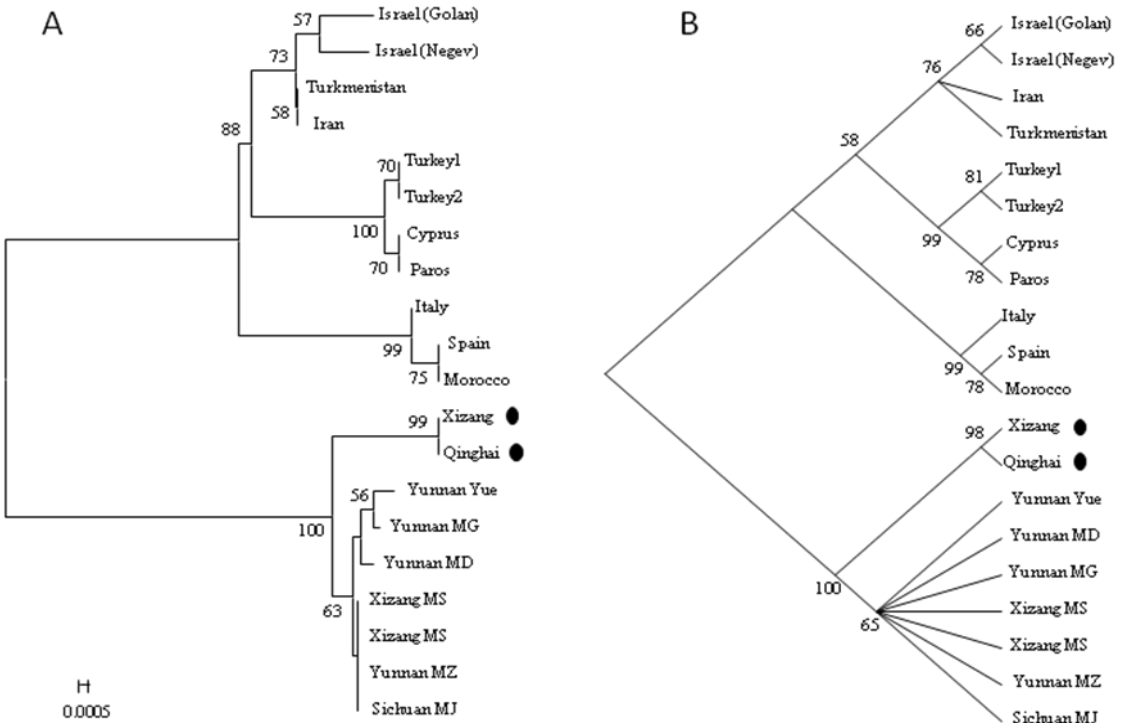


Figure 25: The NJ tree and the ML tree inferred from the ITS data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

5.6. Chloroplast DNA sequencing

I continued sequencing of *Mandragora* samples that had been started by Tu Tieyao. All new sequences obtained in this study were submitted to GenBank (Table 8). The total length of the combined chloroplast DNA (cpDNA) sequences covered 11811 bp. The sequence nucleotide polymorphism is given in Table 11 (in the Appendix) and summarized in Table 9. Overall, there were 135 nucleotide changes in the cpDNA sequences, all of them informative, and 32 indels. Eleven indels were specific for Tibet and one for Israel. Six indels were present in Tibetan and Israeli plants but not in Europeans; four indels were present in Israelis and Europeans, but not in Tibetan plants; and two indels were shared by Europeans and Tibetans, but not Israelis. One indel was missing only in Morocco, Spain and Italy.

The Neighbor Joining and Maximum Likelihood trees of every cpDNA region separately are shown in Figure 26 through Figure 33. Plants from Israel were closely related to plants from Turkmenistan and Iran, and were clustered together as a subclade within a clade including all European plants as another subclade; while Tibetan plants constituted a very distinct separate clade. Only in rpS16-trnK region plants from Israel, Turkmenistan and Iran were clustered together with all European plants (Figure 33).

For three cp regions (trnL-trnF, trnC-psbM and Fv) European plants were subdivided into two groups (Figure 27, Figure 31 and Figure 32). The first group included plants from Morocco, Spain and Italy and the second group included the other Europe samples. Besides, for the ndhF region the European samples were subdivided into three groups, the first one including Morocco, Spain and Italy, the second one Turkey, and the third one Cyprus, Paros and Crete (Figure 30).

In addition, for five cp regions Tibetan plants had an internal division. For atpB region (Figure 26) one group included plants from Yunnan (MZ and MD) and Sichuan province (MJ) and the second group included the remainder Tibetan plants. For ndhF region (Figure 30), the first group included *M. chinghaiensis*; the second group included plants from Yunnan province (MZ, MD and MG); and the third group included the remainder Tibetan plants. For trnC-psbM region (Figure 31) one group included plants from Xizang (MS); the second group included *M. chinghaiensis* and plants from Yunnan (MG and Yue); and the third group included plants from Yunnan

(MZ and MD) and Sichuan (MJ). For FV region (Figure 32) one group included a sample from Xizang (MS) and the second group included the remainder Tibetan plants. For rpS16-trnK region (Figure 33) one group included plants from Yunnan (MD) and Sichuan (MJ); the second group - plants from Yunnan (Yue and MZ) and Xizang (MS); and the third group included the remainder Tibetan plants.

The Neighbor Joining, Maximum Likelihood and Maximum Parsimony trees of combined cpDNA regions are shown in Figure 34 and Figure 35. There were three main clusters in all the trees produced. The first cluster included plants from Israel, Turkmenistan and Iran. The second cluster comprised European plants subdivided into three groups: the first group included plants from Morocco, Spain and Italy, the second group included plants from Turkey, and the third group included plants from Cyprus, Paros and Crete. The third cluster included Tibetan plants with an internal subdivision - the first group included *M. chinghaiensis*; the second group included Tibetan plants from Xizang province (MS); the third group included the remainder Tibetan plants.

Table 9: Summary of the phylogenetic information in *Mandragora* from ITS and cpDNA regions.

Region	Number of variable sites	Number of informative sites [a]	Percentage informative sites [b]	Number of indels	Number of informative indels [a]	Percentage informative indels [b]
ITS	50	47	94	0	0	0
atpB	8	8	100	0	0	0
trnL-trnF	7	7	100	3	3	100
rbcL-accD	4	4	100	0	0	0
trnH-psbA	22	22	100	9	9	100
ndhF	32	32	100	1	1	100
trnC-psbM	17	17	100	4	4	100
FV	34	34	100	11	11	100
rpS16-trnK	11	11	100	4	4	100
Total cpDNA	135	135	100	32	32	100
Total	185	182	98	32	32	100

[a] At a phylogenetically informative site, a nucleotide substitution or indel is shared by two or more species.

[b] Percentage of phylogenetically informative sites among the total number of variable sites or indels.

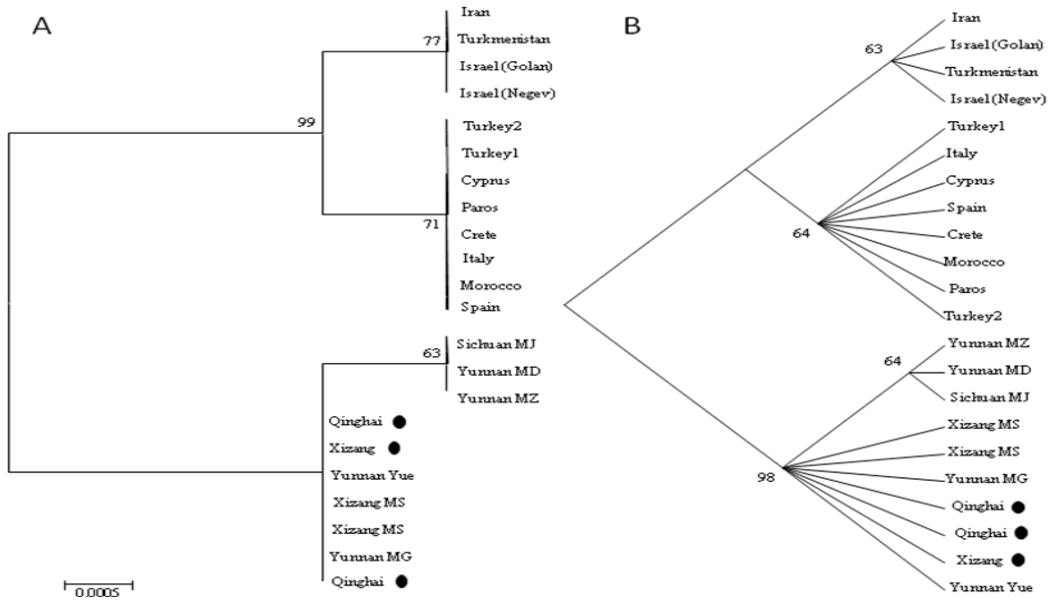


Figure 26: The NJ tree and the ML tree inferred from the atpB data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

