DOLB

Master's thesis :

Karyotype analysis of some endemic plants at El-Jabal El-Akhdar region

BY:HALIMA JEBRIL ADAM

منشورات البرنامج الوطني لتطوير المجلات العلمية

University of Benghazi

Faculty of Arts and Science-Almarj

Department of Botany

Karyotype analysis of some endemic plants at El-Jabal El-Akhdar region

By

Halima Jebril Adam

Supervisor

Dr. Jamila Abd Alhafit Ali Bashasha

(Assistant professor)

This thesis was submitted in partial fulfillment of the requirements for Master's degree of Science in Botany

Date:17/May/2025

Declaration

I am Halima Jebril Adam, and I confirm that the work contained in this thesis unless otherwise referenced, is the researcher's work and has not been previously submitted to meet the requirements of an award at this University or any other higher education or research institution, I furthermore, cede copyright of this thesis in favor of the University of Benghazi.

Halima Jebril Adam

Signature:……..

Date: 17/May/2025

University of Benghazi
Faculty of Arts and Science-Almarj
Department of Botany

Karyotype analysis of some endemic plants at El-Jabal El-Akhdar region

By
Halima Jebril Adam

This thesis has been approved by the examination committee:

Dr. Jamila Abd Alhafit Ali **(**Supervisor) ……..……
University of Benghazi

Dr. Khalid A. M Elbudony **(**Internal examiner) ……..……..……
Faculty of Arts and Science-Almarj

Dr. Ezzudin Shoaip Mohamed Ali (External examiner**)** ……..……
Omar Al-Mukhtar University

Dr. Abdul salam A. Ali
Head of Graduate Studies and Training Office of the Faculty

Dr. Jalal I. Kabaili
Dean of Faculty of Arts and Science-Almarj

Dr. Suliman Rabeh El-Sharef
Head of Graduate Studies and Training Office of the University of Benghazi

17/5/2025

قوله تعالى:

(الْحَمْدُ لِلهِ رَبِ الْعَالَمِينَ)

(سورة الفاتحة، الآية 1)

Dedication

I dedicate this research to:

The soul of **my dear father**;

My **late brothers, Adel and Younis**;

My **mother, Zahra Mohammed**;

Ms. **Ghazala Omar Alsanossi**;

Dr. **Abdullah Alalwany**;

Dr. **Abdulhakim Bani**;

Dr. **Khadija Bauou**;

and my dear friends, **Fatima El-Gassi** and **Om Alsaad Ramadan**.

Acknowledgement

Alhamdulillah (Praise be to Allah), we praise him, seek his help, and seek his forgiveness. We seek refuge in Allah from the evil within ourselves and from our sinful actions. Whoever Allah guides, no one can lead astray, and whoever Allah leaves astray, no one can guide.

Afterwards, praise be to Allah, abundant and pure praise, blessed in its entirety, filling the heavens and the earth. I thank him for his guidance in completing this study and writing this research.

I want to express my deep gratitude to everyone who contributed to the success of this work, starting with my mother and siblings who provided me with support and encouragement throughout my study.

I would like to thank my supervisor Dr. Jamila Bashasha, and I ask Allah to make this work solely for his noble countenance and to grant me and everyone a share in benefit and success.

Table of Contents

Contents	Pages
Dedication	**I**
Acknowledgement	**II**
Table of Contents	**III**
List of Tables	**VI**
List of Figures	**VII**
List of Appendices	**VIII**
List of Abbreviations	**IX**
Abstract	**XI**
1. Introduction	**1**
1.1. The Aim of the Study	**3**
2. Literature Review	**4**
2.1 Karyotyping Criteria	**4**
2.2 Symmetrical and Asymmetrical karyotype	**8**
2.3 Endemic Plants to El-Jabal El-Akhdar	**12**
2.3.1. *Arum cyrenaicum* Hruby	**16**
2.3.2. *Arbutus pavarii* pamp.	**19**
2.3.3. *Origanum cyrenaicum* Bég. & Vacc.	**21**
3. Materials and Methods	**24**
3.1 Sites Description	**24**

3.2 Plant Materials **26**

3.2.1 *Arum cyrenaicum* **26**

3.2.2 *Arbutus pavarii* **28**

3.2.3 *Origanum cyrenaicum* **30**

3.3 Collection and Germination of Seeds **32**

3.3.1 Collection and Germination of Seeds of *Arum cyrenaicum* Hruby **32**

3.3.2 Collection and Germination of Seeds of *Arbutus pavarii* Pamp. **33**

3.3.3 Collection and Germination of Seeds of *Origanum cyrenaicum* Bég. & Vacc. **34**

3.4 Karyotype Analysis **36**

3.4.1. Karyotype of *Arum cyrenaicum* **36**

3.4.2. Karyotype of *Arbutus pavarii* **36**

3.4.3. Karyotype of *Origanum cyrenaicum* **37**

3.5. Software and Photoshop programs **40**

3.5.1. AutoCAD 2022 program **40**

3.5.2. Adobe Photoshop 2020 **41**

3.5.3. ImageJ 1.46 program **42**

3.5.4. PicsArt program **43**

3.5.5. Ideokar software program **43**

4. Results **51**

4.1 *Arum cyrenaicum* Hruby **51**

4.2 *Arbutus pavarii* Pamp. **62**

4.3 *Origanum cyrenaicum* Bég. & Vacc. 70

5. Discussion 78

5.1 Chromosome number and morphology 78

5.1.1 *Arum cyrenaicum* 78

5.1.2 *Arbutus pavarii* 80

5.1.3 *Origanum cyrenaicum* 81

5.2 Chromosome size 83

5.2.1. *Arum cyrenaicum* 83

5.2.2. *Arbutus pavarii* 83

5.2.3. *Origanum cyrenaicum* 83

5.3 Karyotype asymmetry 84

5.3.1. *Arum cyrenaicum* 84

5.3.2. *Arbutus pavarii* 85

5.3.3. *Origanum cyrenaicum* 86

6. Conclusion 88

7. References 89

Appendices 115

Abstract in Arabic language……

List of Tables

NO.	Tables	Pages
1	The ecological characteristics of the two collection sites.	25
2	Chromosomal and karyotype parameters calculated by Ideokar program.	49
3	Classifying chromosome types according to their arm ratio.	50
4	Classification of karyotype asymmetry degrees.	48
5	Karyomorphometric data for the studied plant taxa	53
6	Estimators on a set of 28 chromosomes of *Arum cyrenaicum*	57
6	(Continued)	58
6	(Continued)	59
7	Chromosome measurements of *Arum cyrenaicum* (mean ± SD)	60
7	**(**Continued)	61
8	Estimators on a set of 13 chromosomes of *Arbutus pavarii*	68
9	Chromosome measurements of *Arbutus pavarii* (mean ± SD)	69
10	Estimators on a set of 15 chromosomes of *Origanum cyrenaicum*	76
11	Chromosome measurements of *Origanum cyrenaicum* (mean ± SD)	77

List of Figures

No.	Figures	Pages
1	The two collection sites of plant species samples used in the current study on the map of El-Jabal El-Akhdar region, Libya. (All indicated by arrows)	24
2	*Arum cyrenaicum* Hruby (A), fruits (B), and their seeds (C).	27
3	*Arbutus pavarii* Pamp.(A), fruits (B), and their seeds (C).	29
4	*Origanum cyrenaicum* Bég. & Vacc.(A), inflorescences (B), and their seeds (C).	31
5	The germination of seeds of *A. cyrenaicum* (A), *A. pavarii* (B), and *O. cyrenaicum* (C).	35
6	Cytogenetic procedure for karyotyping the studied plant species	39
7	Original metaphase image (A), Numbering of homologous pairs (B), Manual drawing and arrangement (C), Initial AutoCAD 2020 output (D), Final AutoCAD 2020 drawing (E).	46
8	Chromosome cropping issues using Adobe Photoshop 2020: Altered shape of chromosome1 (A & B), Loss of chromosome segments in chromosome 2 (B), and banding removal difficulties in chromosome 3 (B) (all indicated by arrows).	47
9	Chromosome trimming and separation issues in imageJ (A), Reduced image quality in ImageJ program (B), and PicsArt program (C).	48
10	Metaphase chromosomes spread (A), and karyogram of *Arum cyrenaicum* (B)	54
11	Ideogram of *Arum cyrenaicum*	55
12	Karyotype parameters of *Arum cyrenaicum*	56
13	Original metaphase chromosome image of *Arbutus pavarii* (A), Image processed with ImageJ software (B), Image enhanced using Adobe Photoshop 2020(C), High-resolution image (PicsArt) showing satellites on chromosomes 1 and 2(D).	64
14	Metaphase chromosomes spread (A), and karyogram of *Arbutus pavarii* (B).	65
15	Ideogram of *Arbutus pavarii*	66
16	Karyotype parameters of *Arbutus pavarii*	67
17	Key chromosomal features in *Origanum cyrenaicum*	72
18	Metaphase chromosomes spread (A), and karyogram of *Origanum cyrenaicum* (B).	73
19	Ideogram of *Origanum cyrenaicum*	74
20	Karyotype parameters of *Origanum cyrenaicum*	75

List of Appendices

NO.	Appendices	Pages
1	Check list of the plants endemic to the El-Jabal El-Akhdar region	115
1	**(continued).** Check list of the plants endemic to the El-Jabal El-Akhdar region	116
1	**(continued).** Check list of the plants endemic to the El-Jabal El-Akhdar region	117
1	**(continued).** Check list of the plants endemic to the El-Jabal El-Akhdar region	118
1	**(continued).** Check list of the plants endemic to the El-Jabal El-Akhdar region	119
2	karyotype of some endemic plants in the El-Jabal El-Akhdar region according to IPCN	120
2	**(continued).** karyotype of some endemic plants in the El-Jabal El-Akhdar region according to IPCN	121
3	Chromosome numbers of some species belong to *Arum* genus according to IPCN	122
4	Chromosome numbers of some species belong to *Arbutus* genus according to IPCN	123
5	Chromosome numbers of some species belong to *Origanum* genus according to IPCN	124
5	**(continued).** Chromosome numbers of some species belong to *Origanum* genus according to IPCN	125

List of Abbreviations

Ask%	Arano index of karyotype asymmetry
AR	Arm ratio
AI	Asymmetry index
A	Degree of karyotype asymmetry
A1	Intrachromosomal asymmetry index
A2	Interchromosomal asymmetry index
CI%	Centromere index
Cm	Centimeter
CL	Chromosome Length
ΣCL	Total chromosome length in set
CVcl	Coefficient of variation of chromosome length
CVci	Coefficient of variation of centromere index
d.w	distilled water
Fl	Flowering period
GBIF	Global Biodiversity Information Facility
Hrs	Hours
HCL	Hydrochloric acid
IPCN	Index to Plant Chromosome Numbers
IRL%	Index of relative length of chromosome
KF	Karyotype Formula
L	Long arm of chromosome
ΣL	Total length of long arms in chromosome set
M	Metacentric chromosome
m	metacentric chromosome
m	minute
n	(Gametic) Haploid chromosome number of a taxon

2*n*	Somatic chromosome number
NORs	Nucleolar Organizing Regions
POWO	Plants of the World Online
SAT	Satellite chromosome
S	Short arm of chromosome
ΣS	Total length of short arms in chromosome set
sCL	Standard deviation of chromosome length
sCI	Standard deviation of centromere index
Sm	Submetacentric chromosome
St	Subtelocentric chromosome
S%	Symmetry index
T	Telocentric chromosome
TF%	Total form percentage of homologous chromosome pairs
THCL	Total haploid length of chromosome complement
t	Acrocentric
μm	Micrometer (Micron)
Vern	Vernacular name
V	Volume
W	Wight
x	Basic chromosome number

Abstract

This research established the chromosome number and karyotypes for three endemic species: *Arum cyrenaicum* (Araceae) and *Arbutus pavarii* (Ericaceae), which were gathered from the Tolmitha site, as well as *Origanum cyrenaicum* (Lamiaceae), collected from the Al-Gubba site in the El-Jabal El-Akhdar region. These plants have significant nutritional and medicinal value. Identifying their chromosomes and establishing their karyotypes provides valuable and important genetic information. The range of chromosome length(**CL**), arm ratio (**AR**), centromere index (**CI%**), symmetry index (**S%**), the degree of karyotype asymmetry (**A**), Arano index of karyotype asymmetry (**Ask%**), total form percentage of homologous chromosome pairs (**TF%**), intrachromosomal asymmetry index ($\mathbf{A_1}$) interchromosomal asymmetry index($\mathbf{A_2}$), coefficient variation of chromosome length($\mathbf{CV_{CL}}$), coefficient variation of centromere index($\mathbf{CV_{CI}}$), and asymmetry index (**AI**) were analyzed across all species under investigation. The results showed that the chromosome numbers and karyotype formula (KF) of *Arum cyrenaicum* 2*n*=4*x*=56 (6M+38m+10sm+2st(2SAT), *Arbutus pavarii* 2*n*=2*x*=26(4M+16m+6sm(4SAT),and *Origanum cyrenaicum*2*n*=2*x*=30 (6M+16m+8sm(3SAT). Furthermore, the chromosome size of *Arum cyrenaicum* was medium to small-sized, with lengths ranging from the largest 4.72±.21µm to the smallest 2.88±.04µm, while *Origanum cyrenaicum* and *Arbutus pavarii* had small-sized chromosomes, with lengths ranging from 3.19±.01µm to 1.98±.02µm and from 3.09±.03µm to 1.98±.03µm, respectively. According to Stebbins' karyotype asymmetry classification, the karyotypes of *Arum cyrenaicum*, *Arbutus pavarii,* and *Origanum cyrenaicum* were **1A** type. The karyotypes of *Arum cyrenaicum*, *Arbutus pavarii*, and *Origanum cyrenaicum* are symmetrical.
Satellites were observed on two chromosome pairs of *Arum cyrenaicum*, four chromosome pairs of *Arbutus pavarii*, and three chromosome pairs of *Origanum cyrenaicum*. The karyotypes of these three species are recorded for the first time, and these findings contribute to a scientific understanding of these plants from a genetic perspective.

Key wards: Chromosome numbers, karyotype, *Arum cyrenaicum*, *Arbutus pavarii*, *Origanum cyrenaicum*, El-Jabal El-Akhdar.

1. Introduction

Libya is one of the largest countries in North Africa, covering an area of about 1.7 million km^2. Its territory is mainly covered by desert or semi-deserts, and Mediterranean vegetation is restricted to the coastal region (Thor & Nascimbene, 2010). The important areas for plant diversity in Libya are the coastal strip and the mountains of the Mediterranean coast (Radford, 2011).

El-Jabal El-Akhdar is considered the most significant biodiversity area in Libya, although it constitutes only 1% of the country's area (Radford, 2011). El-Jabal El-Akhdar represents the only natural forest in the distance extending from Tunisia in the west to Palestine in the east (GEA, 2010). It is located in the northeastern part of Libya. It is, furthermore, an ecologically significant region characterized by a Mediterranean climate (Elshatshat, 2009). This region is considered the most important area for agriculture (e.g., fruits, cereals, and vegetables) in Libya.

The Libyan flora consists of 2,118 species belonging to 864 genera and 161 families. Of these, 2,088 species, 844 genera, and 145 families are Angiosperms; 15 species of 8 genera and 6 families are Gymnosperms; and 15 species of 12 genera and 10 families are Pteridophyta (Mahklouf & Etayeb, 2018). El-Jabal El-Akhdar is one of the largest vegetation areas, containing 70% of the Libyan plant species (over 1,350 species) (Saaed *et al*., 2022).

Endemic species, constituting valuable floristic elements, are those confined to a particular geographic region. Their identification, conservation, and genetic resources are of significant interest to the scientific community (Ghaffari *et al.,* 2005). Endemism can be explained based on cytological and morphological data, geological factors, and phylogeny (Ferreira & Boldrini, 2011). Endemic plant species often display polyploidy, a phenomenon characterized by the duplication of chromosome sets, which has the potential to cause significant ecological shifts for Angiosperms (Villa *et al*., 2022). Polyploidy is an important mechanism in plant speciation and evolution, occurring in two ways: autopolyploidy, involving genome duplication within a single species; and allopolyploidy, involving genome duplication between species (Martin *et al*., 2022).

Generally, the flora of Libya has a low percentage of endemic species-no more than 7%. The total number of endemic plant species in Libya is around 80-81, and they are distributed in four regions of endemism in Libya. These regions include: (i) El-Jabal El- El-Akhdar with 44 endemic species, (ii) the coastal belt including the Jabal Nafusah

and Marmarica plateau with 26 endemic species, (iii) the center of the Sahara with 8 to 9 species, (iv) the plateaus of Ghat, Tebesti, and Aweinat with 2 species (Mahklouf & Etayeb, 2018).

It is worth noting that 50% of the total number of endemic plants in Libya are found in the El-Jabal El-Akhdar region, which has consequently been described as one of the most important centers of endemism in Libya, and they are identical to the plants of the Mediterranean basin. Some reports mention that between 100 and 140 species, subspecies, or varieties are endemic to El-Jabal El-Akhdar (WCMC, 1992; Elshatshat, 2009).