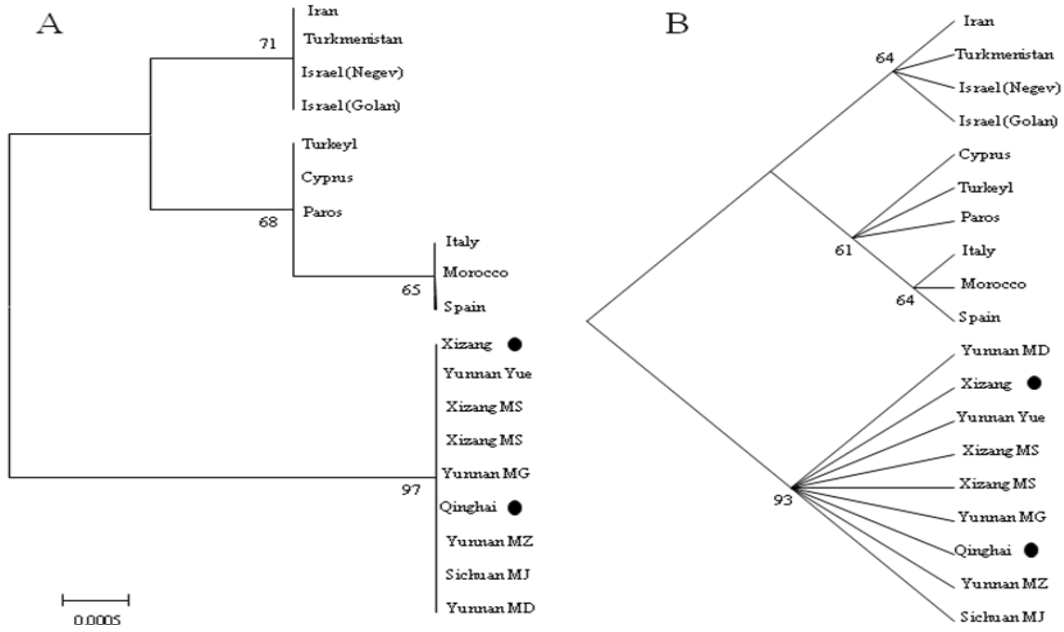


Figure 27: The NJ tree and the ML tree inferred from the trnL-trnF data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

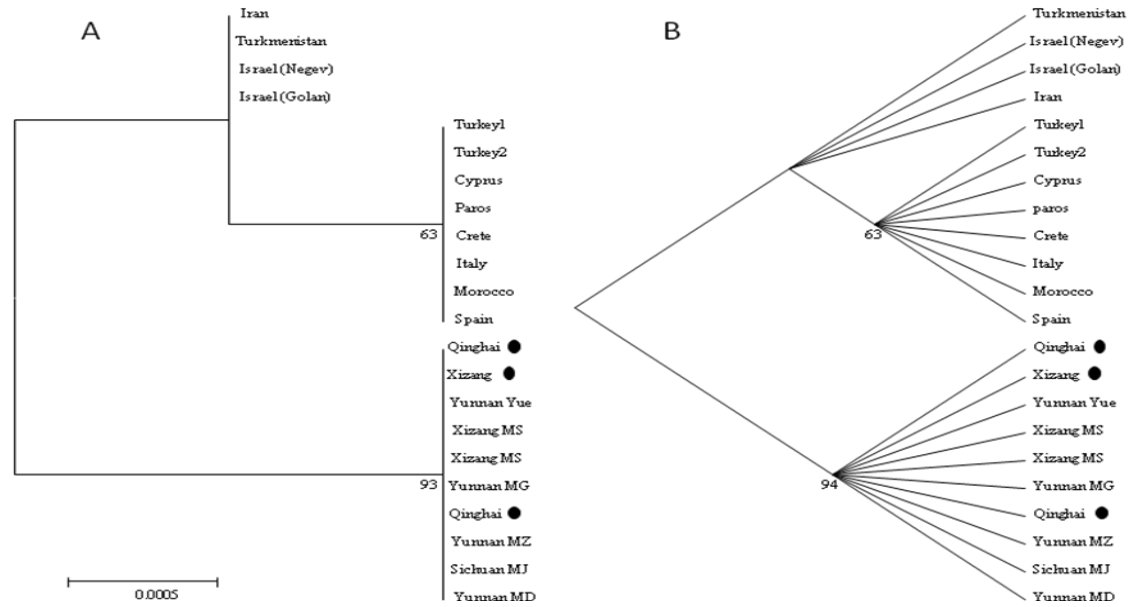


Figure 28: The NJ tree and the ML tree inferred from the rbcL-accD data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

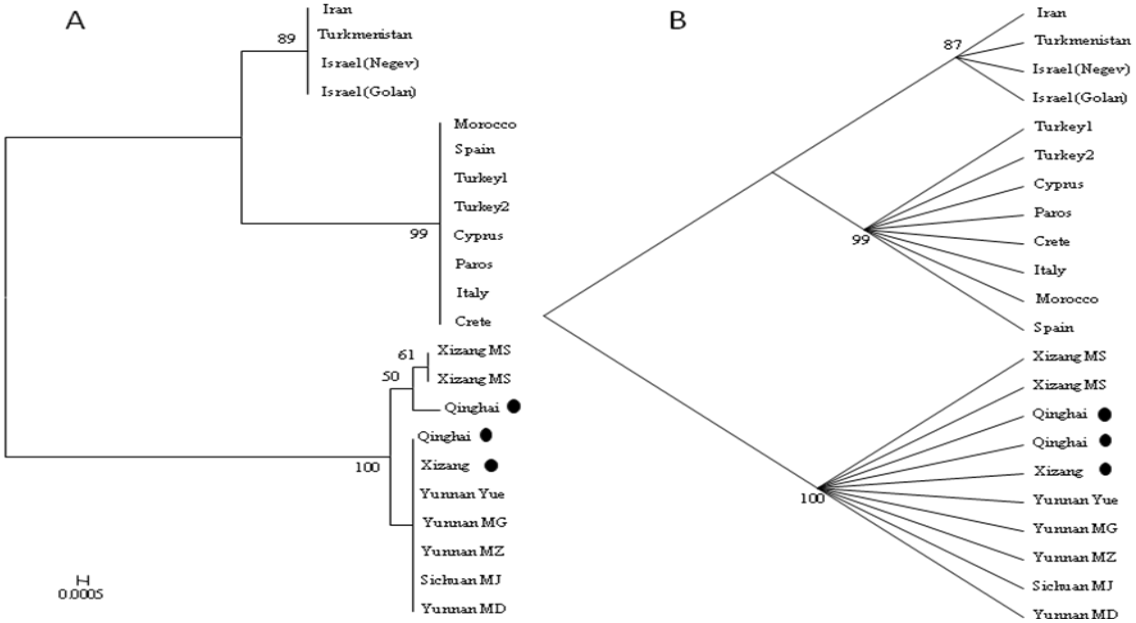


Figure 29: The NJ tree and the ML tree inferred from the trnH-psbA data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

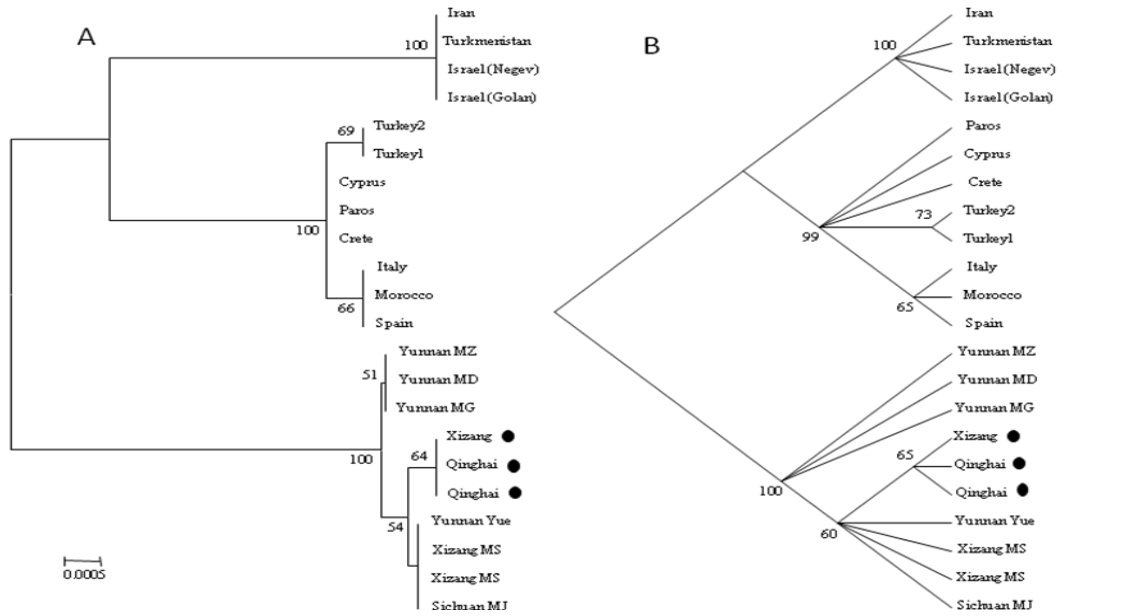


Figure 30: The NJ tree and the ML tree inferred from the ndhF data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

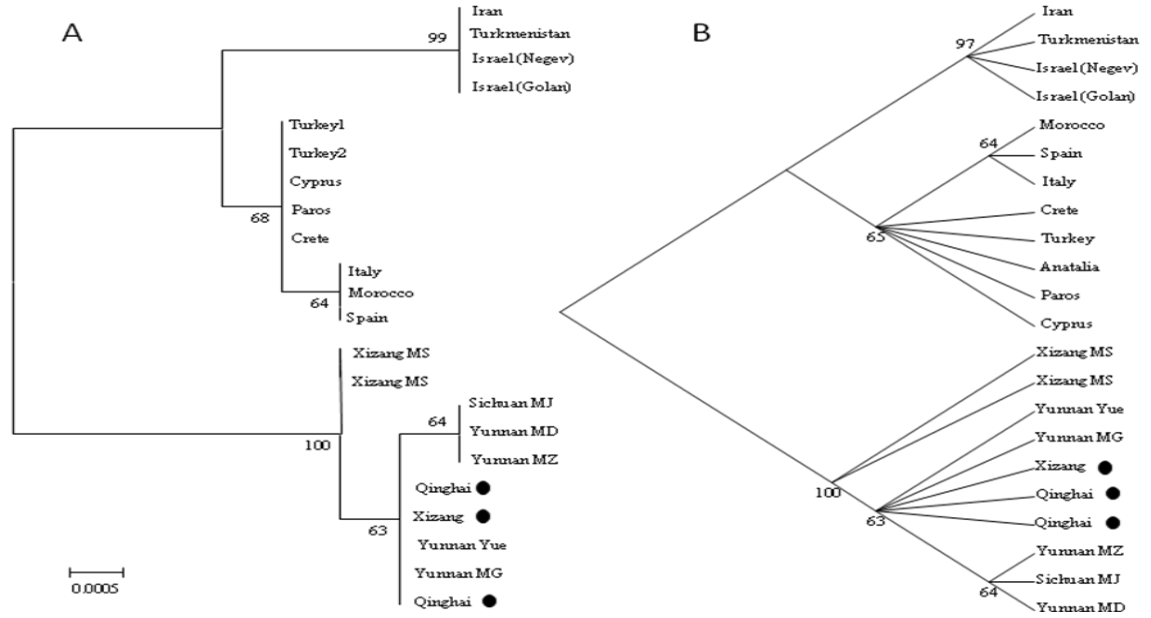


Figure 31: The NJ tree and the ML tree inferred from the trnC-psbM data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

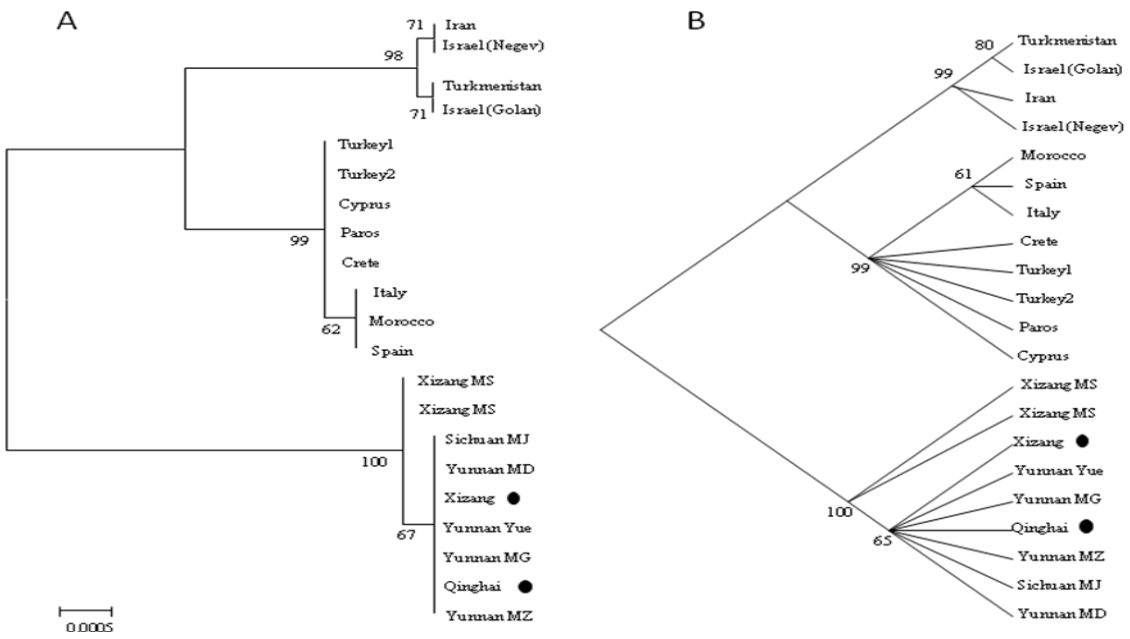


Figure 32: The NJ tree and the ML tree inferred from the FV data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

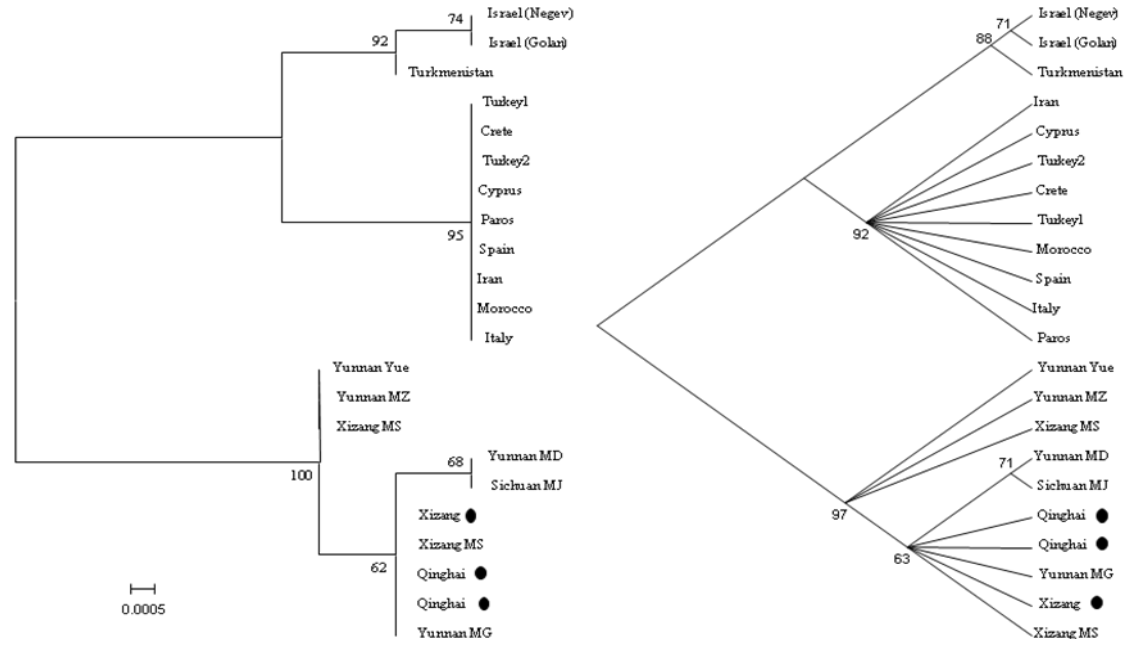


Figure 33: The NJ tree and the ML tree inferred from the rpS16-trnK data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