For any organism, karyological data represent essential information and provide important characteristics for plant systematics and evolutionary analysis (Hörandl *et al*., 2005). Variations in chromosome number and karyotypic characteristics (such as chromosome size, chromosome morphology, ploidy levels, and karyotype coefficient of variation) are the main mechanisms governing species diversification (Guerra, 2008; Martin *et al*., 2015). These characteristics are considered vital sources of taxonomic and evolutionary information, as they serve as powerful tools for the authentication and identification of plant species after morphological studies (Badr & El-Shazly, 2021; Singh *et al*., 2023).

Although modern biosystematic investigations are mainly based on molecular methods, information on chromosome morphology is a powerful method for characterizing genomes (Crawford *et al*., 2005). Analyses of plant chromosome count data have been used for decades to understand phylogenetic relationships among plants and the processes leading to evolutionary diversification (Alberto *et al*., 2003; Rivero *et al*.,2019). However, according to the Chromosome Counts Database (CCDB), chromosome number data are known for only 20% of plant species (Rice *et al*., 2014).

1.1. The Aim of the Study:

Karyotype studies have yet to be conducted for many endemic plant species in Libya, particularly in the El-Jabal El-Akhdar region, recognized as an exceptional center of endemism within the country. This region is home to a variety of unique plant species,some of which have significant nutritional and medical importance. Conducting karyological research to provide chromosomal data is essential for identifying these species at the cytological level. Furthermore, these data can also provide valuable information to support future taxonomic and evolutionary studies, such as comparative analyses of chromosomal data and evolutionary behaviors in plants.

Therefore, the aim of this study is to apply karyotype analysis on *Arum cyrenaicum*, *Arbutus pavarii*, and *Origanum cyrenaicum*:

1. To determine the chromosome numbers, sizes, and types of these endemic species;
2. To determine the ploidy level of these species; and
3. To determine the number and sites of satellites and secondary constrictions.

2. Literature Review

2.1. Karyotyping Criteria

Each species has a characteristic chromosome complement; its karyotype can be defined as the phenotypic appearance of the somatic chromosomes, including number, size, and morphology (Levitzky, 1931). A karyotype represents the organism's complete set of chromosomes. The term additionally denotes a laboratory-generated visual representation of an organism's chromosomes, which are extracted from a single cell and systematically organized in numerical order. A karyotype is also defined as the phenotype of the chromosome set that includes the structural characteristics of all the chromosomes in the organism (Devadas *et al*., 2010; Chen *et al*., 2023).

Moreover, karyotype can be defined as the phenotype of the chromosome which includes the chromosome number, chromosome shape, position of the centromere, the distribution of euchromatin and heterochromatin, and the size of the satellites. Each species of creature has a different shape and number of chromosomes, so the karyotype is also different. Abnormalities in the karyotype are related to anatomy, morphology, and physiology. Chromosomal differences describe dissimilarities in the genetic content of an individual (Muliawati *et al*., 2023). A karyotype can be employed to identify irregularities (chromosome abnormalities) in either the number or structure of chromosomes (Preston, 2014).

To create a karyotype, researchers capture an image of the chromosomes from a single cell, subsequently cut them out and arrange them based on criteria such as chromosome number, size, banding patterns, position of the centromere, and the position of the satellites.

The majority of plant and animal species have distinct individuality in their somatic chromosomes, which is demonstrated by their size, shape, location of primary constrictions or centromeres, and other characteristics like secondary constrictions and satellites. This was firmly established by the pioneering work of the Russian School of Cytologists, headed by S. Navashin. Furthermore, distantly related species are frequently distinguishably different in those features with large, easily studied chromosomes, while closely related species are typically similar in these ways (Stebbins, 1950).

As a result, the term "karyotype" can be reinterpreted to refer to the somatic chromosomes' phenotypic appearance, while the term ideogram is used to refer to the diagrammatic representation of the karyotype (Stebbins, 1950). The primary methods through

which karyotypes differ from each other are well described in the classic work of cytogenetics. These distinguishing features used in karyotype are: 1.the chromosome number; 2. the shape of distinct chromosomes within the same complement; 3. the number of satellites and secondary constrictions; 4.the absolute length of chromosomes; and 5. the distribution of chromosomal material with distinct staining properties (Levitzky, 1931; Darlington, 1937). Plant chromosome karyotype analysis is a technology based on metaphase chromosomes as the research object, according to the characteristics of chromosome length, centromere position, ratio of long and short arms, and using banding technology to analyze, compare, sort, and number chromosomes (Senavongse *et al*., 2018).

The karyotype is the end product of numerous forces acting at the structural, organizational, and functional levels of the genome in an effort to enable it to couple with meiosis, mitosis, and the various functional states of the interphase cells throughout the organism's life. It is the genetic material itself and provides a global view of the genome organization, which sets it apart from other phenotypic aspects of plants. Certain karyotype traits are distinctive indications of sterility and meiotic aberration, as well as reproductive barriers or a unidirectional evolutionary process (Guerra, 2008).

The discovery of the constant species-specific numbers of chromosomes by Strasburger (1910) leads to the question of whether the karyotype might provide information about the systematic position of a species. The use of karyological data in taxonomy is known as cytotaxonomy, which helps to assess the genetic relationship among species or populations and to better understand the way they diverged from each other (Greilhuber & Ehrendorfer, 1988; Dobigny *et al*., 2004). Cytotaxonomy is a branch of cytogenetics, focused on the systematic and evolutionary comparison of karyological characteristics (Siljak-Yakovlev & Peruzzi, 2012). Chromosome studies can yield a variety of information, including chromosome number, karyotype structure, karyotype asymmetry, chromosome banding, FISH, GISH, and chromosome painting (Stace, 2000; Levin, 2002; Graphodatsky *et al*., 2011; Guerra, 2012).

All the reports concerning plant systematic studies need to explore the genetic constitution of the taxon under study, and particularly its chromosome complement, together with its morphologic and ecologic description, to strengthen the uniqueness of the new entity (Gianfranco *et al*., 2008).

Karyomorphology is a powerful approach to obtain useful basic comparative information in systematic studies (Astuti *et al*., 2017), including chromosome number, morphology, and chromosomal measurements (Peruzzi *et al*., 2024), which also helps evaluate genetic relationships among taxonomic categories and their divergence (Venkatesh *et al*., 2019; Chen *et al*., 2023).

Chromosome numbers are a useful systematic character, as similar chromosome numbers not only may indicate close relationships, but by studying the karyotype of different species, their position in the evolutionary process can be revealed (Greilhuber,1995).

The chromosome number is the simplest karyotype parameter, but it still has some unique appeal for cytotaxonomists. It is the quickest, least expensive, and most straightforward method of obtaining any significant information about a species' genome. For practically every family and the majority of plant genera, the most well-known cytotaxonomic datum is the number of chromosomes (Guerra, 2008).

The majority of flowering plants always exhibit chromosome number = 2*n* in any type of cytological test, whereas an odd or unexpected somatic chromosome number typically indicates meiotic problems or sterility. This initial karyotype description can be supplemented with a variety of additional karyological data, such as satellite chromosomal position, banding patterns, karyotype symmetry, and chromosome size and shape (Greilhuber, 1995).

Karyotype characters are valuable in resolving taxonomic problems and assessing relationships between species (Sun *et al*., 2019). For example, at the genus level, the genera *Yucca* and *Agave* share similarities in their vegetative characteristics, both possessing long, stiff leaves. However, they differ in the position of the ovary in their flowers: *Yucca* species have an upper ovary, which led to their initial classification in the family *Liliaceae*, while *Agave* species have a lower ovary, placing them in the family *Amaryllidaceae*. In 1933, Mckevely and Sax studied the karyotypes of some species from both genera to compare chromosome number and morphology. They found that both genera possessed similar chromosomal patterns, with five large chromosomes and 25 small chromosomes. Accordingly, they reclassified both genera within the family *Agavaceae*.

At the sectional level, Maire (1958) classified the Libyan-endemic *Allium ruhmerianum* alongside *Allium chamaemoly* within the section Molium. The chromosome complement of *A. chamaemoly* is ($2n = 2x = 16$), consisting of seven metacentric pairs and one telocentric satellite pair (Mossa & Scrugli, 1970; Garbari, 1975). In contrast, Bartolo *et al*. (1984) reported that *A. ruhmerianum* has a chromosome complement of ($2n = 3x = 33$), with all chromosomes being metacentric-three of which bear satellites. Therefore, its karyotype distinct from the derived karyotype of *A. chamaemoly.* Consequently, Bartolo *et al*. (1984) reclassified the Libyan-endemic *A. ruhmerianum* into the section *Rhynchocarpum*.

Also, at the species level, some chromosome characters, such as satellites, may be used to identify species in certain genera. However, all species in the genus have the same chromosome numbers; hence, it could not be used to identify species (Yu *et al*., 2009). Saensouk *et al*. (2019) found that clear satellites appeared in *Colocasia gigantea*, which can be distinguished from *C. fallex* and *C. lihengiae*, even with the same chromosome numbers ($2n = 28$).

2.2. Symmetrical and Asymmetrical Karyotype

The symmetric karyotype exhibits minimal differences in chromosome size, while the asymmetric karyotype has significant differences between large and small chromosome sizes. The size of the chromosome set is nearly equal in symmetrical karyotyping and not in asymmetrical karyotyping. The primary distinctions are based on centromere position, metacentric centromeres, chromosome components, size, and shape. More metacentric chromosomes are found in symmetric karyotypes, whereas asymmetric karyotypes have fewer metacentric chromosomes (Stebbins, 1971).

Interchromosomal and intrachromosomal asymmetries are the two types of estimation that compose the concept of karyotype of asymmetry. The study of intrachromosomal and interchromosomal karyotype asymmetry is one of the most popular, inexpensive, and extensively applied methods in comparative cytogenetics, particularly among botanists (Peruzzi & Eroğlu, 2013).

Levitsky (1931) was the first to develop the idea of karyotype asymmetry, which is defined as a karyotype characterized by the predominance of chromosomes with terminal/subterminal centromeres (intrachromosomal asymmetry) and highly heterogeneous chromosome sizes (interchromosomal asymmetry).

Levan *et al.* (1964) proposed a classification of chromosomes based on the ratio between the long and short chromosome arms, differentiated by the position of the centromere. According to their classification scheme, individual chromosomes can be categorized as metacentric (M/m), submetacentric (Sm), subtelocentric (St), acrocentric (t), and telocentric (T). A symmetrical karyotype is characterized by the predominance of metacentric and submetacentric chromosomes of approximately the same size. Asymmetry can increase when the centromere position shifts from median/submedian to terminal or subterminal positions, or due to the accumulation of differences in chromosome size within the chromosome complement, resulting in increased heterogeneity in the karyotype (Stebbins,1971).

Stebbins (1971) later proposed a quantitative method for estimating karyotype asymmetry in twelve categories. This method included four classes (from 1 to 4) distinguished based on the ratio of the difference between the largest and smallest chromosomes, as well as the proportion of metacentric chromosomes with an arm ratio of less than 2:1. These were then combined with three classes (from A to C) that were

defined by the increasing ratio between the largest and smallest chromosomes in a complement. The classification of Stebbins is widely used to assess karyotype symmetry and describe karyotypic relationships between different taxa.

In the years that followed, other researchers proposed quantitative estimation methods for interchromosomal asymmetry, which is caused by heterogeneity in chromosome sizes within a complement. This is the case with the Rec index (Greilhuber & Speta, 1976; Venora *et al*., 2002), the R ratio (Siljak-Yakovlev,1996), and the CV_{CL} (Lavania & Srivastava, 1992; Watanabe *et al*., 1999; Paszko, 2006). The latter, actually a Coefficient of Variation, is a statistically correct parameter that can capture even minor differences in chromosome sizes in a complement.

The quantitative estimation of intrachromosomal asymmetry caused by centromere position is more complex and debatable. To address this issue, the first quantitative index proposed was the TF% (Huziwara,1962), based on the total percentage of homologous chromosome pairs (TF%) ranging from 0 to 50, to analyze karyotypes. Another karyotype asymmetry index called the Arano asymmetry karyotype index (Ask%) was proposed by Arano (1963) to determine the phylogenetic relationships within the genera.

Greilhuber and Speta (1976) developed two indices to evaluate karyotype asymmetry: (1) the karyotype symmetry index and (2) the chromosomal size resemblance index. Venora *et al*. (2002) later renamed these as the Syi and Rec indices, respectively. Both indices have been widely applied to estimate karyotype asymmetry and analyze evolutionary relationships among species.

Romero-Zarco (1986) introduced an alternative approach for assessing karyotype asymmetry through quantitative measurement and graphical representation. He proposed two numerical parameters to estimate karyotype asymmetry:

A_1 (intrachromosomal asymmetry), ranging from 0 to 1, evaluates the variation in centromere position within chromosome complement, and A_2 (interchromosomal asymmetry) evaluates the variation in chromosome length.

He stated that these indices independent of chromosome numbers, and the values of A_1 and A_2 are considered to be close to 0 when the karyotype contains metacentric chromosomes and near 1 when all chromosomes are telocentric. Therefore, higher values of A_1 and A_2 indicate greater karyotype asymmetry, while values closer to zero indicate a more symmetrical karyotype.

Mugnier and Siljak-Yakovlev (1987) used the asymmetry index (AsI), which appears to be synonymous with Arano's (1963) Ask% index. Lavania and Srivastava (1992) proposed the dispersion index (DI), a parameter calculated as the ratio between the centromeric gradient (CG) and the coefficient of variation of chromosome length (CV).

Watanabe *et al*. (1999) introduced two indices: the index of karyotype symmetry (S%), which ranges from 0 to 100, and the degree of karyotype asymmetry (A), which ranges from 0 to 1. Paszko (2006) proposed three indices: the asymmetry index (AI), the coefficient of variation for chromosome pairs length (CV_{CL}), and the coefficient of variation for centromere index (CV_{CI}). According to Zuo and Yuan (2011), the coefficient of variation of the centromere index (CV_{CI}) is the best parameter that provides information about centromere heterogeneity.

The AI index provides a measure of the heterogeneity of chromosome length and centromeric position in a given karyotype and is similar to the dispersion index (DI) (Lavania & Srivastava, 1992) in the sense that the DI index is a broad measure, not intended to provde information about the asymmetry of a particular karyotype (Paszko,2006). Paszko (2006) proposed that higher values of the AI index indicate higher levels of karyotypic asymmetry, while lower values indicate greater karyotype symmetry. In addition, the (Ask%), (A), (A_1), and (A_2) all have a perfect negative correlation with three other indices: the TF%, S%, and CI%.

Two indices, the Rec and the A_2, were created to assess the variation in chromosome length in a complement. The A_2 index is a relative standard deviation of chromosome length and, from the statistical point of view, is a sensible parameter that adequately assesses the relative variation in chromosome length in a complement. The Rec index is a wrong parameter and does not reflect relationships between karyotypes too closely or at all. The DI index was developed by Lavania and Srivastava (1992) in order to give a single value that evaluated the karyotype asymmetry. It was the first attempt to create one karyotype asymmetry index, but one of two parameters used, the centromeric gradient (CG), cannot correctly assess the variation in centromeric position. As a result, the DI index is unable to evaluate karyotype asymmetry (Paszko,2006).

Paszko (2006) claimed that the latter, a Coefficient of Variation of Centromeric Index, was the only statistically valid parameter. However, her proposal has recently been strongly criticized by Zuo and Yuan (2011), who demonstrated that CV_{CI} can only measure the relative variation (heterogeneity) among centromere positions in a karyotype; it cannot capture and quantitatively express the original meaning of karyotype asymmetry (i.e. the prevalence of telocentric-subtelocentric chromosomes). Therefore, the issue of accurately estimating intrachromosomal asymmetry remains unresolved.

Lastly, some authors attempted to integrate the two types of asymmetry into a single index, including Paszko (2006) with AI and Lavania and Srivastava (1992) with DI. However, Paszko (2006) and Peruzzi *et al*. (2009) both harshly criticized these indices, so it is imperative that their use be discouraged.

For interchromosomal asymmetry, the use of the coefficient of variation of chromosome length, a powerful statistical parameter, has been confirmed (Peruzzi & Eroğlu, 2013). The new mean centromeric asymmetry is the most appropriate parameter for the intrachromosomal asymmetry. That is, the centromeric asymmetry for each chromosome in a complement can be easily obtained by calculating the difference between the relative lengths of the long and short arms. Also, the coefficient of variation of centromeric index, which has been heavily criticized in recent literature, is an additional karyological parameter that is not properly associated with karyotype asymmetry. The above-mentioned parameters show what to measure and how to accurately infer karyotype asymmetry. They can be the basic measurement tools for researchers dealing with cytotaxonomy (Peruzzi & Eroğlu, 2013).

One of the cheapest, most popular, and most preferred methods in comparative cytotaxonomy is that concerning karyotype asymmetry. Significant information about karyotypic phylogeny and speciation can be found in chromosomal data, especially karyotype asymmetry. The general morphology of chromosomes can be well expressed by karyotype asymmetry (Eroğlu, 2024).

2.3. Endemic Plants to El-Jabal El-Akhdar

El-Jabal El-Akhdar Plateau is a longitudinal terrace running west to east, parallel to the Mediterranean Sea. These terraces vary in width from place to place. This plateau is located in Cyrenaica, northeastern Libya, between latitudes 32° and 33° north and longitudes 20° and 23° east. El-Jabal El-Akhdar is bordered by the Mediterranean Sea to the north and west, the Butnan Plateau to the east, and the Great Desert to the south. The area of El-Jabal El-Akhdar is not precisely defined due to the lack of clear geographical features marking its borders, but the area of the hills is estimated at approximately 19,200 square kilometers (Leuenberger, 1965). This area is unique as it contains a variety of natural environments resulting from its exceptional geological, topographical, and climatic characteristics. It extends along the coast belt for about 300 km and arises to approximately 881 m above sea level(Ali, 2024).

The high concentration of endemic species in El-Jabal El-Akhdar may be attributed to its unique physiographic and climatic conditions, which differ significantly from most of the country. Climatically, the region has a Mediterranean summer-dry climate, with spring being the main growing season. The rocky, stony terrain is crossed by numerous wadis (valleys), receiving 250-600 mm of annual rainfall, with soils of terra rossa or heavy clay type. Biogeographically, the area forms a unique habitat island, isolated by the Mediterranean Sea to the north and west, and the desert to the south (Sharaf, 1971; El-Zwaam, 1995; Hunt *et al*., 2024). These factors have provided an ecological refuge, that contributes to the restricted distribution of many endemic taxa (Qaiser & El-Gadi, 1984; Al-Sodany *et al*., 2003; El-Mokasabi, 2010).

El-Jabal El-Akhdar is considered the largest and most significant Important Plant Areas (IPA) in Libya. The unique physiographic and climatic isolation of the Cyrenaican mountains from the rest of Libya has resulted in El-Jabal El-Akhdar harboring 75-80% of the Libyan flora, including a significant proportion of the country's endemic plant species, despite representing only 1% of Libya's total land area (Radford, 2011).

Plants are essential to life and play a vital role in all ecosystems. Despite their importance, plant biodiversity is under increasing threat worldwide, with the number of threatened species rising dramatically each year (FAO, 2019). The extinction of natural populations - or even entire species- is often linked to the habitat destruction and alteration due to human overexploitation, and more recently, pollution and climate

change, resulting in the loss of genetic diversity (Corlett & Bigger, 2017; Coelho *et al*, 2020). Many of these species are endemic and exist in limited and fragmented wild populations (Reed *et al*., 2011).

The concept of endemism dates back to Augustin Pyramus de Candolle's *Géographie Botanique* in the early nineteenth century. Endemism refers to the distribution of a taxon restricted to a particular geographical area of the world, where it naturally occurs. In other words, an endemic species is one that is unique and highly adapted to a specific geographic region (Isik, 2011; Foggi, 2014). Areas where the distribution ranges of two or more taxa overlap are called "areas of endemism" (Morrone, 2008). Endemism can occur on various spatial scales, ranging from continents to islands or mountaintops.

The term "endemic" may apply to any taxonomic level that is restricted to a specific biogeographic unit. Endemic species are typically concentrated in a limited number of taxa and do not represent a random taxonomic distribution (Dhar, 2002). Endemics may be categorized as local occurs on a variety of spatial scales, from areas as large as continents to small areas as islands or mountain tops. Endemic species can be categorized as local (restricted to a small area), provincial (limited to the borders of a province), national (limited to the borders of a country), regional (limited to a geographical region), or continental (limited to a continent) based on their distribution scale (Ladle & Whittaker, 2011).

Most endemic species share traits that make them particularly vulnerable to both natural and human- induced threats. These include limited range, few and small populations, declining population size, overcollection, restricted reproductive capacity, habitat specificity, and dependence on stable environments. The more such traits a species exhibits, the more vulnerable it becomes to extinction (Isik, 2011; IUCN, 2019).

Karyotyping is considered one of the most important analytical tools used in studying endemism (Siljak-Yakovlev & Peruzzi, 2012; Sun *et al*., 2019). The technique was first applied to endemic flora by Chiarugi (1949). Favarger and Contandriopoulus (1961) proposed four categories of endemism based on ploidy levels: paleoendemics (diploids or paleopolyploids), patroendemics (ancient diploid endemics originated from their progenitor polyploid taxa), schizoendemics (retaining the same chromosome number as their progenitor), and apoendemics (inversely to patroendemics, apoendemics are polyploid derivatives from widespread diploids). Stebbins and Major(1965), on the other hand,

suggested two categories of endemism based on geographical distribution: paleo-endemics (ancient taxa with ancient origins that have become confined to a specific region and a relict distribution, whose endemism resulted from the loss of habitat), and neo-endemics (recently originated taxa that have not spread to other regions).

Endemic plants of a country carry a unique genetic diversity within the country's flora, and the Libya's flora is not particularly rich in endemic species. There are no endemic plant families in Libya, but three endemic genera exist, each represented by a single species: *Pachyctenium mirabile* (Umbiliferae), *Libyella cyrenaica* (Gramineae), and *Oudneya africana* (Cruciferae). The first two genera are found in the El-Jabal El-Akhdar region, whereas the third is confined to the desert region (Mahklof & Etayeb, 2018).

El-Jabal El-Akhdar, considered the most important natural habitat in Libya, faces numerous environmental challenges. Currently, there is an imminent risk of genetic erosion of endemic species due to factors such as heavy grazing, the collection of medicinal and woody plants for local use and trade, over-cultivation, recurrent drought conditions, and other hazards that frequently occur in the El-Jabal El-Akhdar region (El-Darier & El-Mogaspi, 2009).

The chromosome is a unique, definite and stable structure in any living organism, and its form, number, and size have been used alongside morphological and ecological differences to circumscribe populations of plants (Adeigbe *et al*., 2013). The flora of the El-Jabal El-Akhdar region is distinguished by its rich diversity of endemic plants. However, the cytological data from this region is not comprehensively studied, hence there is scarcity in data concerning it. This highlights the need to document chromosomal diversity at the regional level. Chromosomal data are essential for differentiating between species, understanding the relationships between species through chromosomal similarities and variations, and for species identification. A list of endemic plant species names and their geographical distributions in the El-Jabal El-Akhdar region is documented in Appendix 1 (Ali & Jafri, 1976-1989; Dobignard & Chatelain, 2010-2013; POWO, 2024).

The flora of the El-Jabal El-Akhdar region includes 61 endemic species (54 species and 7 subspecies) belonging to 48 genera and 28 families, with the scientific names of 15 endemic species updated. The scientific names of 7 families have also been updated.

The dominant families in this region are Lamiaceae (12 species, 19.67% of endemics), and Asteraceae (10 species, 16.39% of endemics). The families Crassulaceae, Plumbaginaceae, and Caryophyllaceae are represented by 3 species each (4.92% of endemics), while Apiaceae, Poaceae, Geraniaceae, Asparagaceae, Caprifoliaceae, Plantaginaceae, and Iridaceae are represented by 2 species each (3.28% of endemics). Furthermore, the families Orchidaceae, Amaryllidaceae, Araceae, Convolvulaceae, Liliaceae, Ericaceae, Euphorbiaceae, Hypericaceae, Orobanchaceae, Polygalaceae, Primulaceae, Rubiaceae, Ranunculaceae, Rhamnaceae, Santalaceae, and Papaveraceae are each represented by one species (1.64% of endemics).

The karyotype data and ploidy levels obtained from the Index to Plant Chromosome Numbers (IPCN, 2024) indicate significant chromosomal diversity in the El-Jabal El-Akhdar region (Appendix 2). However, the information about chromosome numbers and karyotypes for endemic plant species in this region is quite limited, with only 27.87% of endemic species having published chromosome number records. The endemic plants that were previously studied karyologically belong to a variety of families. The most studied families in the region include: Asteraceae (7 endemic species, 11.48%), Plumbaginaceae (3 endemic species, 4.92%), Asparagaceae (2 endemic species, 3.28%), and Primulaceae (1 endemic species, 1.64%), as well as Geraniaceae (1 endemic species, 1.64%), Araceae (1 endemic species, 1.64%), Amaryllidaceae (1 endemic species, 1.64%), and Ranunculaceae (1 endemic species, 1.64%).

In each species, x is the basic chromosome number, n is the gametic chromosome number and $2n$ is the zygotic or somatic chromosome number (Mirzaghaderi & Marzangi, 2015). Chromosome numbers observed in this region ranged from $2n$ = 8 to 96, with the mean chromosome number of $2n = 28.65 \pm 26.53$. *Cyclamen rohlfsainum* has the highest chromosome number ($2n$ = 96), and *Bellevalia cyrenaica* the lowest ($2n$ = 8) among previously studied endemic species. The frequency of species likely of diploid was 14.75%, triploid was 3.28%, tetraploid was 4.92%, and hexaploid was 4.92%. The diploid ratio was notably higher in the flora of the region. Observed basic chromosome numbers include (x) = 4,5,7,9,11, and 14 (Legro,1959; Guittonneau & Le Houérou,1968; Marchant, 1973; Boyce, 1989; Bartolo *et al*.,1984; Brullo *et al*.,1990).

Species within the same genus can be differentiated by their chromosome numbers and ploidy levels even without additional karyological data (Rivero *et al*., 2019). Brullo *et al*. (1990) reported chromosomal diversity (polyploidy) in three Libyan endemic species from the genus *Limonium*: *L. teuchirae* (2*n* = 3*x* = 27) (triploid), *L. subrotundifolium* (2*n* = 4*x* = 32) (tetraploid), and *L. cyrenaicum* (2*n* = 6*x* = 54) (hexaploid). These chromosomal variations are useful in differentiating these species and indicate that polyploidy plays a minor role in the speciation of this genus. Brullo *et al*. (1990) also observed that, despite sharing the same chromosome number (2*n* = 18), the karyotype formulas of *Anthemis taubertii* and *Anthemis cyrenaica* differ.

Additionally, Bartolo *et al*. (1984) found that the karyotype of *Allium ruhmerianum* is symmetrical, composed exclusively of metacentric chromosomes, whereas asymmetrical karyotypes characterize *Bellevalia cyrenaica*, *Prospero cyrenaicum*, *Anthemis taubertii*, *Anthemis cyrenaica*, and *Picris mauginiana* (Bartolo *et al*., 1984; Brullo *et al*., 1990).

Satellite chromosome patterns also vary significantly among species: *Cyclamen rohlfsainum* possesses four satellites (Legro, 1959), *Allium ruhmerianum* has satellites on three metacentric chromosomes; *Scilla cyrenaica* shows a macro-satellite on one acrocentric chromosome; and *Bellevalia cyrenaica* has satellites on two metacentric and two submetacentric chromosomes (Bartolo *et al*., 1984). Despite these findings, comprehensive karyotypic data remain limited for most regional endemics. Chromosomal analysis of cytological preparations involves the calculation of karyotypic parameters and generation of ideograms (Mirzaghaderi & Marzangi, 2015). Published literature includes only chromosome measurements (1-2 μm) for *Cyclamen rohlfsainum* (Legro,1959) and ideograms for three endemic species: *Allium ruhmerianum*, *Scilla cyrenaica*, and *Bellevalia cyrenaica* (Bartolo *et al*., 1984).

2.3.1. *Arum cyrenaicum* Hruby

Arum cyrenaicum Hruby is famous in Libya for its use in folk medicine. It has been used traditionally as a remedy for dermatitis, psoriasis, diarrhea, and diabetes (El-Mokasabi, 2014; Al-Traboulsi & Alaib, 2021). Phytochemical analysis of *A. cyrenaicum* revealed the presence of many compounds like flavonoids, alkaloids, terpenes, carbohydrates and sterols (Abdel-Karim *et al*., 2018). Many studies have provided evidence for the antioxidant and antimicrobial activities of *A. cyrenaicum* (Abdulrraziq & Salih, 2021; Ben Ramadan *et al*., 2021).

Arum cyrenaicum Hruby is an annual plant, belonging to Araceae which is a large plant family that comprises 140 genera and about 4,075 known species (Christenhüsz & Byng, 2016; Saensouk *et al*., 2022). In Libya, there are 3 genera: *Arum*, *Biarum*, and *Arisarum* (Jafri & EL-Gadi, 1977). The genus *Arum* which comprises 38 species, is native to Europe, Asia, and North Africa (The Plant List, 2024).

There are diploid and polyploidy types in the genus *Arum*. Polyploidy refers to origanisms that consist of three or more complete sets of chromosomes. Polyploidy is widespread in nature, and it is estimated that approximately 70% of plants in nature have undergone polyploidy in their evolution and approximately 35% of angiosperms have a polyploid origin (Villa *et al*., 2022; Cui *et al*., 2023).

From a karyological point of view, the basic chromosome number for the *Arum* genus is $x = 14$ (Petersen, 1993), with most of the species are diploid ($2n = 2x = 28$) rather than tetraploid ($2n = 4x = 56$) and hexaploid ($2n = 6x = 84$) (Prime, 1980; Turco *et al*., 2014) (Appendix 3). Polyploidy, or whole genome duplication (WGD), is a ubiquitous feature of plant species evolution, and all groups of green plants have one or more events of WGD in their ancestry (Soltis *et al.*, 2015; Chanderbali *et al.*, 2022). However, despite being widely recognized as an important process for plant evolution and ecology, the extent to which polyploidy represents a general mechanism in the emergence of endemic species throughout the angiosperms has yet to be investigated.

According to reports, the majority of polyploid *Arum* taxa cover larger geographic areas than their diploid counterparts (Bedalov, 1981). *Arum italicum* is found throughout the Mediterranean region, the Atlantic coast, and the Caucasus (Bedalov, 1975). Central and Western Europe are home to *Arum maculatum* (Meusel *et al*, 1965; Terpo, 1973; Bedalov, 1981). Therefore, the ability of *Arum italicum* and *Arum maculatum* to colonize new areas may account for their wider geographic range in comparison to diploids like *Arum pictum*, or *Arum orientale* (Prime, 1980). However, the tetraploid *Arum apulum* has a very limited distribution, limited to Southern Italy (Bianco *et al*., 1994), while the diploid *Arum alpinum* has a very wide distribution (Bedalov & Fischer, 1995).

The previous studies showed only chromosome numbers of many *Arum* species (Marchant, 1973; Boyce, 1989; Bedalov & Drenkovski, 1997; Bedalov *et al*., 2006; Christou *et al*., 2008; Hand, 2015). While karyotype descriptions of some *Arum* species were studied by several workers. For example, D'Emerico *et al*. (1993) found that the *A. orientale*, *A. alpinum*, *A. nigrum*, and *A. pictum* are diploid, and *A. maculatum* is tetraploid, also their results showed that the karyotypes of *A. orientale*, *A. alpinum*, and *A. nigrum* were very similar, and the karyotype of *A. maculatum* shows great similarities to the karyotypes of *A. orientale* and *A. alpinum*. Furthermore, chromosome sizes vary between 5.40 and 3.10 µm for *A. orientale*, 4.80 to 2.95 µm for *A. alpinum*, 6.40 to 3.42 µm for *A. nigrum*, 5.00 to 2.65 µm for *A. pictum* and 4.80 to 2.00 µm for *A. maculatum*.

The diploid karyotypes of *A. orientale*, *A. alpinum, A. nigrum,* and *A. pictum* are characterized by numerous pairs of submetacentric and subtelocentric chromosomes, with high value of asymmetry indices 61%, 61%, 60%, and 65%, respectively (D'Emerico *et al*., 1993). Turco *et al*. (2014) studied the karyomorphological characteristics of *A. italicum*, *A. maculatum*, and *A. apulum*, and found both *A. maculatum* and *A. apulum* are tetraploid, while *A. italicum* is hexaploid, and their results showed that the karyotype morphology of *A. italicum* and *A. maculatum* is similar. On the other hand, *A. italicum*, shows a more asymmetrical karyotype, with a higher intrachromosomal asymmetry index (A_1 = 0.43) than *A. maculatum* (A_1 = 0.39). By contrast, *A. apulum* possesses the most symmetrical karyotype of the three (A_1 = 0.32).

The use of chromosome counts to determine the limits of poorly defined species has proved useful in the study of *Arum* (Boyce, 1993). For example, *Arum cyrenaicum* an endemic Libyan species first validly described by Hruby (1912) was previously misclassified under multiple names. Cosson (1875) identified it as *A. hygrophilum*, while Durand and Barratte (1910) reclassified it as *A. hygrophilum var. rupicolum*, and Pampanini (1931) identified it as *A. pictum*.

Bedalov (1973, 1975a, 1975b, 1981) observed that species with horizontal-rhizomatous tubers (e.g., *A. italicum* with $2n = 84$) are mostly polyploid, whereas those with vertical-discoid tubers (e.g., *A. hygrophilum* and *A. pictum* with $2n = 28$) are mostly diploid. However, exceptions exist, such as *A. alpinum* (horizontal-rhizomatous tubered species with $2n = 28$; Bedalov, 1983; Boyce, 1989). In contrast, the Italian endemic *A. apulum* and

the Libyan endemic *A. cyrenaicum* (both vertical-discoid tuber species) are tetraploid (2*n* = 56) (Boyce, 1989).

Boyce (1989) classified *A. hygrophilum* in the subsection *Hygrophila*, *A. pictum* in the subgenus *Gymnomesium*, and *A. italicum* in the section *Arum*. Meanwhile, *A. cyrenaicum* and *A. apulum* were placed in subsection *Dischroochiton* based on tuber morphology, flowering characteristics, and supported this morphologically-based separation by chromosome number.