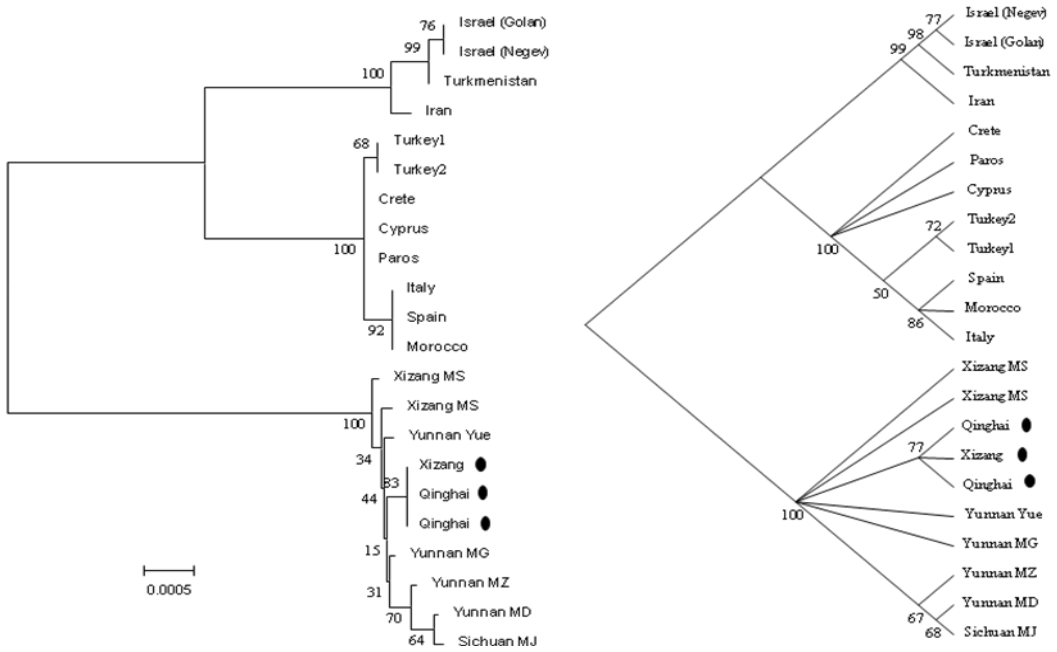


Figure 34: The NJ tree and the ML tree inferred from the combined cpDNA data: A- Evolutionary relationships of taxa by Neighbor Joining method; B- Molecular Phylogenetic analysis by Maximum Likelihood method. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches. Scale bar indicates an evolutionary distance of 0.0005 nucleotide substitution per position in the sequence.

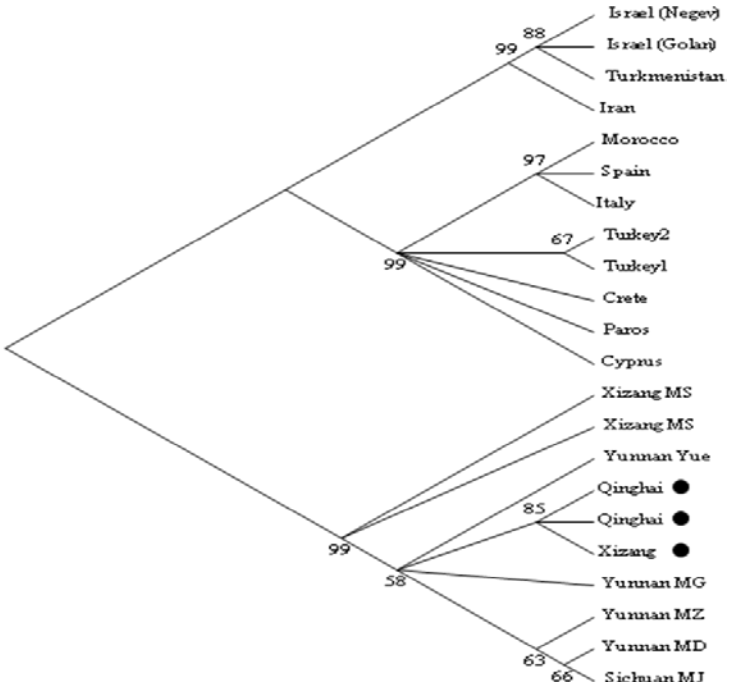


Figure 35: The Maximum Parsimony tree inferred from the combined cpDNA data. Circles denote *M. chinghaiensis*. Bootstrap values from 1000 replicates are shown next to the branches.

6. Discussion

The long history of mythology and medicinal use of the *Mandragora* and the variable morphology and phenology have created confused classification of *Mandragora*. According to the identification of Ungricht *et al.* (1998) and Akhani and Ghorbani (2003) the current taxonomy of the genus *Mandragora* (Solanaceae) includes three recognized species: the first species is *M. turcomanica* Mizg. from Turkmenistan and Iran region; the second species is *M. officinarum* L. from the Mediterranean (Europe and Israel) region; and the third species is *M. caulescens* C.B. Clark from Tibet region (*M. chinghaiensis* and *M. caulescens* are plants from Tibet that were merged into one species in this taxonomy). This taxonomy was based on morphometric analysis of herbarium specimens, phenology, habitat and distribution.

The working hypothesis of this work was that *Mandragora* of different geographic origin have different ploidy level and that the current genus taxonomy is out-dated and needs to be revised. Another hypothesis was that different *Mandragora* plants possess sufficient variation in fruit size and taste for successful domestication. In this study I investigated in detail the phylogeny and taxonomy of the genus *Mandragora* and revised it based on morphometrics (fruit, seed and flower morphology), FACS, AFLP and sequencing of cpDNA and ITS.

6.1. Morphology and FACS

Mandragora plants of different geographic origin were successfully self-pollinated so it seems that there is no self incompatibility and all plants can be self-pollinated with a different percentage of fruit setting. However the percentage of fruit set was not high, probably because the plant has protogyny (Bernhardt and Dafni, 2000). Additionally, the *Mandragora* plants were successfully cross-pollinated with pollen of plants of different country origin and the results show that there is no reproductive isolation and nearly all plants can be cross-pollinated.

In order to estimate the domestication potential of *Mandragora* fruits the maximum values were given. Fruits from crosses of Israeli or Turkmenian accessions produced bigger fruits, and one fruit of Turkmenistan origin that was collected from a plant grown in the field in Sde Boqer (Figure 16 D) was very large (55 mm in diameter). This can indicate that *Mandragora* fruits can reach this size and have potential for domestication. Beside large fruit size, *Mandragora* plants from Israel

have good taste and therefore crosses of Israeli accessions with even larger Turkmenian plants are most suitable for domestication.

The flower traits that were measured and the distribution of flowering onset were variable among years and origins and further examination and comparison of flowers from Tibet (*M. chinghaiensis* and *M. caulescens*) and other flowers from the rest Europe (Italy, Paros and Crete) should be done in order to complete this work.

According to the fruit and seed morphology results there are three *Mandragora* groups. The first group includes plants from Israel, Turkmenistan and Iran that have heavier, larger and globular fruit with the largest seed; the second group includes plants from Europe that have smaller, lightweight and ellipsoid fruit with medium size seed; and the third group includes plants from Tibet, with the smallest seed and different seed coat morphology.

According to the literature, Tibetan fruit is medium size (10-25 mm in diameter), relative to other *Mandragora* fruits origins, and globular. Tibetan plants have a stem (Figure 5 and Figure 6) (*M. chinghaiensis* has shorter stem than *M. caulescens* - Figure 6) and their leaves are arranged in a cluster on the top stem, in contrast to other *Mandragora* plants that are stem-less (Table 1). The Tibetan *Mandragora* flowering period lasts from April to September and fruiting is from August to October, in contrast to other *Mandragora* origins that flower from September-October to March-April and set fruit till June. Besides this, Tibetan *Mandragora* is dormant during winter, while plants of non-Tibetan origin are dormant during summer (Ungricht *et al.*, 1998).

Additionally, Tibetan plants inhabit high-elevation cold meadow steppes and desert steppes at around 4000 m, whose climate is dramatically different from the Mediterranean climate in the European part of *Mandragora* range including Israel (altitude below 1000 m) and arid continental climate of Turkmenistan (altitude about 1000 m) (Ungricht *et al.*, 1998).

These results match and expand the previous work of Tu *et al.* (2010) who showed that *Mandragora* plants from Israel and Turkmenistan are clustered together as a subclade close to plants from Turkey, Morocco and Italy cluster as another subclade (Figure 37). The separation of Tibet from the other origins corresponds to the current taxonomy of Ungricht *et al.* (1998).