2.3.2. *Arbutus pavarii* Pamp.

Durand and Barratte (1910) studied the morphology of Libyan *Arbutus* but noted no morphological variation between it and *Arbutus unedo*, a species widespread across multiple regions (e.g., Italy, Tunisia, Algeria, Morocco, Lebanon, Syria, Spain, Greece, Portugal, France, and the United Kingdom). Later, the Italian botanist Pampanini (1936) re-examined a specimen (n° 5836) found in Florentine University in Italy, collected in the 1933 from Wadi El-Fahaga (between El Garib and Tolmitha) and identified distinct differences from *A. unedo* in flowering branches, fruit shape, and bark morphology. Consequently, Pampanini (1936) reclassified the specimen as the Libyan endemic *A. pavarii*. This endemism has been further supported by a subsequent study (Cuccuini *et al*., 2015) based on re-evaluations of its morphological data.

In Libya, the fruits of *Arbutus pavarii* are used for relief and protection against a number of diseases in folk medicine, such as antiseptic, diuretic and laxative properties. The leaves have also been used traditionally as a remedy for urinary tract infections, constipation, and gastritis (El-Darier & El-Mogaspi, 2009; Al-Traboulsi & Alaib, 2021).

The phytochemical studies on *A. pavarii* revealed the presence of active compounds such as phenolic acid, flavonoids, tannins, glycosides, arbutin, ursolic acid, betulinic acid methyl ester, sterols and some vitamins like vitamin A, C, and E (Elshatshat & Elshibani, 2020; Al Groshi *et al*., 2022). There are many scientific studies that have provided evidence for the antioxidant, antimicrobial, and anticancer activities of *A. pavarii* (Abdulrrazeq & Salih, 2020b; Al Groshi *et al*., 2022).

Arbutus pavarii belongs to Ericaceae that is a family of flowering plants, commonly known as the heath or heather family, and contains a morphologically diverse range of taxa, including herbs, shrubs, and trees. The Ericaceae family is considered large with 4250

known species spread across 124 genera (Christenhüsz & Byng, 2016). In Libya, it is represented by two genera: *Erica* and *Arbutus* as well as three species (Jafri & EL-Gadi, 1978).

Arbutus is a genus that includes 12 species, growing in the Americas and the Mediterranean area. The American species are: *A. arizonica* (*Gray*) (Mexico and USA), *A. menziesii* (USA), *A. madrensis*, *A. occidentalis*, *A. tessellata*, *A. mollis*, *A. bicolor* (Mexico), and *A. xalapensis* (El Salvador, Guatemala, Honduras, Texas, Arizona, and Mexico). Whereas in the Mediterranean region, there are 4 species: *A. unedo* and *A. andrachne* (eastern Mediterranean region), *A. canariensis* (Canary Islands), and *A. pavarii* (Coasts of Libya) (Torres *et al*., 2002; González-Elizondo *et al*., 2012).

The Ericaceae has been relatively under-studied cytologically and the only large genera in the Ericaceae studied in detail are *Rhododendron*, *Erica*, and *Gaultheria* (Jones & Brighton, 1972; Middleton & Wilcock, 1990; Choi *et al*., 2022). In spite of this, Raven (1975) was able to offer some ideas regarding the Ericales and Ericaceae's ancestral basic numbers. According to his theory, the Ericales had a basic chromosome number of $x = 6$ (like the Epacridaceae), from which the Ericaceae were descended with a basic number of $x = 12$. This has frequently resulted in dysploid derivatives, particularly $x = 11$ and $x = 13$.

Although the genus of *Arbutus* includes a few species disturbed in the Mediterranean and American areas. However, previous chromosome studies on some *Arbutus* species were insufficient and mainly limited to determining the chromosome numbers, and do not show the morphological characteristics of the chromosome set of the species studied or the characteristics of the karyotypes (Sealy & Webb, 1950; Darlington & Wylie, 1955; Taylor & Taylor, 1977; Martins *et al*., 2022). All of these previous data showed a consistent basic chromosome number (x)=13 with diploid species ($2n=2x= 26$) (Appendix 4).

Natural polyploidy has not been reported in *Arbutus* ($n = 13$). Also, polyploidy has not been documented in *Erica* which belong to Ericaceae and chromosome counts which are constant at $n = 12$ for all species with the exception of the European species *Erica spiculifolia* that is $n = 18$ (Nelson & Oliver, 2005).

Attempts to obtain *Arbutus* tetraploid plants have been carried out by many researchers (Martins *et al*., 2022). The first attempt was done by Antunes (2010) using nodal segments micro-propagated *in vitro* that were treated with two c-mitotic agents (colchicine and oryzalin) in solid and liquid media. However, most of the treated plants remained diploid, some became mixoploid and only a tetraploid was obtained on solid medium containing 125 µM oryzalin. A second attempt was carried out under similar conditions by (Martins & Canhoto, 2014). Several mixoploid plants were obtained, but only three plants were found to be tetraploid after a treatment with oryzalin.

The third attempt was made by Martins *et al*. (2022) who counted chromosomes in somatic-embryonic cells of root tips that had been treated with 0.05% (w/v) colchicine for 3 h at 25 °C, in the dark. They reported that despite the reduced dimensions of the chromosomes, the expected chromosome number of $2n = 26$ was found in the seedling cells, demonstrating that no chromosomal changes occurred during regeneration through somatic embryogenesis. Taken together, these findings suggest chromosome duplication is difficult to achieve when *A. unedo* and a tetraploid genome is very unstable. This explains the lack of natural polyploidy, and therefore the absence of polyploid species, in the genus *Arbutus* within the family Ericaceae.

2.3.3. *Origanum cyrenaicum* Bég. & Vacc.

Origanum cyrenaicum was first described as a Libyan endemic species by Béguinot and Vaccari (1913) and later confirmed by Pampanini (1931). Itswaart (1975) classified this taxon as *O. akhdarensis*, while Brullo and Furnari (1979) subsequently reclassified it as *Amaracus cyrenaicum*. Jafri and EL-Gadi (1984) and El Rabiae *et al*. (2024) reinstated the original designation (*Origanum cyrenaicum*) through taxonomic revisions.

Origanum cyrenaicum is used in Libyan folk medicine as an expectorant, carminative, stomachic, and for menstrual cramps. It is also used to inhale the steam from boiled leaves during flu to facilitate breathing and for ear congestion (Agiel & Mericli, 2017). The phytochemical analysis of the oils from *O. cyrenaicum* leaves revealed the presence of active compounds such as D-germacren-4-ol, epizonarene, linalool, thymol, terpineol, camphene, trans-caryophyllene, and β-phellandrene (Elabbar *et al*., 2014).

O. cyrenaicum is a member of Lamiaceae family. Lamiaceae, generally known as the mint family, have long been known for their aromatic oils, which have played a significant role in culinary, medicinal, and horticultural aspects of human history. As currently circumscribed, Lamiaceae comprises more than 230 genera and over 7000 species (Zhao *et al*., 2021). The genus *Origanum* L. placed in the family Lamiaceae, contains 43 species and 20 hybrids (Martin *et al*., 2020). In Libya, it is represented by 22 genera and 65 species: *Mentha*, *Ajuga*, *Teucrium*, *Rosmarinus*, *Salvia*, *Coleus*, *Lavandula*, *Marrubium*, *Sideritis*, *Scutellaria*, *Prasium*, *Lamium*, *Phlomis*, *Ballota*, *Stachys*, *Ocimum*, *Calamintha*, *Thymus*, *Nepeta*, *Satureja*, *Micromeria*, and *Origanum* (Jafri & EL-Gadi, 1984).

The application of the Image Analysis System in the karyotyping of plant taxa characterized by small and often indistinguishable somatic chromosomes (Fukui, 1998; Iijima *et al*., 1991) has garnered interest for examining chromosome morphology within the genus *Origanum*. Previous studies on the karyotype analyses within the genus *Origanum* were limited by determination of chromosome numbers, and the lack of sufficient data on the karyomorphology of this genus is probably due to the small size of its chromosomes (Bakha *et al*., 2017; Martin *et al*., 2020). It was reported that the main diploid chromosome numbers were $2n$ = 28, 30 and 32 (Lepper, 1970; Von Bothmer, 1970; Gill,1981; Fernandes & Leitão, 1984; Magulaev, 1984; Ayyangar & Vembu, 1985; Pastor *et al*., 1990; Khatoon & Ali, 1993; Bastida & Talavera, 1994; Markova & Goranova, 1995; Dobea *et al*., 1997; Kitiki, 1997; Balim & Kesercioğlu, 1998; Yildiz & Gücel, 2006; Bakha *et al*., 2017; Dirmenci *et al.*, 2018a, 2018b; Dirmenci *et al*., 2019; Martin *et al*., 2020), with the basic chromosome number of x = 15, and no polyploidization pattern was observed in the genus *Origanum* (Bakha *et al*., 2017) (Appendix 5).

Bakha *et al*. (2017) studied the chromosome number of five Moroccan taxa of the genus *Origanum* occurring in the wild in addition to the exotic species *O. onites*. All investigated taxa are diploid with chromosome number of $2n$ = 30. Moreover, one B chromosome and 6 satellite chromosomes were observed in *O. elongatum*.

Martin *et al.* (2020) examined the chromosome numbers and structures of some *Origanum* L. taxa that grow in Turkey. According to findings, *O. sipyleum* has the smallest chromosome length, measuring (0.32µm), while *O. minutiflorum* has the longest chromosome length (2.02µm) among the studied species. *O. vulgare* subsp. *hirtum* has

the smallest total haploid length (10.08 µm), while *O. haussknechtii* Boiss has the largest value (22.00 µm). The smallest mean length is 0.33 µm in *O. vulgare* subsp. *hirtum* and *O. saccatum*. The largest mean length is 0.74 µm in *O. sipyleum*. The centromeric position in the Turkish *Origanum* species could not be clearly observed because the chromosomes were generally very small, while the total chromosome length was measurable (Martin *et al.*, 2020). Their chromosome counts analysis confirm the speciation of *Origanum* members via homoploid hybridization.

3. Materials and Methods

3.1 Sites Description

The natural habitats of the plant species used in the present study were: 1) the southern heights of Tolmitha region, and 2) the forest lands near Al-Gubba region. Both sites are located in El-Jabal El-Akhdar region, Cyrenaica, northeastern Libya (Fig.1).

Figure 1. The two collection sites of plant species samples used in the current study on the map of El-Jabal El-Akhdar region, Libya. (All indicated by arrows)

Tolmitha site is characterized by reddish clay soil with relatively dense vegetation dominated by *Juniperus phoenicea*, *Pistacia lentiscus*, *Ceratonia siliqua*, and *Olea europaea*. Meanwhile, Al-Gubba site is characterized by a shallow calcareous soil with scattered vegetation primarily consisting of *Pistacia lentiscus* and *Juniperus phoenicea*. The ecological characteristics of the two collection sites was described in Table 1.

Table 1. The ecological characteristics of the two collection sites

Site	Latitude (N)	Longitude (E)	Altitude (M)	Rainfall (mm/y) (2021-2024)
Tolmitha	″3243′51	″210′24	129	525.41
Al-Gubba	″32765′0	″222′429	590	408.66

Source: prepared by the researcher based on data from:

Accurate Altimeter App & NASA/POWER SRB/FLASH Flux/MERRA2/GEOS (5.12.4).

3.2 Plant Materials

The three plant species (*Arum cyrenaicum, Arbutus pavarii* and *Origanum cyrenaicum*) used in the current study are at the forefront of the plant species endemic to El-Jabal El-Akhdar and are of utmost importance as they are threatened in their ecological habitats.

3.2.1 *Arum cyrenaicum*

Arum cyrenaicum (vernacular name: Renish) is an annual herbaceous plant with discoid-shaped tubers, which grows during the early fall season. It has simple sagittate and hastate leaves with long petioles (Fig. 2). The plant produces a single inflorescence, which is sail-shaped and dark purple in color. The inflorescence consists of two parts: the spathe and the spadix, blooms during March-April. The fruits of *A. cyrenaicum* are red berries when ripe, and the seeds have an ovate shape (Abdulrraziq & Salih, 2020a). *A. cyrenaicum* was reported as endemic species to the El-Jabal El-Akhdar region in Libya (Jafri & EL-Gadi, 1977). Its classification as follows:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Liliopsida
Order: Alismatales
Family: Araceae
Genus: *Arum*
Species: *Arum cyrenaicum* Hruby (GBIF, 2025).

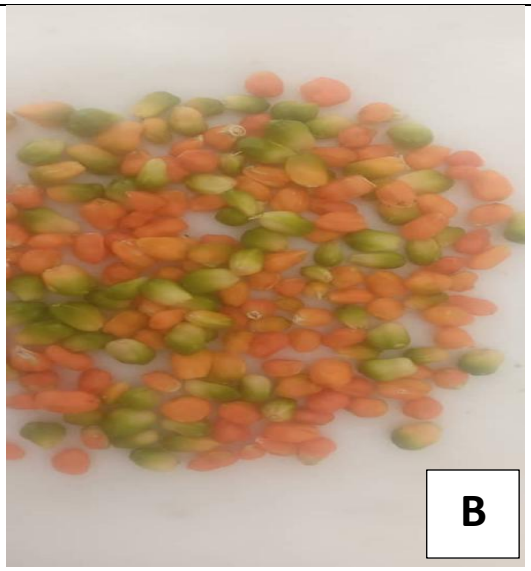


Figure 2. *Arum cyrenaicum* Hruby (A), fruits (B), and their seeds (C).

3.2.2 *Arbutus pavarii*

Arbutus pavarii (vernacular name: Shmari) is an evergreen shrub or small tree, 1.5-3m tall. The bark is reddish-brown, the leaves are lanceolate to ovate in shape, and the flowers are drooping bells approximately 5cm, generally white or pinkish (Fig. 3). The fruits are spherical and take around 8 months to ripen, which is why they are still on the tree when it flowers. They are irregular, with a diameter of 15-20mm, and contain many seeds. Their flowering occur during October-February (Jafri & EL-Gadi, 1978; Elshatshat, 2009), and reported as endemic species to the El-Jabal El-Akhdar region in Libya (Cuccuini *et al*., 2015). Its classification as follows:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Ericales

Family: Ericaceae

Genus: *Arbutus*

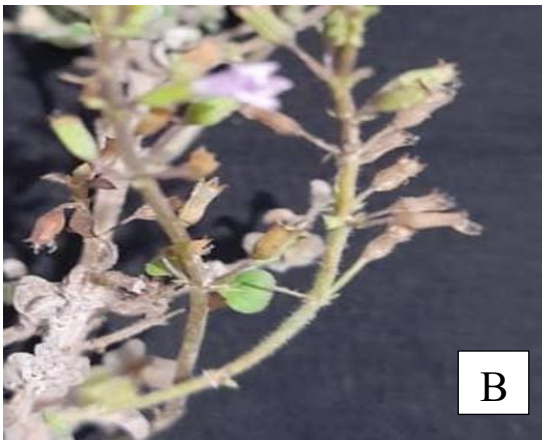
Species: *Arbutus pavarii* Pamp. (GBIF, 2025).

Figure 3. *Arbutus pavarii* Pamp.(A), fruits (B), and their seeds (C).

3.2.3 *Origanum cyrenaicum*

Origanum cyrenaicum (vernacular name: Martwosha) is a perennial evergreen shrub with an erect stem bearing branches measuring 10-20 cm in length. The leaves are small, ovate-orbiculate, and more or less covered by hirsute (Fig.4). The inflorescences are usually smaller than the leaves and the small bracts are purplish and ovate-lanceolate, and blooms during October-January. Additionally, the bracts contain many seeds, and the corolla is pinkish and twice as long as the calyx (Jafri & EL-Gadi, 1984). *O. cyrenaicum* was reported as endemic species to the El-Jabal El-Akhdar region in Libya (Jafri & EL-Gadi, 1984). Its classification as follows:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Origanum*
Species: *Origanum cyrenaicum* Bég. &Vacc. (GBIF, 2025).



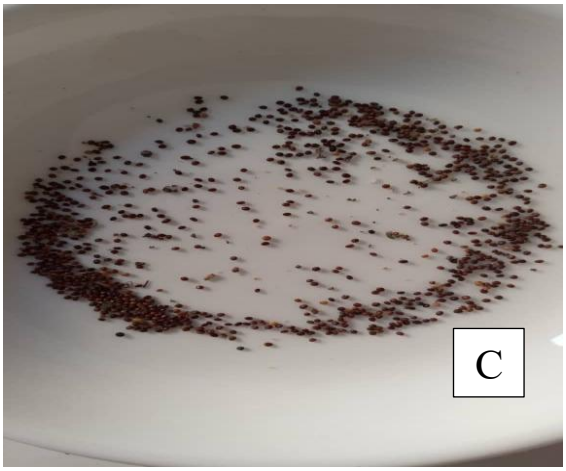


Figure 4. *Origanum cyrenaicum* Bég. & Vacc.(A), inflorescences (B), and their seeds (C).

3.3 Collection and Germination of Seeds

The plant materials used in this study were collected from their natural ecological habitats. Moreover, the seeds were collected from mature fruits and inflorescences, since collecting them before full ripening may result in incomplete embryo development and poor germination. Early collection can also reduce the amount of stored nutrients in the seeds, leading to a lower germination rate.

3.3.1. Collection and Germination of Seeds of *Arum cyrenaicum* Hruby

Mature fruits of *Arum cyrenaicum* were obtained from the ancient ruins of Ptolemais, about 2 kilometers from the Tolmitha region, on 17 May, 2022. The seeds of *Arum cyrenaicum* were carefully extracted from the fruits, then thoroughly washed with distilled water to remove any pulp, and stored at 4°C until use. A total of ten pre-germination treatments were applied, and these treatments were designed to evaluate the effects of physical, chemical, and temperature factors known to influence seed dormancy germination. The treatments were as follows:

1. **Soaking the seeds in distilled water** for time intervals of 1, 2, 3, 4, and 5 days, followed by placement on moist filter paper in Petri dishes at room temperature.

2. **Mechanical scarification** with a sharp tool, followed by soaking in distilled water for the same time intervals as above, then incubated at room temperature.

3. **Scarification and soaking** (1 day), followed by placement in moist paper towels and stored at 4°C.

4. **Soaking in sulfuric acid** at 90% and 50% concentrations for time intervals of 5, 10, 15, and 20 minutes with continuous stirring to prevent seed clumping due to testadegradation. Seeds were then rinsed with distilled water for 30 minutes to remove any residual acid before conducting germination tests.

5. **Rubbing with sandpaper**, soaking in distilled water for one day, incubated at room temperature.

6. **Sandpaper abrasion**, then followed by sulfuric acid (90%) for varying durations (5,10, 15, and 20 minutes); incubated at both room temperature and 4°C.

7. **Two-days Soaking**, **scarification**, and incubation in air-conditioned room at 16-26°C.

8. **Soaking (2 days)**, **scarification**, then immersion in sulfuric acid at concentrations of 90% and 50% for time intervals of 5, 10, and15 minutes with continuous stirring, followed by a 30-minute distilled water rinse.

9. **Soaking (1 day)**, scarification, and incubation on moist paper towels stored at 4°C.

10. **Boiling water treatment**: Soaking the seeds in distilled boiling water at 100°C for 5, 10, and 15 minutes, followed by cooling at room temperature.

All of these treatments failed to induce seed germination in *A. cyrenaicum* seeds-except one: Soaking the seeds in distilled water for one day, followed by mechanical scarification using a sharp tool, and incubation on moist filter paper in a Petri dish at a temperature range between 12°C and 15°C. Under this condition, germination occurred successfully within two weeks (see Fig. 5A), indicating its effectiveness in breaking dormancy and promoting germination. Some *A. cyrenaicum* seeds were also tested for germination over a period of nearly three months.

3.3.2. Collection and Germination of Seeds of *Arbutus pavarii* Pamp.

Mature fruits of *Arbutus pavarii* were collected from wadi Emleka, approximately 19 kilometers from the Tolmitha region, on 11 November, 2021.

The seeds of *A. pavarii* were extracted from the fruits and thoroughly washed with distilled water to remove any remaining fruit pulp. Subsequently, some seeds were stored at room temperature, while others were refrigerated. The seeds were then soaked in distilled water for one day before being placed on wet filter paper in Petri dishes. Germination was conducted at room temperature for a period of seven days (Fig. 5B). At high ambient temperatures, seeds were soaked in distilled water for one day, then placed on moistened paper towels inside a container and kept at 4°C to facilitate germination. Additionally, during the germination period, some *A. pavarii* seeds were tested for germination over an extended duration of two month and three weeks.

3.3.3. Collection and Germination of Seeds of *Origanum cyrenaicum* Bég & Vacc.

The mature inflorescences of *Origanum cyrenaicum*, bearing fully developed seeds, were collected from wadi Bouhalfaya, located roughly 3 kilometers from the Al-Gubba region, on 6 November, 2022.

The seeds of *O. cyrenaicum*, which are very small in size, were extracted from the inflorescences and washed with distilled water. They were then stored at room temperature. Afterwards, the seeds were soaked in distilled water for one day and subsequently placed on wet filter paper in a Petri dish at room temperature for two days to germinated (Fig. 5C).

At high ambient temperatures, successful germination of all *O. cyrenaicum* seeds proved difficult under normal conditions. Initial attempts to germinate the seeds on moisted filter paper were unsuccessful, as the filter paper tended to rot at higher temperatures, which adversely affected seed viability and germination. Therefore, the use of wet filter paper was deliberately avoided, and instead, seeds were germinated in containers containing a small amount of distilled water, sufficient to maintain moisture without fully submerging the seeds. The containers were then placed in a refrigerator at a temperature ranging between 4 and 10°C. The seeds were kept there for three to four days as part of a cold stratification treatment that help to break dormancy and enhance germination. Some *O. cyrenaicum* seeds were also tested for germination over an extended period of up to four months.

Figure 5. The germination of seeds of *A. cyrenaicum* (A), *A. pavarii* (B), and *O. cyrenaicum* (C).

3.4 Karyotype Analysis

The karyotype analysis was performed using actively dividing meristematic cells obtained from the root tips of germinated seeds. The general procedure included germination of seeds, selection of root tips with optimal growth, pre-treatment with 0.1% colchicine to arrest cells at metaphase, fixation in Carnoy's solution, hydrolysis in 1N hydrochloric acid, and staining with aceto-orcein. Permanent slides were prepared using the cellophane method. Chromosome spreads were observed and photographed using an Olypmus CX21 light microscope equipped with a Y.W 5.0 camera.

The overall workflow applied to all studied plant species is summarized in (Fig. 6). The following subsections provide a detailed description of the procedure as applied to each species individually.

3.4.1. Karyotype of *Arum cyrenaicum*

The root tips ranging from 0.5 to 0.8cm in length were soaked in 0.1% Colchicine for 6 hours at room temperature. Afterwards, the root tips were washed with 45% acetic acid, and then fixed in (Carnoy's solution I) (ethanol: glacial acetic acid=3:1, v/v) for 24 hours, and stored in 70% ethanol at 4°C until they were used. Subsequently, the fixed root tips were hydrolyzed in 1N HCL at 60°C for 20 minutes and then were soaked in cold distilled water for 20min. They were then stained with 2% aceto-orcein (2 g orcein in 100 ml of 45% glacial acetic acid) for 20 minutes, and the growing tip of the root was cut off using a sharp tool and placed on a glass slide. A drop of 45% acetic acid was placed on the root tip, followed by the placement of a cover slip. Afterwards, the slide was placed between folded filter paper, then gently squashed using the thumb, and the pointed end of a pencil was passed over the cover slip in a zigzag motion to eliminate air bubbles, separate the cells, and facilitate the observation of chromosomes.

3.4.2. Karyotype of *Arbutus pavarii*

Root tips of the germinated seeds ranging from 0.5 to 0.6cm in length were treated with 0.1% Colchicine solution for 3hrs at room temperature, and the root tips were transferred to a fixative solution (Carnoy's solution I) (ethanol: glacial acetic acid=3:1, v/v) in vials

for 24 hours, and then stored in 70% ethanol at 4°C until they were used. The fixed roots were hydrolyzed in 1N HCl at 60°C for 30 to 32 minutes, followed by a 20-minute soak in cold distilled water (Optional). They were then stained in 2% aceto-orcein (2 g orcein in 100 ml of 45% glacial acetic acid) for 17-20 minutes.

The root tips of *A. pavarii* are very small and difficult to squash, so a modified method was used for preparing the slides. In this method, the stained meristematic growing tip of the root was placed on a glass slide and cut using a sharp tool. A small drop of distilled water was added to the sample, followed by the placement of a small square piece of cellophane nylon. A cover slip was then carefully placed on the cellophane.

To squash the sample, the pointed end of a pencil was used to gently tap the cover slip. Afterwards, the slide was placed between folded filter paper (or paper towels), and gentle pressure was applied using the thumb and the pointed end of a pencil was passed over the cover slip in a zigzag motion. Once squashed, the cover slip was removed, and the cellophane nylon containing the squashed sample was lifted and inverted onto a second clean slide. A new cover slip was placed. Additionally, a cover slip was placed on the orginal slide from which the cellophane had been removed. This technique proved effective for squashing the delicate root tips of *A. pavarii*, allowing for elimination of air bubbles, good spreading of cells, and clear visualization of chromosomes-while avoiding breakage of the cover slip.

3.4.3. Karyotype of *Origanum cyrenaicum*

Germinated roots ranging from 0.3 to 0.4cm in length were treated with a 0.1% Colchicine solution for 3 hours at room temperature to halt mitosis. Following treatment, the root tips were fixed in (Carnoy's solution I) (ethanol: glacial acetic acid=3:1, v/v) for 24 hours. Subsequently, the root tips were stored in 70% ethanol at 4°C until use (optional). The fixed roots were hydrolyzed in 1N HCl at 60°C for 30 to 32 minutes, followed by soaking in cold distilled water for 20 minutes.

Due to the extremely small size of *O. cyrenaicum* seeds-approximately the size of a grain of sand-cytological slides preparation required particular care. Two to three germinated seeds were placed on a single slide, and the seed coat was carefully removed using a sharp tool and light pressure to ensure separation of the germinated root from the rest of the seed.

A drop of 2% aceto-orcein stain (2 g orcein in 100 ml of 45% glacial acetic acid) was then applied to the germinated roots and left for 15-20 minutes. Subsequently, a small drop of distilled water was added to the germinated root tips, followed by placement of a small square piece of cellophane nylon over root tips. While the cellophane remained in place, it was quickly rinsed two consecutive times using carnoy's solution or 70% ethanol.

A cover slip was then gently placed over the cellophane. To squash the root tips, the slide was tapped lightly using the pointed end of a pencil. It was then placed between two sheets of filter paper, and zigzag pressure was applied using the pencil tip. The cover slip was carefully removed, and the cellophane containing the squashed root tips was delicately lifted and inverted onto a second clean slide. A new cover slip was placed on this second slide, and another cover slip was also placed on the original slide. This modified method helped delicately flatten the root tips without damaging the cells, allowing proper chromosome spreading and clear microscopic observation.

Chromosome counting, measurement of chromosome lengths, and karyotype analysis were conducted using slides containing chromosomes at the metaphase stage of mitosis. Photographs were captured with a YW5.0M digital camera attached to an Olympus CX21 optical microscope, operating at a magnification of 100x with immersion oil, and at least ten slides with well-spread and clearly observable morphologies were considered.

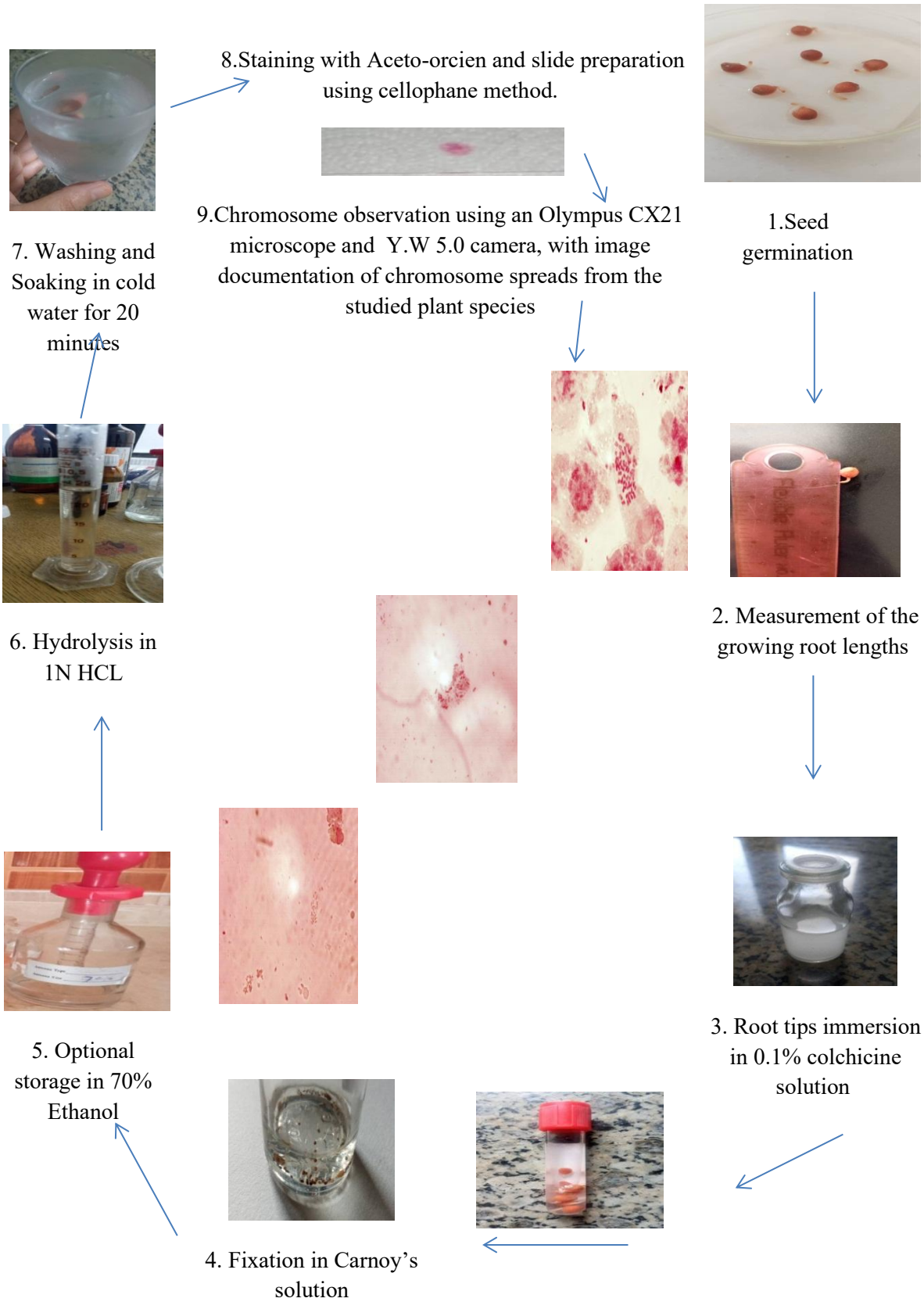


Figure 6. Cytogenetic procedure for karyotyping the studied plant species.

3.5. Software and Photoshop programs

Due to the precise and complex nature of chromosome studies, it was essential to utilize digital and technical tools that enhance image quality and allow for more objective and accurate analysis. Therefore, a set of software programs was used to assist in cropping and arranging chromosomes to generate the karyogram. These programs also supported the measurement of chromosome lengths and the calculation of karyotypic parameters, which contributed to a precise karyotype analysis. This step is particularly important, as karyotype studies rely heavily on digital image editing and analysis tools, including software originally developed for engineering scientific, and design purposes. Such tools provide flexible and accurate image processing capabilities that support cytological research.

The use of various software programs for processing chromosome images were incorporated gradually throughout the practical work. A single metaphase image (Fig. 7A) was initially used as a test case across multiple programs. The image was processed in sequence using AutoCAD 2022, Adobe Photoshop 2020, ImageJ, and PicsArt. Each software was assessed based on image quality, ease of use, tool availability, and its overall effectiveness in cropping and arranging chromosomes.

3.5.1. AutoCAD 2022 program

Paper copies were printed from metaphase image (Fig. 7A) to visually identify homologous chromosomes. Pairs of similar chromosomes were assigned matching numbers, as illustrated in Fig. 7B. These chromosomes were then manually redrawn using carbon paper, cut out, and arranged into homologous pairs, forming Fig. 7C. This image was subsequently imported into AutoCAD (2022), developed by Autodesk; a software widely used in engineering and architectural design. Although primarily intended for technical applications, AutoCAD (2022) includes several features that proved useful in this biological context. These include precision drawing tools based on vector graphics, full control over dimensions, lines, and layer management, support for various formats such as images and PDFs, and a flexible, customizable interface that allows efficient access to drawing tools.

Although AutoCAD is not specifically designed for biological research, it was effectively used here to practice drawing and organizing chromosomes into homologous

pairs. The initial output (Fig. 7D) lacked accuracy and precision; however, with continued training, the quality of the chromosome drawings improved significantly, resulting in (Fig. 7E). Despite its primary application in engineering disciplines, AutoCAD supported the conceptual understanding of karyotypic arrangement, which is essential in cytogenetic analysis, as each species has a unique chromosomal set that must be organized in homologous pairs for proper study.

3.5.2. Adobe Photoshop 2020 program

The same image (Fig. 7A) was then processed using Adobe Photoshop 2020. This program includes a wide range of tools for image processing, such as opacity control, layer management, cropping tools, and brushes for color blending and image smoothing. It was necessary to study the features of each tool and practice using them in order to apply them effectively for chromosome cropping, and this process generally takes around one to two months.

Despite its advanced capabilities, the use of this program was difficult and presented several challenges. For example, adjusting settings for tools such as "opacity" and the "Crop Tool" required training to determine the most suitable values for accurately cropping chromosomes. Additionally, placing marks on the edges of the chromosome to be cropped, as shown in (Fig.8A), led to various problems. Some chromosomes lost their original shape after marking the edges, such as chromosome 1 (Fig. 8A and 8B). In other cases, parts of the chromosomes were lost due to adhesion between them, which later affected chromosome length measurements, as seen in chromosome 2 (Fig. 8B).