The seed morphology agrees with Zhang *et al.* (2005) (Figure 36) who described *Mandragora* seeds as 'ovoid, ellipsoid or reniform' and distinguished between the seeds of Himalayan mountain plants: *M. chinghaiensis* (from Xining, China) and *M. caulescens* (from Xizang, Tibet) to the seeds of Mediterranean origin: *M. officinarum* (probably from Israel) and *M. autumnalis* (from Crete) according to the seed coat morphology. They characterized *M. chinghaiensis* (Figure 36: 13, 14) and *M. caulescens* (Figure 36: 63) as small seeds (2.6–2.8 x 1.6–1.8 mm), having polygonal isodiametric testal cells with long and straight lateral cell walls, while *M. officinarum* (Figure 36: 61) and *M. autumnalis* (Figure 36: 62) were characterized as larger seeds (3.0–6.5 x 2–5 mm) having isodiametric spermoderm cells, with slightly curved lateral walls. However, they emphasized that *M. officinarum* seeds were especially large (4–6.5 x 4–5.5 mm) than *M. autumnalis* but did not separate these two groups, probably because their seed coat morphology appeared similar to each other. In contrast to the latter conclusion, I think that consistent difference in size of seeds between Israeli and west-Mediterranean samples, especially when combined with other results presented in this work; suggest a taxonomic separation of these two groups.

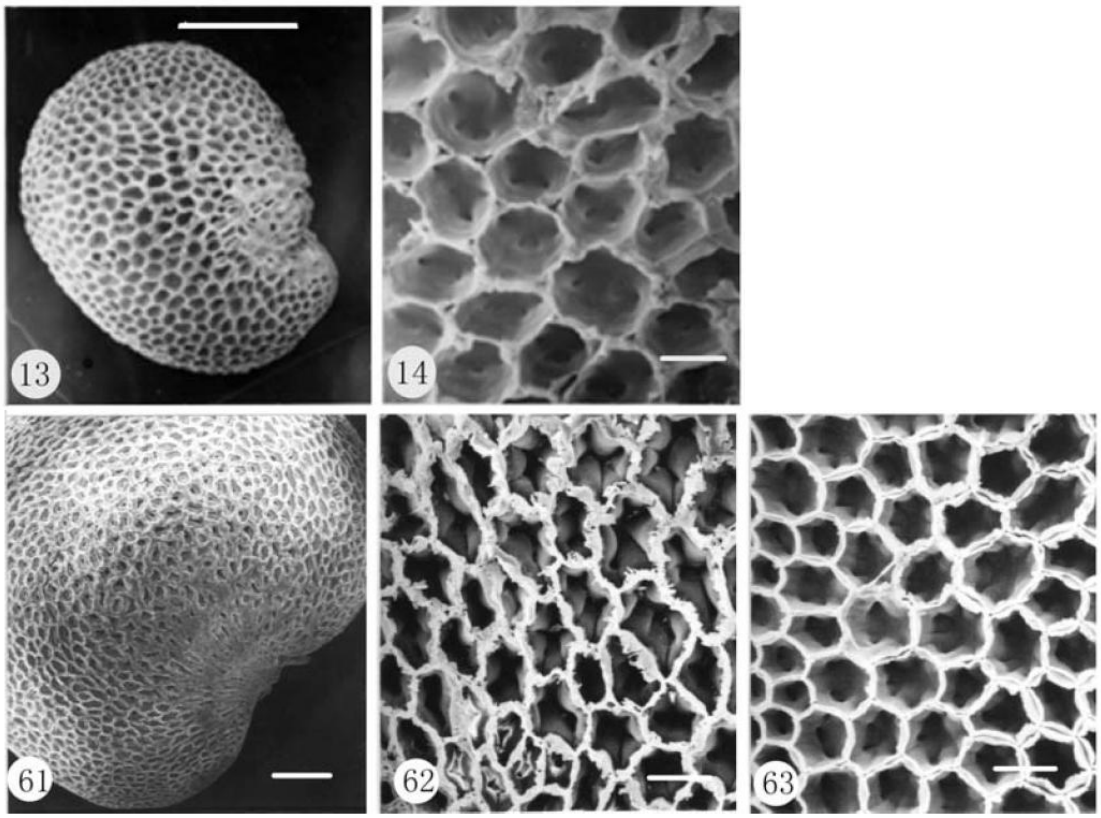


Figure 36: SEM observation of *Mandragora* in the literature: 13, 14 - *M. chinghaiensis*; 61 - *M. officinarum*; 62 -*M. autumnalis*; 63 - *M. caulescens*. Scale bar: 13, 61= 420 µm; 14, 62, 63= 60 µm. (Adapted from Zhang *et al.*, 2005).

According to FACS analysis, it seems that *Mandragora* plants from Israel, Turkmenistan and Iran have higher ploidy level and represent a different taxonomic group than *Mandragora* plants from Morocco, Spain, Cyprus, Turkey and Italy. However, we still do not know the ploidy level or chromosome number in the samples analyzed. Additionally, FACS analysis of plants from Tibet is needed.

The FACS results agree with the data on fruit and seed morphology, suggesting that plants from Israel, Turkmenistan and Iran differ from plants from Europe, and refute the current classification of Ungricht *et al.* (1998) and Akhani and Ghorbani (2003). The results also support the hypothesis that higher ploidy level is associated with larger fruit size (Singh and Wafai, 1984; Eckardt, 2004). It can be explained by the fact that increase in nuclear DNA has been associated with an increase in cell volume, and as a result the whole organ is larger (Hilu, 1993).

6.2. Molecular variation

The use of eight cpDNA sequence regions and an ITS sequence region that produced over 12,500 bp of sequence, 182 informative sites and 32 indels, in addition to AFLP analysis, provides a level of tree resolution and clade support confidence unmatched by single or two-sequence studies.

Three major clusters were recognized according to AFLP, ITS and cpDNA sequencing. The first cluster consisted of plants from Israel, Turkmenistan and Iran. The second cluster comprised plants from Europe. The third cluster included plants from Tibet.

These results agree with fruit and seed morphology and FACS that distinguish *Mandragora* plants from Israel, Turkmenistan and Iran from European plants. This division matches and provides further details to the previous work of Tu *et al.* (2010) who showed that plants from Israel and Turkmenistan were clustered together as a subclade within a clade including European plants from Turkey, Morocco and Italy as another subclade and Tibetan plants were a distinct separate clade (Figure 37).

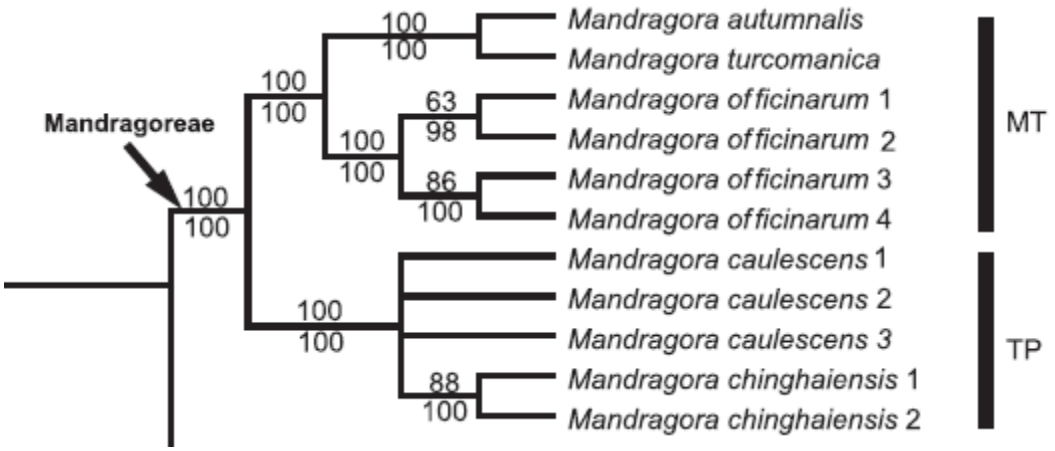


Figure 37: Maximum Parsimony tree of *Mandragora* based on the combined sequence data of six plastid markers, adapted from Tu *et al.*, 2010. *M. autumnalis* = Israel (Golan); *M. turcomanica* = Turkmenistan*; M. officinarum* 1, 2 = Turkey, 3 = Italy and 4 = Morocco. TP= Tibetan Plateau area; MP=Mediterranean-Turanian area. (adapted from Tu *et al*. (2010)

The AFLP, ITS and cpDNA results also suggest that western Mediterranean range represents two groups: one includes Morocco, Spain and Italy and the other Turkey, Cyprus, Paros and Crete. This supports and expands previous work of Tu *et al.* (2010) that differentiate Turkey from Morocco and Italy (Figure 37).

Eleven nucleotides regions that were found only in Tibetan *Mandragora* indicate the long evolutionary distance of Tibetan *Mandragora* from the congeners (Tajima and Nei, 1984). Tu *et al.* (2010) suggested that the global environmental changes in Eurasia caused by the uplift of the Tibetan plateau in the Tertiary were responsible for early separation of the Tibetan clade (Figure 37).