There was also difficulty in utilizing tools such as the Brush Tool, Blur Tool, and Smudge Tool to remove banding or striation effects from the image, as shown in chromosome 3 (Fig. 8B). These smoothing tools negatively affect image quality and lead to the loss of important chromosomal details, such as the location of the centromere, secondary constriction, or satellites, if present.

Overall, Adobe Photoshop 2020 is an effective program, but it is not the most suitable option for cytological analysis due to its complexity, the time required for training, and its limited precision when cropping chromosomes, which are delicate structures that must retain their original morphology.

3.5.3. ImageJ 1.46r program

The same image (Fig. 7A) was then processed using ImageJ 1.46r software (Java 1.6.0_20) (Rasband, 2012), a widely used open-source program developed by the National Institutes of Health (NIH,USA). It allows users to analyze and process scientific images and measure areas and pixel values, which can be converted into specific units based on the researcher's input. ImageJ supports various image formats and is commonly applied in microscopy, cytology, and histological image analysis.

In the current study, ImageJ was used to crop chromosomes; however, the process was slow and time-consuming. The program does not provide a feature to collect and organize the cropped chromosomes within a single file. Additionally, it lacks a tool for removing excess background or edges around each chromosome (Fig. 9A). There was also difficulty separating overlapping or closely adjacent chromosomes, and the overall image quality was relatively low (Fig. 9B), which made precise editing and cropping challenging.

The same metaphase image (Fig. 7A) was processed using ImageJ to remove the lines caused by intense microscope lighting. This was done by selecting the "Process" option from the toolbar, then choosing "Smooth" from the dropdown menu. These lines made it difficult to identify the position of the centromere (primary constriction) for each chromosome, and also hindered the detection of secondary constrictions or satellites, if present.

To preserve the clarity of the image and provide accurate measurements, the image dimensions had to be consistent with the screen resolution. For example, the resolution on the computer used was 720*1280 pixels, so the image had to be of the same or compatible size. The scale of the image was set by importing it into ImageJ, drawing a straight line, and selecting "Analyze" from the toolbar. A dropdown menu appeared, and "Set Scale" was chosen. The program then displayed the scale in pixels. The user would enter the image width (e.g.,720 pixels), a known distance (e.g.,10), and select micron (μm) as the unit. The program automatically calculated the scale as 72 pixels/ μm. Then, from the "Analyze" menu, "Scale Bar" was selected. A scale bar of 10μm was added, with font size 28, black or white color, and the position set to either the top or bottom of the image.

To measure chromosome lengths, a straight line was drawn along each chromosome, then the "Analyze" > "Measure" tool was used, and the chromosome length appeared in micron. After measuring all chromosomes while still inside the cell using ImageJ, the lengths were recorded manually on paper, focusing on the length of each chromosome. This was important because some chromosomes were very similar in shape, size, and centromere position. Therefore, arranging them into pairs was challenging and required precision, focus, and multiple measurements, relying on visual assessment alone was not sufficient.

3.5.4. PicsArt program

The same image, with the same scale, was then transferred to PicsArt program (26.0.5_lite). Several copies of the image were made while adjusting the percentage values of specific tools such as Size, Opacity, and Hardness. Each chromosome was then cropped individually and placed on a white background, which was more suitable for editing. While cropping, attention was given to the presence or absence of secondary constrictions or satellites, and to clearly show the position of the centromere (Fig. 9C).

3.5.5. Ideokar software program

Next, chromosomes that were similar in appearance were arranged in pairs, from the longest to the shortest, according to the lengths measured earlier in imageJ, to construct the karyogram on the PicsArt (Fig. 9C). The karyogram image was then imported into the Ideokar software program (Mirzaghaderi & Marzangi, 2015). The image scale was set to 10 µm using the ruler tool found in the main toolbar of the program.

Each chromosome pair was measured accurately by pressing (Windows & "+") keys simultaneously to zoom in on the chromosome, making it appear clearly. This allowed the researcher to place markers accurately on both ends of the chromosome, determine the centromere position, and mark the presence of satellites or secondary constrictions, if visible. The software displayed live measurement readings. Moreover, the better the image quality, the more accurate the results were.

One of the advantages of Ideokar is that it provides the lengths of the short arm, long arm, centromere position, and total chromosome length. The software also automatically generates an Ideogram after the researcher completes the chromosome measurements. Chromosomes are arranged from largest to smallest in the ideogram. Additionally, this program automatically calculates karyotype parameters without the need for the researcher to use external statistical analysis tools. With its user-friendly interface and built-in scale calibration tools, Ideokar is considered an effective and reliable tool for cytogenetic and chromosomal studies.

Afterward, the chromosomal and karyological measurements were saved in an Excel file, and the mean and standard deviation of the chromosome measurements were calculated using Microsoft Excel 2010.

After nearly three months and 17 days of practical training and continuous work on the same image (Fig. 6A), several programs were tested for image processing and chromosome analysis. These included AutoCAD2022, ImageJ, Adobe Photoshop 2020, and PicsArt. The comparison showed that the best image quality was achieved using PicsArt, as it allowed for improved chromosome clarity and facilitated the individual cropping of each chromosome, with precise control over visual properties such as size, contrast, opacity, and brightness.

The program also helped in clearly identifying the centromere location and verifying the presence or absence of secondary constrictions or satellites, making it the most suitable and reliable tool for processing the remaining images during the study period. Accordingly, PicsArt was adopted as the primary tool for chromosome cropping and karyogram preparation for the rest of the samples used in this research.

The following chromosomal measurements were recorded: chromosome length (CL), arm ratio (AR), Index of relative length of chromosome(IRL%), centromere index (CI%), as well as chromosome type (M/m, sm, st, t, and T) (Table 2 and Table 3). Also, the following karyotype parameters were documented: degree of karyotype asymmetry (A), intrachromosomal asymmetry (A_1), interchromosomal asymmetry (A_2), coefficient of variation of chromosome length (CV_{CL}), coefficient of variation of centromere index (CV_{CI}), Arano index (Ask%), symmetry index (S%), total form percentage of homologous chromosome pairs TF%, and Asymmetry index (AI) (Table 2).

The chromosome type was determined using the centromere position and arm ratio and classified according to Levan *et al.*(1964) (Table 3). The classification of Stebbins (1971) was used to evaluate the degree of karyotype asymmetry (Table 4).

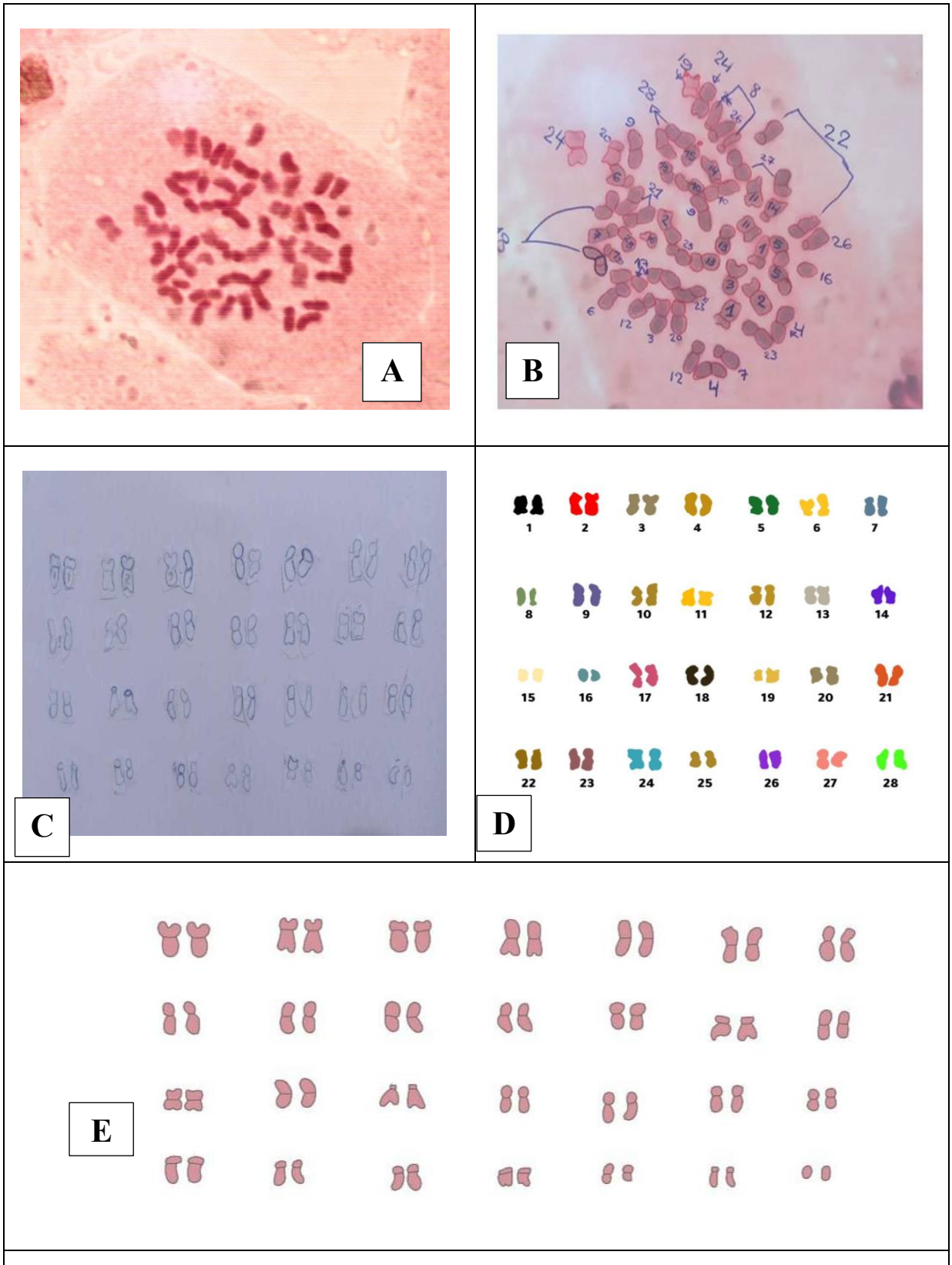


Figure 7. Original metaphase image (A), Numbering of homologous pairs (B), Manual drawing and arrangement (C), Initial AutoCAD 2020 output (D), Final AutoCAD drawing (E).

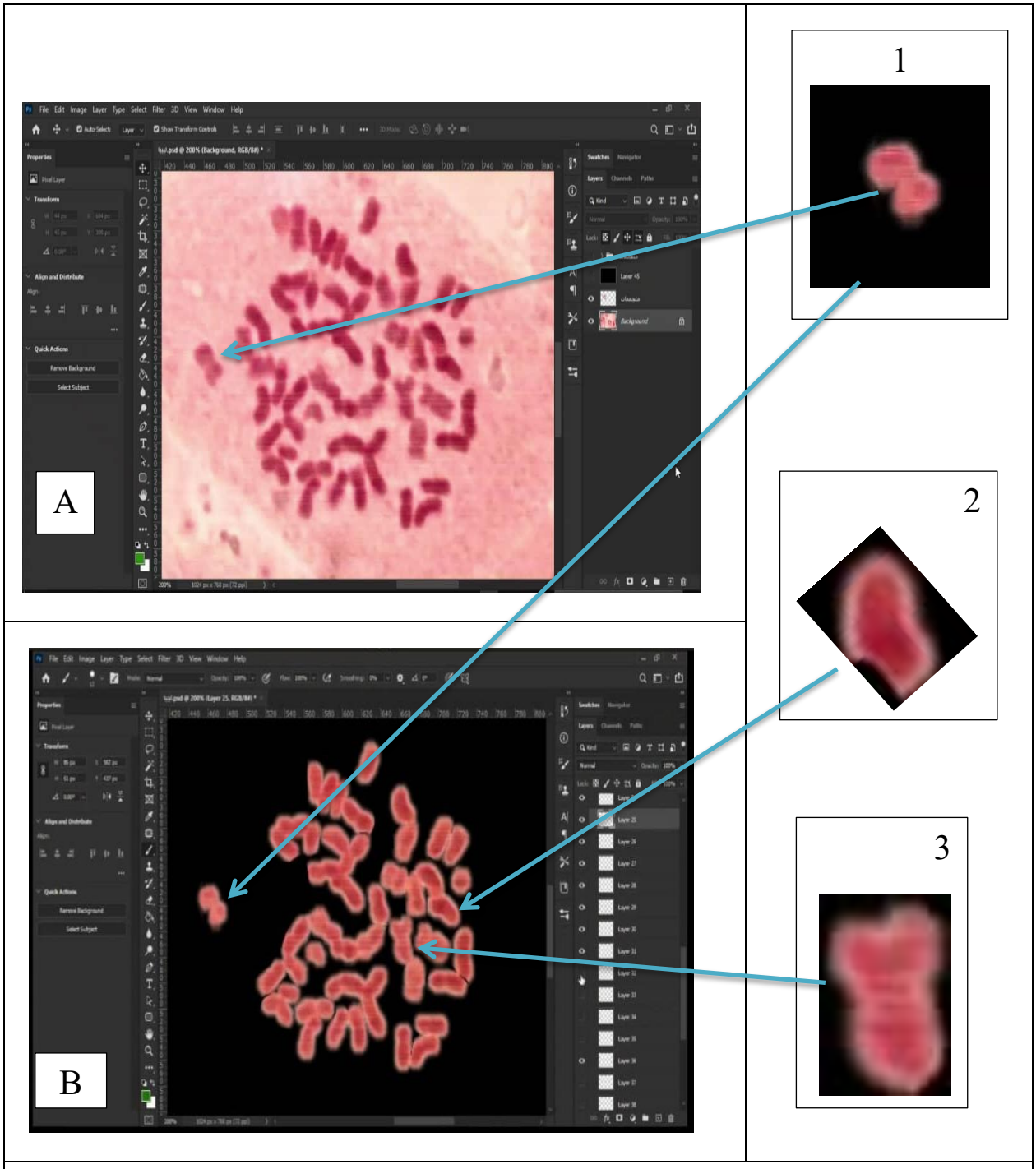
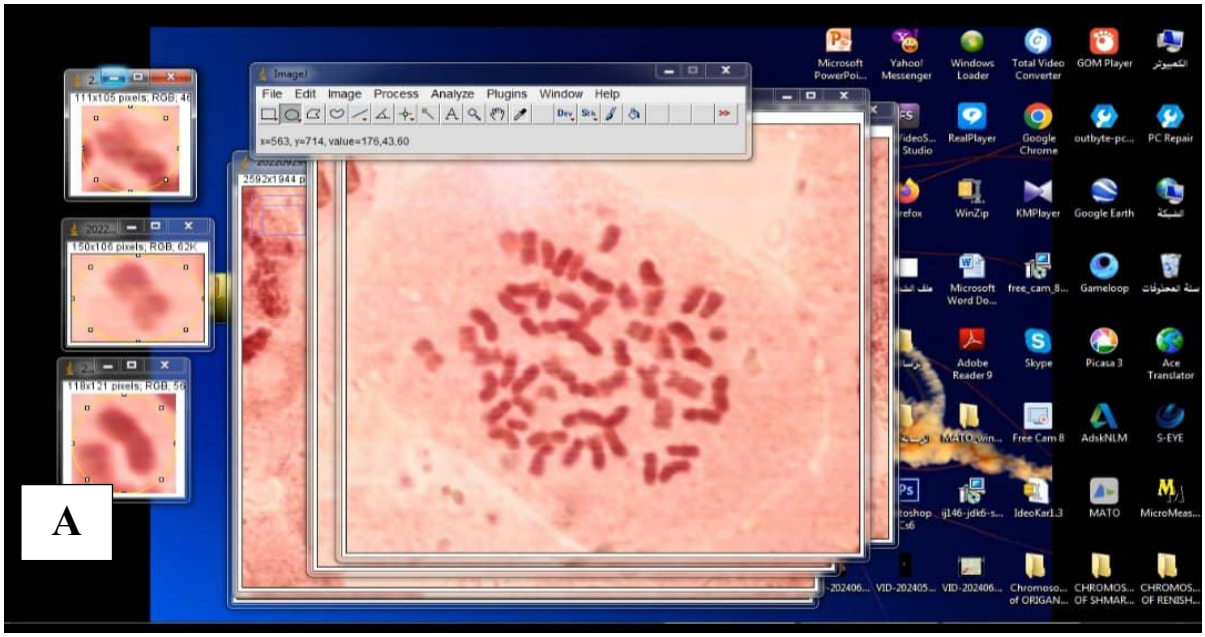
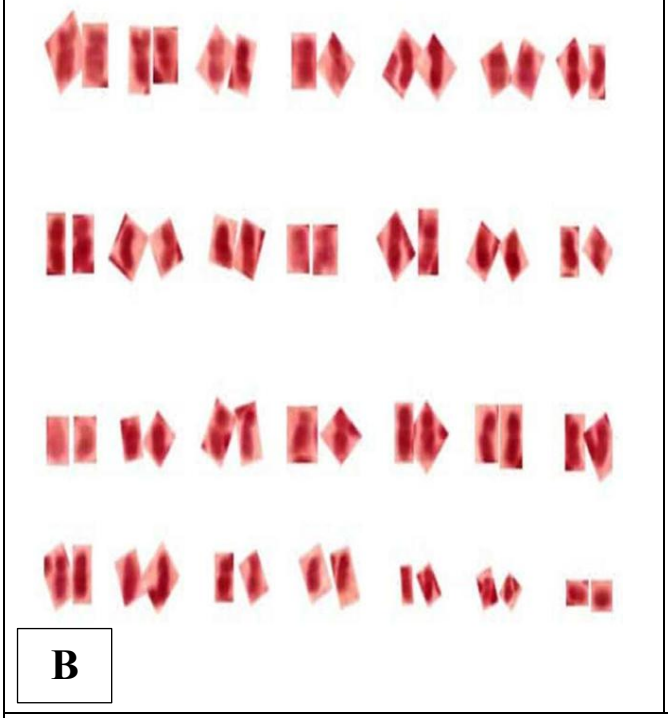


Figure 8. Chromosome cropping issues using Adobe Photoshop 2020: Altered shape of chromosome1 (A & B), Loss of chromosome segments in chromosome 2 (B), and banding removal difficulties in chromosome 3 (B) (all indicated by arrows).