Kuang and Lu (1978) and Tu *et al.* (2010) divided *Mandragora* plants from Tibet into *M. chinghaiensis* and *M. caulescens* (Figure 37). They characterized *M. caulescens* as an herb with elongate stems 50-60 cm long (Figure 5) that grows in forests or shrublands on the mountains of the southeastern Tibetan plateau; whereas *M. chinghaiensis* has rosette leaves and short stems (Figure 6) and is found in the meadow steppes of the inner platform. Ungricht *et al.* (1998) merged these two groups together considering differences to be within the range of variability of each taxon. In this study, the ITS and ndhF sequences (Figure 30) supported the diversion of these two Tibetan *Mandragora* groups, but not the other cpDNA sequences. More information on Tibetan *Mandragora* is needed for distinguishing between *M. chinghaiensis* and *M. caulescens*.

6.3. Concluding remarks

According to the morphometric data (fruit and seed), FACS and three classes of molecular markers (AFLP, cpDNA and ITS), *Mandragora* plants can be classified as belonging to three groups. One group includes plants from Israel, Turkmenistan and Iran, which are characterized by globular and large fruit, with large seed and high DNA content. The second group includes plants from Cyprus, Turkey, Italy, Spain and Morocco, which are characterized by ellipsoid and small fruit, small seed and lower DNA content. Tibetan plants are the third group with very small seed and very distinct ecology and morphology.

Therefore, the recognition of *M. turcomanica* as a separate species (Akhani and Ghorbani, 2003) and of *M. officinarum* as one species including plants from Europe and Israel (Ungricht *et al.*, 1998) are no longer satisfactory. It follows from the new results, that *M. caulescens* is a highly separated clade from *M. officinarum* - *M. turcomanica*, while *M. turcomanica* is not a separate species but a relict population or human introduced *M. officinarum* from Israel. Additionally, *M. officinarum* must be split into two different species – one with a range including Israel and probably adjacent Jordan, Lebanon and Syria; and another one having western Mediterranean distribution.

7. Appendix

Table 10: The nucleotide polymorphism in the ITS sequence. The first row is the reference sequence. Dots denote the same nucleotide as the reference sequence and dashes indicate gaps. *M. ching.* and *M. caul.* are abbreviations of *M. chinghaiensis* and *M. caulescens*, respectively.

polymorphic sites:	**ITS 692bp**																													
Sample' origin	48	49	65	72	81	88	91	111	132	140	143	152	189	192	194	200	204	206	222	233	240	273	283	287	405	411	430	436	439
Israel (Golan)	C	A	A	T	A	C	G	C	G	A	T	A	A	A	G	G	A	G	A	A	C	A	C	G	G	G	G	C	G
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	A	.	G	T	.	.	.	A	.	.	.	A
Turkey2	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	A	.	G	T	.	.	.	A	.	.	.	A
Cyprus	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	C	.	G	T	.	.	.	A	.	.	.	A
Paros	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	C	.	G	T	.	.	.	A	.	.	.	A
Italy	.	.	.	C	.	.	.	.	.	.	.	.	C	.	A	A	G	.	.	G	.	.	.	.	.	A	.	.	.
Spain	.	.	.	C	.	T	.	.	.	.	.	.	C	.	A	A	G	.	.	G	.	.	.	.	.	A	.	.	.
Morocco	.	.	.	C	.	T	.	.	.	.	.	.	C	.	A	A	G	.	.	G	.	.	.	.	.	A	.	.	.
M. ching. Xizang 2	T	G	G	.	G	.	A	T	A	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. ching. Qinghai MC	T	G	G	.	G	.	A	T	A	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Xizang MS1	T	G	G	.	G	.	.	T	.	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Xizang MS2	T	G	G	.	G	.	.	T	.	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Yunnan Yue	T	G	G	.	G	.	.	T	.	.	A	G	C	G	A	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Yunnan MG	T	G	G	.	G	.	.	T	.	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Yunnan MZ	T	G	G	.	G	.	.	T	.	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Yunnan MD	T	G	G	.	G	.	.	T	.	.	A	G	C	G	A	.	.	.	C	G	.	G	T	A	.	.	T	A	.
M. caul. Sichuan MJ	T	G	G	.	G	.	.	T	.	.	A	G	C	G	.	.	.	.	C	G	.	G	T	A	.	.	T	A	.

polymorphic sites:	**ITS cont.**																				
Sample' origin	440	445	455	457	458	459	461	497	508	526	527	528	529	540	542	547	573	576	598	630	644
Israel (Golan)	C	C	G	G	C	G	G	C	-	G	G	G	G	A	A	A	A	G	G	G	G
Israel (Negev)	.	G	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Iran	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Turkey1	.	.	.	A	.	.	.	T	.	.	.	.	C	G	.	.	.	.	.	.	.
Turkey2	.	.	.	A	.	.	.	T	.	.	.	.	C	G	.	.	.	.	.	.	.
Cyprus	.	.	.	A	.	.	.	T	.	.	.	.	C	G	.	.	.	.	.	.	.
Paros	.	.	.	A	.	.	.	T	.	.	.	.	C	G	.	.	.	.	.	.	.
Italy	.	.	.	A	G	.	A	.	.	.	.	.	C	G	.	.	.	.	.	.	A
Spain	.	.	.	A	.	.	A	.	.	.	.	.	C	G	.	.	.	.	.	.	A
Morocco	.	.	.	A	.	.	A	.	.	.	.	.	C	G	.	.	.	.	.	.	A
M. ching. Qinghai 2	T	.	A	A	.	.	.	.	G	A	C	A	C	G	G	T	G	A	A	.	.
M. ching. Xizang MC	T	.	A	A	.	.	.	.	G	A	C	A	C	G	G	T	G	A	A	.	.
M. caul. Xizang MS1	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	.	.
M. caul. Xizang MS2	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	.	.
M. caul. Yunnan Yue	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	A	.
M. caul. Yunnan MG	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	A	.
M. caul. Yunnan MZ	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	.	.
M. caul. Yunnan MD	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	.	.
M. caul. Sichuan MJ	.	.	A	A	.	T	.	.	G	A	C	.	C	G	G	T	G	A	A	.	.

Table 11: The nucleotide polymorphism of the cpDNA sequence. The first row is the reference sequence. Dots denote the same nucleotide as the reference sequence and dashes indicate gaps. *M. ching.* and *M. caul.* are abbreviations of *M. chinghaiensis* and *M. caulescens*, respectively. Empty rows are regions that failed to sequence.

polymorphic sites:	**atpB 1091bp**								**trnL trnF 950bp**										**rbcL accD 1392bp**			
Sample' origin	35	78	120	306	477	609	891	1029	139	317-318	351-373	438	476	521	603	613	615	709	87	711	747	1018
Israel (Golan)	C	A	C	A	C	G	A	G	A	-	-	A	C	A	C	T	C	T	A	A	T	C
Israel (Negev)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey 1	.	.	.	T	.	.	.	A	.	.	.	.	.	C	.	C	.	.	.	C	.	.
Turkey 2	.	.	.	T	.	.	.	A											.	C	.	.
Cyprus	.	.	.	T	.	.	.	A	.	.	.	.	.	C	.	C	.	.	.	C	.	.
Paros	.	.	.	T	.	.	.	A	.	.	.	.	.	C	.	C	.	.	.	C	.	.
Crete	.	.	.	T	.	.	.	A											.	C	.	.
Italy	.	.	.	T	.	.	.	A	.	.	.	.	.	C	.	C	.	G	.	C	.	.
Spain	.	.	.	T	.	.	.	A	.	.	.	.	.	C	.	C	.	G	.	C	.	.
Morocco	.	.	.	T	.	.	.	A	.	.	.	.	.	C	.	C	.	G	.	C	.	.
M. ching. Qinghai 1	T	G	.	.	T	A	C	A											G	.	C	A
M. ching. Qinghai 2	T	G	.	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. ching. Xizang MC	T	G	.	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Xizang MS1	T	G	.	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Xizang MS2	T	G	.	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Yunnan Yue	T	G	.	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Yunnan MG	T	G	.	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Yunnan MZ	T	G	A	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Yunnan MD	T	G	A	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A
M. caul. Sichuan MJ	T	G	A	.	T	A	C	A	-	TT	TTCTCCATTGAAGAAAGAATCGA	G	T	C	G	.	A	.	G	.	C	A