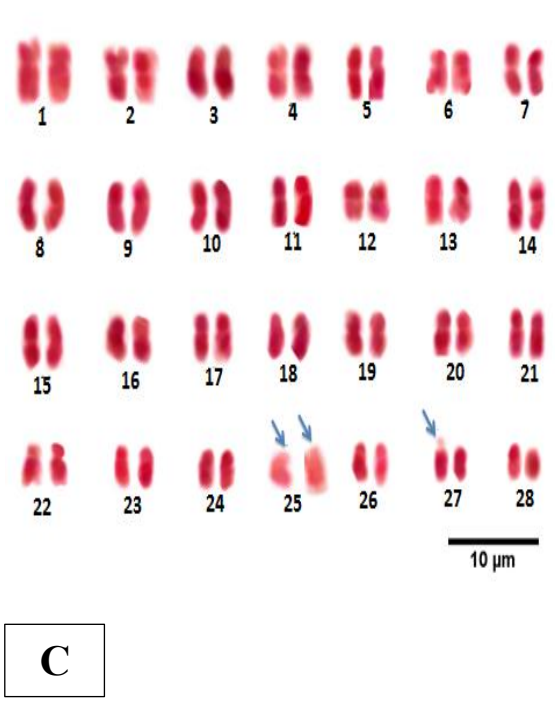


Figure9. Chromosome trimming and separation issues in imageJ (A), Reduced image quality in ImageJ program (B), and PicsArt program (C).

Table 2. Chromosomal and karyotype parameters calculated by Ideokar program.

Chromosomal and karyotype parameters	Formula	Reference
Arm ratio	$\mathrm{AR} = \mathrm{L}/\mathrm{S}$	(Levan *et al*.,1964)
Chromosome length	$\mathrm{CL} = \mathrm{L} + \mathrm{S}$	
Index of relative length of chromosome	$\mathrm{IRL\%} = \left[\mathrm{CL}/\sum \mathrm{CL}\right] \times 100$	
Centromere index	$\mathrm{CI\%} = \mathrm{S}/\mathrm{CL} \times 100$	(Levan *et al*.,1964)
Total form percentage of homologous chromosome pairs	$\mathrm{TF\%} = \left(\sum \mathrm{S} / \sum \mathrm{CL}\right) \times 100$	(Huziwara,1962)
Arano index of karyotype asymmetry	$\mathrm{Ask\%} = \left(\sum \mathrm{L} / \sum \mathrm{CL}\right) \times 100$	(Arano,1963)
Chromosome type	Defined terms in Table 3	(Levan *et al*.,1964)
Stebbins asymmetry classification	$\mathrm{A} - \mathrm{C}, 1 - 4$ (see Table 4)	(Stebbins,1971)
Intrachromosomal asymmetry index	$\mathrm{A}_1 = \left[\sum_{i=1}^{n} (S_i/L_i)/n\right]$	(Romero-Zarco,1986)
Interchromosomal asymmetry index	$\mathrm{A}_2 = \mathrm{S}_{\mathrm{CL}}/\mathrm{X}_{\mathrm{CL}}$	(Romero-Zarco,1986)
Symmetry index	$\mathrm{S\%} = (\mathrm{CL}_{\min}/\mathrm{CL}_{\max}) \times 100$	(Watanabe *et al*.,1999)
Degree of karyotype asymmetry	$\mathrm{A} = \left[\sum_{i=1}^{n} (l_i - S_i)/(L_i + S_i)\right]/n$	(Watanabe *et al*.,1999)
Coefficient of variation of chromosome length (a measure of interchromosomal asymmetry)	$\mathrm{CV}_{\mathrm{CL}} = (\mathrm{S}_{\mathrm{CL}}/\mathrm{X}_{\mathrm{CL}}) \times 100 = \mathrm{A}_2 \times 100$	(Paszko,2006)
Coefficient of variation of the centromere index (a measure of centromere position heterogeneity in the karyotype)	$\mathrm{CV}_{\mathrm{CI}} = (\mathrm{S}_{\mathrm{CI}} / \mathrm{X}_{\mathrm{CI}})/100$	(Paszko,2006)
Asymmetry index	$\mathrm{AI} = (\mathrm{CV}_{\mathrm{CL}} \times \mathrm{CV}_{\mathrm{CL}})/100$	(Paszko,2006)

Table 3. Classifying chromosome types according to their arm ratio.

Term	Location	AR(arm ratio)	CI×100
M	Median point	(1.00-1.04)	50-49
m	median region	(1.05-1.67)	48-39.5
sm	submedian region	(1.7-3.00)	39.4-25
st	subterminal region	(3.01-7.00)	25-12.5
t	terminal region	(7.00-39)	12.5-2.5
T	Terminal point	∞	0

After Levan *et al*. (1964)

Table 4. Classification of karyotype asymmetry degrees.

Ratio	Proportion of chromosomes with arm ratio< 2:1			
Largest / Smallest	1.00 (1)	0.99-0.50 (2)	0.51-0.01 (3)	0.00 (4)
<2:1 (A)	1A	2A	3A	4A
2:1-4:1 (B)	1B	2B	3B	4B
>4:1 (C)	1C	2C	3C	4C

After Stebbins (1971)

4. Results

The number, size, and asymmetry of chromosomes are important parameters to elucidate the phylogenetic relationships of species (Eroğlu *et al*., 2013; Peruzzi & Altinordu, 2014). Chromosomal asymmetry indices have been widely used in interpreting and classifying plant karyotypes (Muliawati *et al*., 2023).

This study provides new cytological information on three endemic plants at El-Jabal El-Akhdar (*Arum cyrenaicum*, *Arbutus pavarii*, and *Origanum cyrenaicum*). The chromosome numbers and karyotypes analysis of all obtained species are shown in Tables 5-11 and Figures 10-20.

4.1 *Arum cyrenaicum* Hruby

In *A. cyrenaicum* is tetraploidy with a basic chromosome number of $x = 14$, having $2n = 4x = 56$ (Fig. 10), and the ideogram is shown in (Fig. 11). The karyotype analysis of this species revealed the existence of three distinct types of chromosomes, with the metacentric (M-m) type being more frequent than the submetacentric (sm) and subtelocentric (st) types. The karyotype formula (KF) includes 22 M-m pairs, 5 sm pairs, and 1 st pair. The formula was 6M + 38m + 10sm + 2st (2SAT). To the best of the researcher's knowledge, this is the first study to document the karyotype of *Arum cyrenaicum*, which is considered an endemic species of El-Jabal El-Akhdar.

The analysis of 28 chromosomes from *A. cyrenaicum* revealed significant variation in the arm lengths and their ratios. The average length of the short arm (S) was 1.62 μm, while the average length of the long arm (L) was 2.20 μm, resulting in an average total chromosome length of 3.82 μm. The S/L ratio, which indicates the degree of symmetry between the two arms, ranged from 0.28 (chromosome 18) to 1.000 (chromosomes 2), with an overall average of 0.74.

Further analysis using the $(L - S)/(L + S)$ index, which reflects the relative difference between arm lengths, revealed a mean value of 0.15, reinforcing the presence of moderate arm length; a symmetry in most chromosomes. The $(L - S)/L$ index also averaged 0.24, which similarly supports this observation.

Out of the 28 chromosomes, three chromosomes (2, 4 and 10) showed perfect symmetry ($S \approx L$), classifying them as metacentric(M). In contrast, several chromosomes, such as 3, 7, 18, 23, 25, and 26, had noticeably low S/L ratios (below 0.65),

indicating submetacentric to telocentric configurations. Chromosome 18 was identified as the most asymmetrical, with an S/L ratio of 0.28 and a (L − S)/(L + S) value of 0.56 (Table 6).

The karyotype of this species is classified as a symmetry type 1A according to Stebbins classification. The average value of the intrachromosomal asymmetry index (A_1) and the average value of the interchromosomal asymmetry index (A_2) for *A.cyrenaicum* are 0.26 and 0.19, respectively. The value of the coefficient of variation of chromosome length (CV_{CL} = 19.31) and the value of the coefficient of variation of the centromere index (CV_{CI} = 5.82). The values of the asymmetry index (AI) = 1.12, Arano asymmetry index (Ask%) = 57.46, and the degree of karyotype asymmetry (A) = 0.15, the values of the total symmetry index (S%) = 61.01, and the total form percentage of homologous chromosome pairs (TF%) = 42.31 (Table 5) (Fig.12).

The relative length of the chromosomes, indicated by the index of relative length (IRL%), ranged from 2.53% to 4.29%. The centromere index (CI%) ranged from 21.94% to 50.00%, with an average centromere position of 43.68%. The arm ratio (AR) ranged from 1.00 to 3.55, with an average arm ratio of 1.42 (Table 7).

Table 5. Karyomorphometric data for the studied plant taxa

Plant Taxa	Ch. No (2n)	Karyotype Formula	Ch. Size variation (µm)	THCL	CVci	Interchromosomal Index		Intrachromosomal Index				Symmetry Index S%	Asymmetry Index AI
						CVcl	A_2	TF%	Ask%	A	A1		
A. cyrenaicum	56	6M+38m+10sm+2S	2.88 – 4.72	107.21 ±1.51	5.82 ±.45	13.55 ±.195	0.19 ±1.62	42.31 ±.56	56.131 ±.091	0.15 ±.39	0.26 ±.40	61.01 ±.19	1.12 ±.00
A. pavarii	26	4M+16m+6sm+4S	1.98 – 3.09	33.2 ±.43	4.53 ±.318	17.36 ±.09	0.17 ±.00	42.59 ±1.27	57.46 ±1.47	0.15 ±.95	0.25 ±.03	64.38 ±.80	0.79 ±.05
O. cyrenaicum	30	6M+16m+8sm+3S	1.98 - 3.19	39.56 ±.72	5.58 ±.04	22.48 ±.00	0.22 ±.00	42.49 ±.51	57.529 ±.405	0.15 ±.01	0.25 ±.03	62.07 ±.90	1.25 ±.01

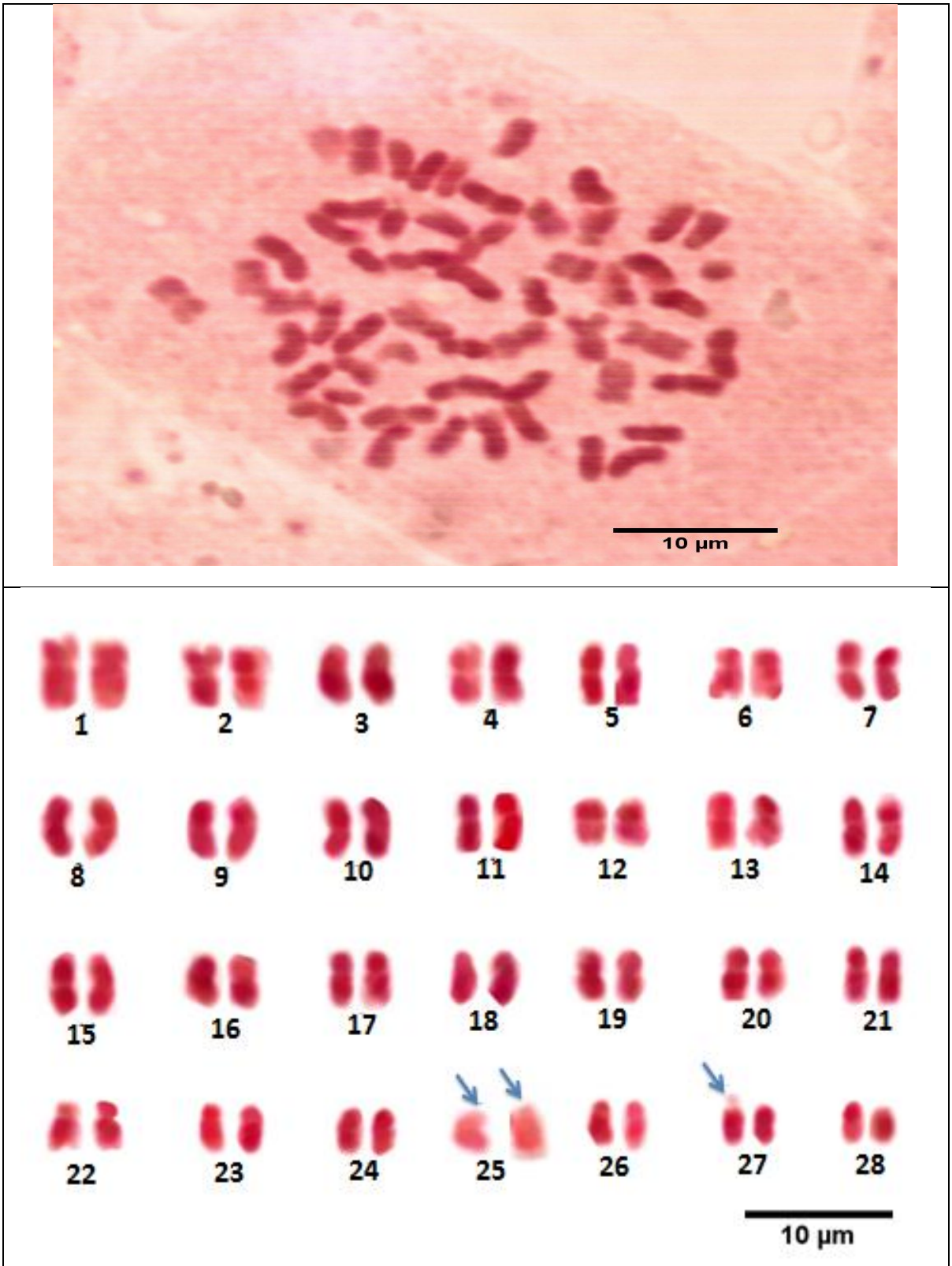


Figure 10. Metaphase chromosomes spread (A), and karyogram of *Arum cyrenaicum* (B)