polymorphic sites:	**trnH psbA 503bp**																						
Sample' origin	23	131-134	135-139	141	142	179-181	189-194	246	268	277	283	301	311	317	318	321	343-344	345	346	347-350	352	353	359
Israel (Golan)	T	CTTT	TTATT	A	T	TTT	-	C	A	G	T	G	C	C	T	T	AA	-	-	-	A	G	T
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
Turkey2	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
Cyprus	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
Paros	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
Crete	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
Italy	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	A	.	.	.	C	T	.
Spain	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
Morocco	.	-	-	.	.	-	.	T	.	.	G	.	.	A	.	.	-	.	.	.	C	T	.
M. ching. Qinghai 1	.	.	-	T	A	.	AGATAT	T	T	T	.	A	T	.	C	C	.	A	.	GAAA	.	A	G
M. ching. Qinghai 2	G	.	-	T	A	.	AGATAT	T	T	T	.	A	T	.	C	C	.	A	.	GAAA	.	A	G
M. ching. Xizang MC	.	.	-	T	A	.	AGATAT	T	T	T	.	A	T	.	C	C	.	A	A	GAAA	.	A	G
M. caul. Xizang MS1	G	.	-	T	A	.	.	T	T	T	.	.	T	.	C	C	.	.	.	GAAA	.	A	G
M. caul. Xizang MS2	G	.	-	T	A	.	.	T	T	T	.	.	T	.	C	C	.	.	.	GAAA	.	A	G
M. caul. Yunnan Yue	.	.	-	T	A	TT	AGATAT	T	T	T	.	A	T	.	C	C	.	A	A	GAAA	.	A	G
M. caul. Yunnan MG	.	.	-	T	A	.	.	T	T	T	.	A	T	.	C	C	.	A	.	GAAA	.	A	G
M. caul. Yunnan MZ	.	.	-	T	A	.	AGATAT	T	T	T	.	A	T	.	C	C	.	A	A	GAAA	.	A	G
M. caul. Yunnan MD	.	.	-	T	A	.	AGATAT	T	T	T	.	A	T	.	C	C	.	A	.	GAAA	.	A	G
M. caul. Sichuan MJ	.	.	-	T	A	TT	AGATAT	T	T	T	.	A	T	.	C	C	.	A	.	GAAA	.	A	G

polymorphic sites:	**trnH psbA cont.**									**ndhF 1999bp**													
Sample' origin	375-379	380-401	418	426	436	448	454	480	490	210	262	372	411	412	453	477	546	562	616	813	900	946	1118
Israel (Golan)	TCTTT	-	G	C	T	T	A	A	-	G	C	G	G	A	T	A	A	C	C	T	T	C	T
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	-	.	.	.	.	.	.	G	.	.	A	.	A	C	.	G	G	.	.	C	.	T	.
Turkey2	-	.	.	.	.	.	.	G	.	.	A	.	A	C	.	G	G	.	.	C	.	T	.
Cyprus	-	.	.	.	.	.	.	G	.	.	A	.	A	C	.	G	G	.	.	C	.	T	.
Paros	-	.	.	.	.	.	.	G	.	.	A	.	A	C	.	G	G	.	.	C	.	T	.
Crete	-	.	.	.	.	.	.	G	.	.	A	.	A	C	.	G	G	.	.	C	.	T	.
Italy	-	.	.	.	.	.	.	G	.	C	A	.	A	C	.	G	G	.	.	C	.	T	.
Spain	-	.	.	.	.	.	.	G	.	C	A	.	A	C	.	G	G	.	.	C	.	T	.
Morocco	-	.	.	.	.	.	.	G	.	C	A	.	A	C	.	G	G	.	.	C	.	T	.
M. ching. Qinghai 1	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. ching. Qinghai 2	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. ching. Xizang MC	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Xizang MS1	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Xizang MS2	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Yunnan Yue	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Yunnan MG	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Yunnan MZ	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Yunnan MD	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C
M. caul. Sichuan MJ	.	GAAATAATAATAGAATATCATT	T	A	C	G	C	.	G	.	A	T	.	C	A	.	G	A	G	C	C	.	C

polymorphic sites:	**ndhF cont.**																		
Sample' origin	1118	1134	1194	1207	1239	1255	1314	1334	1434	1448-1453	1460	1585	1644	1680	1752	1891	1902	1962	1980
Israel (Golan)	T	C	T	A	A	G	A	A	T	TTTTTT	C	T	G	C	T	A	A	T	T
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	.	A	C	C	C	.	G	.	.	.	A	T	A	.	G	C	.	.	.
Turkey2	.	A	C	C	C	.	G	.	.	.	A	T	A	.	G	C	.	.	.
Cyprus	.	A	C	C	C	.	G	.	.	.	.	T	A	.	G	C	.	.	.
Paros	.	A	C	C	C	.	G	.	.	.	.	T	A	.	G	C	.	.	.
Crete	.	A	C	C	C	.	G	.	.	.	.	T	A	.	G	C	.	.	.
Italy	.	A	C	C	C	.	G	.	.	.	.	T	A	.	G	C	.	.	.
Spain	.	A	C	C	C	.	G	.	.	.	.	T	A	.	G	C	.	.	.
Morocco	.	A	C	C	C	.	G	.	.	.	.	T	A	.	G	C	.	.	.
M. ching. Qinghai 1	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	G
M. ching. Qinghai 2	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	G
M. ching. Xizang MC	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	G
M. caul. Xizang MS1	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	A
M. caul. Xizang MS2	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	A
M. caul. Yunnan Yue	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	A
M. caul. Yunnan MG	C	.	C	C	C	A	G	A	C	-	.	G	.	T	.	C	G	G	A
M. caul. Yunnan MZ	C	.	C	C	C	A	G	A	C	-	.	G	.	T	.	C	G	G	A
M. caul. Yunnan MD	C	.	C	C	C	A	G	A	C	-	.	G	.	T	.	C	G	G	A
M. caul. Sichuan MJ	C	.	C	C	C	A	G	C	C	-	.	G	.	T	.	C	G	G	A

polymorphic sites:	**trnC psbM 1850bp**																					
Sample' origin	16-34	97	109	219	226	410	425	518	605	894	962	1006	1077	1200	1229	1264	1337-1342	1353	1361	1374	1825
Israel (Golan)	TGTTGATATAACCCGCCGA	G	T	A	G	C	T	G	T	T	C	T	A	C	T	A	ACTTCT	A	-	G	C
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	-	C	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	A	A	.
Turkey2	-	C	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	A	A	.
Cyprus	-	C	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	A	A	.
Paros	-	C	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	A	A	.
Crete	-	C	.	.	T	.	.	.	.	C	T	.	.	.	.	.	.	.	A	A	.
Italy	-	C	.	.	T	.	.	.	.	C	T	.	G	.	.	.	.	.	A	A	.
Spain	-	C	.	.	T	.	.	.	.	C	T	.	G	.	.	.	.	.	A	A	.
Morocco	-	C	.	.	T	.	.	.	.	C	T	.	G	.	.	.	.	.	A	A	.
M. ching. Qinghai 1	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	.	-	C	.	A	A
M. ching. Qinghai 2	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	.	-	C	.	A	A
M. ching. Xizang MC	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	.	-	C	.	A	A
M. caul. Xizang MS1	.	.	-	T	T	T	C	T	.	C	T	C	.	T	C	.	-	C	.	A	A
M. caul. Xizang MS2	.	.	-	T	T	T	C	T	.	C	T	C	.	T	C	.	-	C	.	A	A
M. caul. Yunnan Yue	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	.	-	C	.	A	A
M. caul. Yunnan MG	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	.	-	C	.	A	A
M. caul. Yunnan MZ	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	C	-	C	.	A	A
M. caul. Yunnan MD	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	C	-	C	.	A	A
M. caul. Sichuan MJ	.	.	-	T	T	T	C	T	A	C	T	C	.	T	C	C	-	C	.	A	A

polymorphic sites:	**FV**	**3358bp**																					
Sample' origin	27	34	35	106	170-175	201-203	274	295	505	559	597	623-627	656	777	915	1422	1426	1934	2234	2473-2480	2574	2584	2596-2600
Israel (Golan)	T	A	A	G	AAAGAA	-	A	T	G	C	T	AGAAC	T	A	C	C	T	A	A	ATACTTAT	G	C	-
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Turkey2	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Cyprus	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Paros	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Crete	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Italy	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Spain	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
Morocco	.	C	G	.	.	.	.	C	.	.	.	-	.	.	T	.	C	G	.	.	.	G	TTATC
M. ching. Qinghai 2	A	C	G	A	-	TTT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. ching. Xizang MC	A	C	G	A	-	TT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Xizang MS1	A	C	G	.	-	TT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Xizang MS2	A	C	G	.	-	TTT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Yunnan Yue	A	C	G	A	-	TTT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Yunnan MG	A	C	G	A	-	TT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Yunnan MZ	A	C	G	A	-	TTT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Yunnan MD	A	C	G	A	-	TTT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC
M. caul. Sichuan MJ	A	C	G	A	-	TTT	G	C	A	T	G	.	G	G	T	A	.	.	C	-	C	G	TTATC

polymorphic sites:	**FV cont.**																				
Sample' origin	2622-2627	2639	2644	2693-2695	2712	2720	2763	2781	2788	2799	2800-2809	2810-2815	2876	2877	2941	3010	3014	3070	3252	3344	3348-3352
Israel (Golan)	-	A	T	-	G	C	G	T	T	-	-	-	G	G	A	T	C	G	G	A	TTATA
Israel (Negev)	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Iran	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkey1	.	.	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	.
Turkey2	.	.	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	.
Cyprus	.	.	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	.
Paros	.	.	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	.
Crete	.	.	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	.
Italy	.	T	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	-
Spain	.	T	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	-
Morocco	.	T	G	.	A	.	.	G	.	A	ATTATTATTT	.	.	T	C	.	.	.	.	C	-
M. ching. Qinghai 2	TTAGAA	.	.	TTT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. ching. Xizang MC	TTAGAA	.	.	TT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Xizang MS1	TTAGAA	.	.	T	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Xizang MS2	TTAGAA	.	.	T	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Yunnan Yue	TTAGAA	.	.	TTT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Yunnan MG	TTAGAA	.	.	TT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Yunnan MZ	TTAGAA	.	.	TTT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Yunnan MD	TTAGAA	.	.	TTT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.
M. caul. Sichuan MJ	TTAGAA	.	.	TTT	A	T	A	.	C	C	ATTATAATTT	AATATT	A	.	C	C	T	T	T	C	.

polymorphic sites:	**rpS16 trnK 668bp**																					
Sample' origin	214	308	334	338	339	340	372	380	381	382-384	385	386	387	388	432-433	487	512	522-549	568	574	590	591
Israel (Golan)	T	T	C	T	T	A	C	T	A	-	-	-	A	-	TT	T	A	-	T	G	A	G
Israel (Negev)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Turkmenistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.
Iran	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Turkey1	.	.	A	.	G	G	.	-	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Turkey2	.	.	A	.	G	G	.	-	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Cyprus	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Paros	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Crete	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Italy	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Spain	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
Morocco	.	.	A	.	G	G	.	.	.	.	.	.	.	.	-	G	.	.	G	.	.	.
M. ching. Qinghai 1	G	C	A	G	C	A	T	.	T	TTT	T	.	.	T	.	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. ching. Qinghai 2	G	C	A	G	C	A	T	.	T	TTT	T	.	.	T	.	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. ching. Xizang MC	G	C	A	G	C	A	T	.	T	TTT	T	.	.	T	.	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. caul. Xizang MS1	G	C	A	G	C	A	T	.	T	-	A	T	.	.	-	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. caul. Xizang MS2	G	C	A	G	C	A	T	.	T	-	A	T	.	.	-	.	.	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. caul. Yunnan Yue	G	C	A	G	C	A	T	.	T	TTT	A	T	.	.	T	.	.	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. caul. Yunnan MG	G	C	A	G	C	A	T	.	T	T	A	T	.	.	T	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. caul. Yunnan MZ	G	C	A	G	C	A	T	.	T	TTT	A	T	.	.	T	.	.	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	.	T
M. caul. Yunnan MD	G	C	A	G	C	A	T	.	T	-	-	-	-	-	T	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	T	T
M. caul. Sichuan MJ	G	C	A	G	C	A	T	.	T	TT	A	T	-	T	T	.	C	AATGAAGTGGATCTATTTCGTTTTATTT	G	T	T	T

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9. תקציר

מטרת מחקר זה היתה לבחון מחדש את הקשרים הפילוגנטיים בין מינים שונים של הסוג דודא (*Mandragora*). זאת, בהתבסס על ריצוף שמונה אזורים בדנ״א הכלורופלסטי, ריצוף אזור ITS בדנ״א הגרעיני, AFLP ומדדים מורפולוגיים שונים (פרחים, פירות וזרעים). בנוסף, איפיון השונות הפנוטיפית והכלאות עצמיות וחיצוניות נעשו כצעד ראשון בתהליך ביות פרי הדודא, ו-FACS שימש להערכת רמת הפלואידיה של הדודא וזיקתה לתפוצה הגיאוגרפית של הצמח.

על פי הנתונים המורפולוגיים (פירות וזרעים) ואנליזת FACS, צמחי הדודא יכולים להתחלק לשלוש קבוצות. הקבוצה הראשונה כוללת צמחי דודא מישראל, טורקמניסטן ואירן, אשר מאופיינים בפירות גדולים ועגולים, בעלי זרעים גדולים ותכולת דנ״א גדולה. הקבוצה השניה כוללת צמחים מאירופה (קפריסין, טורקיה, איטליה, מרוקו וספרד) המאופיינים בפירות קטנים, מאורכים ודמויי אליפסה עם זרעים בגודל בינוני ותכולת דנ״א קטנה. צמחי הדודא מטיבט הם הקבוצה השלישית ולהם זרעים קטנים מאד ומורפולוגיה שונה של השכבה החיצונית של הזרע.

התוצאות של AFLP ושל ריצוף דנ״א כלורופלסטי ו-ITS קרובות מאוד ומראות הבדלים גדולים יותר בין צמחי דודא מאירופה לישראל מאשר בין צמחי דודא מישראל לטורקמניסטן ואירן. מכאן יש להסיק כי בניגוד למסקנות המחקרים הקודמים בנושא (Ungricht *et al.*, 1998 and Akhani and Ghorbani, 2003), *M. officinarum* הכולל צמחים אירופיים וישראלים, אינו מין יחיד כמו שחשבו בעבר. בנוסף, הזיהוי של *M. turcomanica* כמין בפני עצמו לא נתמך ע״י ממצאי העבודה המוצגים כאן, ונראה שהוא קרוב מאוד לצמחים הישראלים.

אם כן, נראה שהטקסונומיה הנוכחית של הסוג דודא (סולניים) הכוללת שלושה מינים (*M. turcomanica* Mizg. מאזור מרכז אסיה ואירן, *M. officinarum* L. מאזור הים התיכון ו- *M. caulescens* C.B. Clark מאזור סינו-הימלאיה) חייבת להתעדכן. על פי התוצאות החדשות, *M. caulescens* הוא אכן קבוצה טקסונומית נפרדת לגמרי מ- *M. turcomanica* - *M. officinarum*, ולעומת זאת *M. turcomanica* אינו מין נפרד, אלא תת אוכלוסיה שנשארה מ- *M. officinarum* באזור הזה. *M. officinarum*, הכולל בתוכו את אירופה וישראל, צריך להיות מחולק לשני תתי מינים, על בסיס הפרדה בין ישראל לאירופה. עוד עולה מן המחקר כי צמחי דודא מישראל קרובים מאוד לצמחים מטורקמניסטן ואירן, דבר המעיד על אפשרות למקור של צמחי *M. turcomanica* מתת המין הנמצא בישראל. מלבד זאת, צמחי דודא מישראל וטורקמניסטן שונים בתכולת הדנ״א מהמין הנפוץ באירופה.

מילות מפתח

דודא, טקסונומיה, סיסטמטיקה, פילוגיאוגרפיה, פילוגנטיקה, ביות.

דודאים: פילוגנטיקה וביות

מחקר לשם מילוי חלקי של הדרישות לקבלת תואר "דוקטור לפילוסופיה"

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אישור המנחה _____

אישור המנחה _____

אישור דיקן בית הספר ללימודי מחקר מתקדמים ע"ש קרייטמן _____

תמוז תשע"ב **יוני 2012**

באר שבע

העבודה נעשתה בהדרכת

ד״ר סרגיי וליס

וד״ר חליל קשקוש

במחלקה למדעי החיים

בפקולטה למדעי הטבע

<u>הצהרת תלמיד המחקר עם הגשת עבודת הדוקטור לשיפוט</u>

אני החתום מטה מצהיר/ה בזאת: (אנא סמן):

___ חיברתי את חיבורי בעצמי, להוציא עזרת ההדרכה שקיבלתי מאת מנחה/ים.

___ החומר המדעי הנכלל בעבודה זו הינו פרי מחקרי <u>מתקופת היותי תלמיד/ת מחקר</u>.

___ בעבודה נכלל חומר מחקרי שהוא פרי שיתוף עם אחרים, למעט עזרה טכנית הנהוגה בעבודה ניסיונית. לפי כך מצורפת בזאת הצהרה על תרומתי ותרומת שותפי למחקר, שאושרה על ידם ומוגשת בהסכמתם.

תאריך _____ שם התלמיד/ה _____ חתימה _____