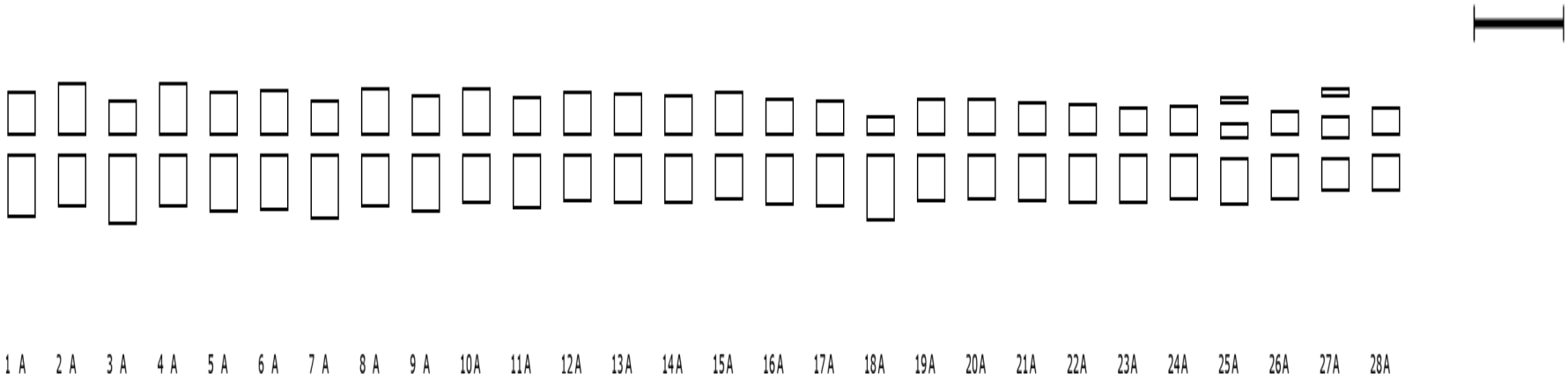


Figure 11. Ideogram of *Arum cyrenaicum*

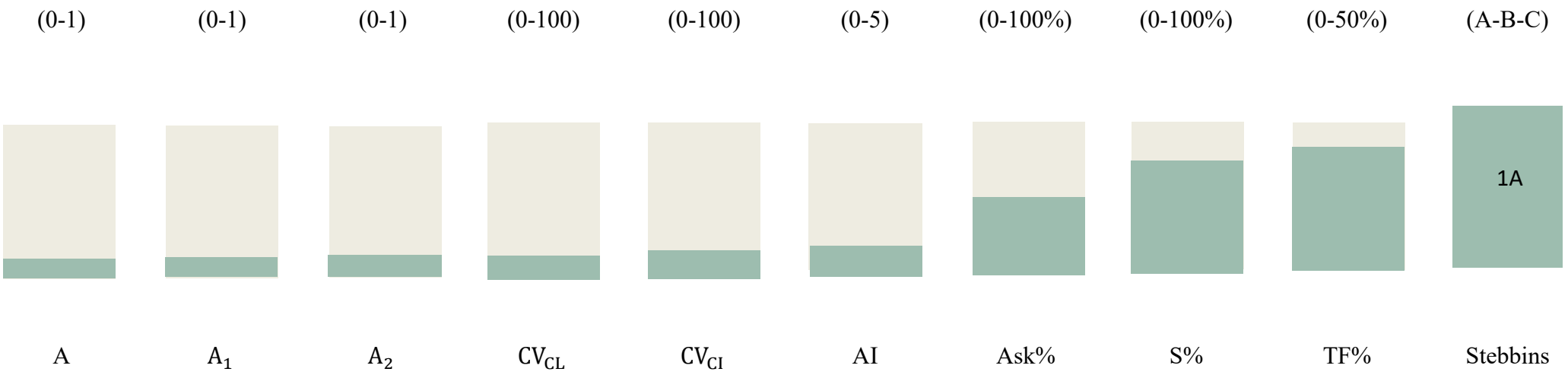


Figure 12. Karyotype parameters of *Arum cyrenaicum*

Table 6. Estimators on a set of 28 chromosomes of *Arum cyrenaicum*

Chromosome No.	S (µm)	L (µm)	(L+S)	S/L	(L-S)	S/(L+S)	L/(L+S)	(L-S)/L	(L-S)/(L+S)
1	1.93±.05	2.79±.16	4.72±.21	0.69±.31	0.86±.11	0.40±.23	0.59±.76	0.3±.68	0.18±.52
2	2.27±.03	2.27±.03	4.54±.06	1.00±1.00	0.00±0.00	0.5±.5	0.5±.5	0.00±0.00	0.00±.00
3	1.53±.04	2.99±.05	4.52±.09	0.51±.8	1.46±.01	0.33±.4	0.66±.5	0.48±.2	0.32±.11
4	2.19±.02	2.25±.06	4.44±.08	0.97±.3	0.06±.04	0.49±.25	0.5±.75	0.01±.67	0.01±.5
5	1.91±.02	2.50±.05	4.41±.08	0.76±.4	0.59±.03	0.43±.25	0.56±.62	0.23±.6	0.13±.37
6	1.97±.06	2.41±.07	4.38±.13	0.81±.85	0.44±.01	0.44±.46	0.55±.53	0.18±.14	0.1±.07
7	1.53±.01	2.77±.02	4.30±.01	0.55±.5	1.24±.01	0.35±1.00	0.64±2.00	0.44±.5	0.28±1.00
8	2.02±.02	2.23±.03	4.25±.01	0.9±.67	0.21±.01	0.47±2.00	0.52±3.00	0.09±.3	0.04±1.00
9	1.73±.02	2.46±.05	4.19±.03	0.7±.4	0.73±.03	0.41±0.67	0.58±1.67	0.29±.6	0.17±1.00
10	2.01±.01	2.08±.02	4.09±.03	0.96±.5	0.07±.01	0.49±.03	0.5±.67	0.03±.5	0.01±.3

Table 6. (Continued)

Chromosome No.	S (µm)	L (µm)	(L+S)	S/L	(L-S)	S/(L+S)	L/(L+S)	(L-S)/L	(L-S)/(L+S)
11	1.64±.00	2.37±.07	4.01±.06	0.69±.00	0.73±.07	0.4±.00	0.59±1.17	0.3±1.00	0.18±1.16
12	1.91±.02	2.02±.02	3.93±.01	0.94±1.00	0.11±.00	0.48±2.00	0.51±2.00	0.05±.00	0.02±.00
13	1.83±.01	2.08±.01	3.91±.03	0.87±1.00	0.25±.00	0.46±.3	0.53±.3	0.12±.00	0.06±.00
14	1.71±.05	2.16±.07	3.87±.02	0.79±.71	0.45±.02	0.44±2.5	0.55±3.5	0.2±.28	0.11±1.00
15	1.87±.04	1.97±.05	3.84±.02	0.94±.8	0.1±.01	0.48±2.00	0.51±2.5	0.05±.2	0.02±.5
16	1.58±.03	2.19±.03	3.77±.01	0.72±1.00	0.61±.00	0.41±3.00	0.58±3.00	0.27±.00	0.16±.00
17	1.55±.06	2.17±.06	3.72±.01	0.71±1.00	0.62±.00	0.41±6.00	0.58±6.00	0.28±.00	0.17±.00
18	0.79±.03	2.81±.03	3.60±.05	0.28±1.00	2.02±.00	0.21±.6	0.78±.6	0.71±.00	0.56±.00
19	1.52±.06	2.04±.06	3.56±.01	0.74±1.00	0.52±.00	0.42±6.00	0.57±6.00	0.25±.00	0.14±.00

Table 6. (Continued)

Chromosome No.	S (µm)	L (µm)	(L+S)	S/L	(L-S)	S/(L+S)	L/(L+S)	(L-S)/L	(L-S)/(L+S)
20	1.54±.03	1.99±.03	3.53±.01	0.77±1.00	0.45±.00	0.43±3.00	0.56±3.00	0.22±.00	0.12±.00
21	1.51±.06	1.95±.11	3.46±.04	0.77±.54	0.44±.05	0.43±1.5	0.56±2.75	0.22±.45	0.12±1.25
22	1.37±.02	2.04±.05	3.41±.02	0.67±.4	0.67±.03	0.4±1.00	0.59±2.5	0.32±.6	0.19±1.5
23	1.24±.02	2.12±.02	3.36±.01	0.58±1.00	0.88±.00	0.36±2.00	0.63±2.00	0.41±.00	0.26±.00
24	1.29±.01	1.97±.01	3.26±.02	0.65±1.00	0.68±.00	0.39±.5	0.6±.5	0.34±.00	0.2±.00
25	1.11±.05	2.04±.06	3.15±.12	0.54±.83	0.93±.01	0.35±.41	0.64±.5	0.45±.17	0.29±.08
26	1.08±.02	2.01±.11	3.09±.14	0.53±.18	0.93±.09	0.34±.14	0.65±.78	0.46±.81	0.3±.64
27	1.41±.03	1.61±.15	3.02±.18	0.87±.2	0.2±.12	0.46±.17	0.53±.83	0.12±.8	0.06±.67
28	1.34±.02	1.54±.03	2.88±.04	0.87±.67	0.2±.01	0.46±.5	0.53±.75	0.12±.3	0.06±.25
Average	1.62±.03	2.20±.05	3.82±.05	0.74±0.64	0.58±.02	0.42±1.3	0.57±1.77	0.24±.31	0.15±.42

Table 7. Chromosome measurements of *Arum cyrenaicum* (mean ± SD)

Chromosome No.	**Relative length%** **(CL/ ΣCL) *100**	**Arm ratio L/S**	**Centromere index%** **S/(S+L)* 100**	**Chromosome type**
1	4.40 ±.15	1.44 ±.04	40.88 ±.15	m
2	4.23 ±.01	1.00 ±.00	50.00 ±.00	M
3	4.21 ±.03	1.96 ±.02	33.84 ±.23	sm
4	4.04 ±.03	1.03 ±.01	49.32 ±.35	M
5	4.11 ±.02	1.31 ±.01	43.31 ±.22	m
6	4.08 ±.07	1.22 ±.00	44.97 ±.03	m
7	4.01 ±.04	1.81 ±.03	35.58 ±.42	sm
8	3.96 ±.04	1.10 ±.01	47.52 ±.55	m
9	3.91 ±.07	1.42 ±.01	41.28 ±.25	m
10	3.81 ±.00	1.03 ±.00	49.14 ±.06	M
11	3.74 ±.02	1.44 ±.04	40.92 ±.73	m
12	3.66 ±.04	1.05 ±.03	48.60 ±.59	m
13	3.64 ±.02	1.13 ±.00	46.80 ±.06	m
14	3.61 ±.02	1.26 ±.08	44.18 ±1.56	m
15	3.58 ±.09	1.05 ±.05	48.69 ±1.17	m

Table 7 (Continued)

Chromosome No.	Relative length% (CL/ ΣCL) *100	Arm ratio L/S	Centromere index% S/(S+L)* 100	Chromosome type
16	3.52 ±.05	1.38 ±.05	41.90 ±.87	m
17	3.47 ±.1	1.40 ±.10	41.67 ±1.74	m
18	3.35 ±.09	3.55 ±.08	21.94 ±.41	St
19	3.32 ±.04	1.34 ±.10	42.69 ±1.85	m
20	3.29 ±.04	1.29 ±.05	43.62 ±1.00	m
21	3.23 ±.07	1.30 ±.12	43.64 ±2.41	m
22	3.18 ±.05	1.48 ±.06	40.17 ±0.97	m
23	3.13 ±.03	1.70 ±.03	36.90 ±.46	sm
24	3.04 ±.01	1.52 ±.02	39.57 ±.26	m
25	2.93 ±.07	1.66 ±.12	35.23 ±.39	sm
26	2.88 ±.09	1.83 ±.06	34.95 ±.77	sm
27	2.82 ±.14	1.14 ±.08	46.68 ±1.90	m
28	2.68 ±.02	1.10 ±.00	46.52 ±0.07	m
Average	3.56 ±.05	1.42 ±.04	42.16 ±0.69	

4.2 *Arbutus pavarii* Pamp.

In the current study, it was challenging to determine the chromosome number and morphology of *Arbutus pavarii* due to the small size of its chromosomes. Image analysis of Fig. 13A was attempted using ImageJ (Fig. 13B) and Adobe Photoshop 2020 (Fig. 13C), but the image quality was insufficient. However, after enhancing Fig.13A using the PicsArt application, which supports image resolution up to 5000 pixels, the chromosome number and morphology of this species were successfully identified. Furthermore, important chromosomal features were revealed, such as the presence of a satellite on the short arm of chromosome1 and another satellite on the long arm of chromosome 2, as shown in Fig. 13D.

The diploid chromosome number ($2n$ = 26) (Fig. 14) was observed in all the investigated population of *A. pavarii*, with the basic chromosome number of x = 13. These results are consistent with the chromosome numbers reported in previous studies of other *Arbutus* species (Darlington & Wylie, 1955; Sealy & Webb, 1959; Taylor & Taylor, 1977; Martins *et al*., 2022), and the ideogram generated automatically using the Ideokar program based on the basic chromosome number (Fig. 15).

Karyotype analysis of this species revealed the presence of two types of chromosomes (m type and sm type), with a higher frequency of m type compared to sm type. The karyotype formula (KF) includes 10 M-m pairs, and 3 sm pairs, and its formula was $2n$ = $2x$ = 26 = 4M+16m+6sm (4SAT) (Fig. 14).

The analysis of 13 chromosomes from *Arbutus pavarii* showed notable variation in the lengths of the short (S) and long (L) arms, as well as in their respective ratios. The average short arm length was approximately 1.08 μm, while the average long arm length was about 1.46 μm, leading to an average total chromosome length of around 2.55 μm.

The S/L ratio, an indicator of arm length symmetry, ranged from values 0.5 (chromosome 7) to 1.00 (chromosome 8), with an overall mean near 0.75. This suggests a spectrum of chromosomal shapes from highly symmetrical to nearly metacentric. Using the (L−S)/(L+S) index to assess arm length disparity, the mean value was around 0.15, indicating moderate symmetry across the chromosomes. The related (L−S)/L index averaged about 0.24, further confirming this trend.

Among the 13 chromosomes, several, such as chromosomes 8 and 13, showed near-equal arm lengths (S ≈ L), consistent with metacentric morphology. Conversely, chromosomes like 3 , 7 and 11 displayed significantly lower S/L ratios (<0.53), reflecting submetacentric types. (Table 8).

The karyotype of this species is classified as 1A type in Stebbins' symmetry classification, and the coefficient of variation of chromosome length (CV_{CL} = 17.36), while the coefficient of variation of centromere index (CV_{CI} = 4.53). The symmetry index (S%) = 64.38, and the total form percentage of homologous chromosome pairs (TF%) = 42.59, while the Arano asymmetry karyotype index (Ask%) = 57.46, the degree of karyotype asymmetry (A)=0.15, intrachromosomal asymmetry index (A_1)=0.25, interchromosomal asymmetry index (A_2)= 0.17, and asymmetry index (AI)= 0.79 (Table 5) (Fig. 16).

The index of chromosome relative length (IRL%) ranged from 5.96 to 9.34%, with an average of 7.69%. The centromere index (CI%) ranged from 33.2 to 50.00 %, with an average centromere position of 42.69%. The arm ratio (AR) ranged in *A.pavarii* from 1.00 to 2.01, with an average of 1.39 (Table 9). The total haploid length of chromosome complement (THCL) of *A.pavarii* = 33.2µm, and the division of the total length into long arms (L) and short arms (S) (14.14µm and 19.08µm, respectively).

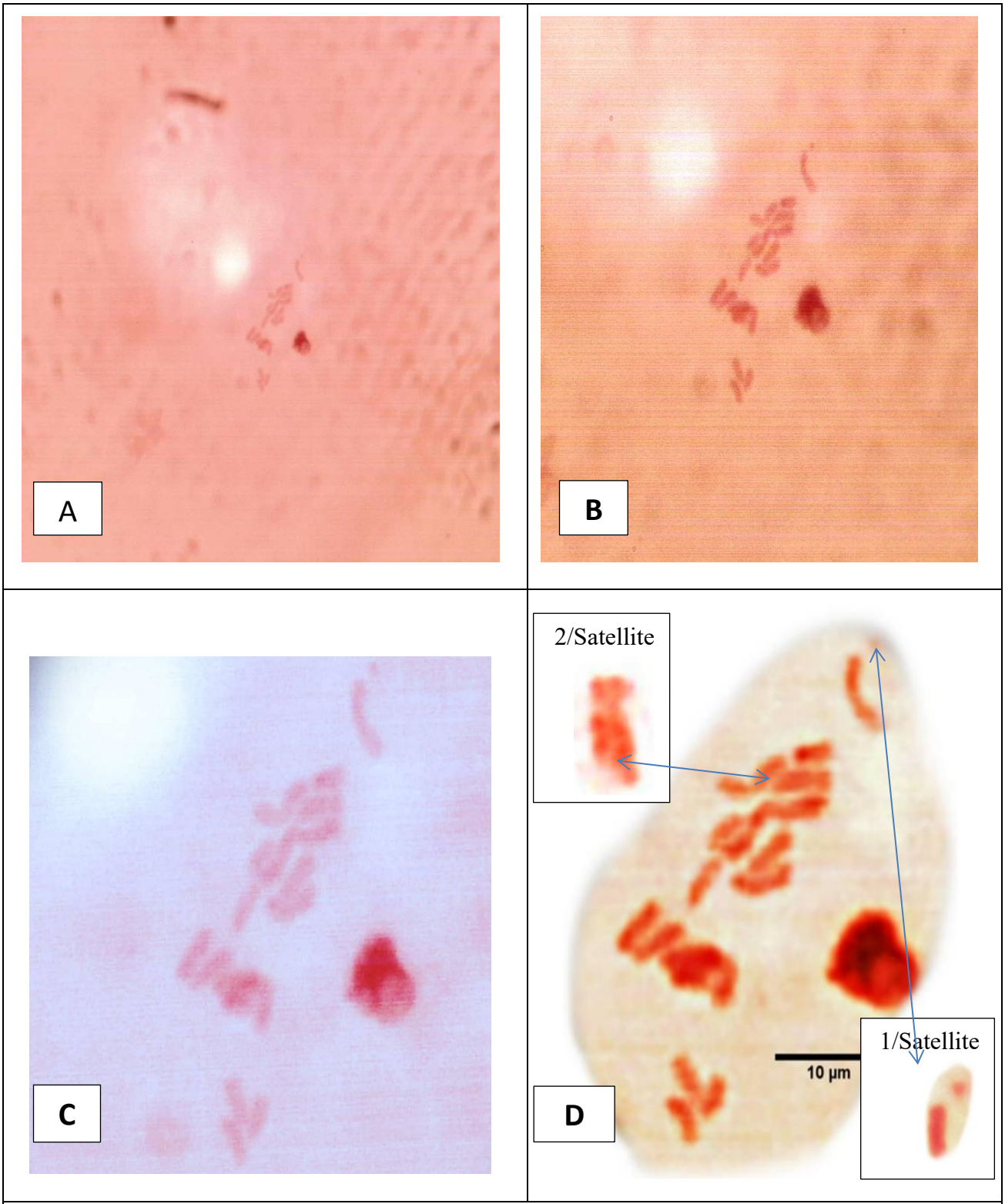


Figure 13. Original metaphase chromosome image of *Arbutus pavarii* (A), Image processed with ImageJ software (B), Image enhanced using Adobe Photoshop 2020(C), High-resolution image (PicsArt) showing satellites on chromosomes 1 and 2(D).

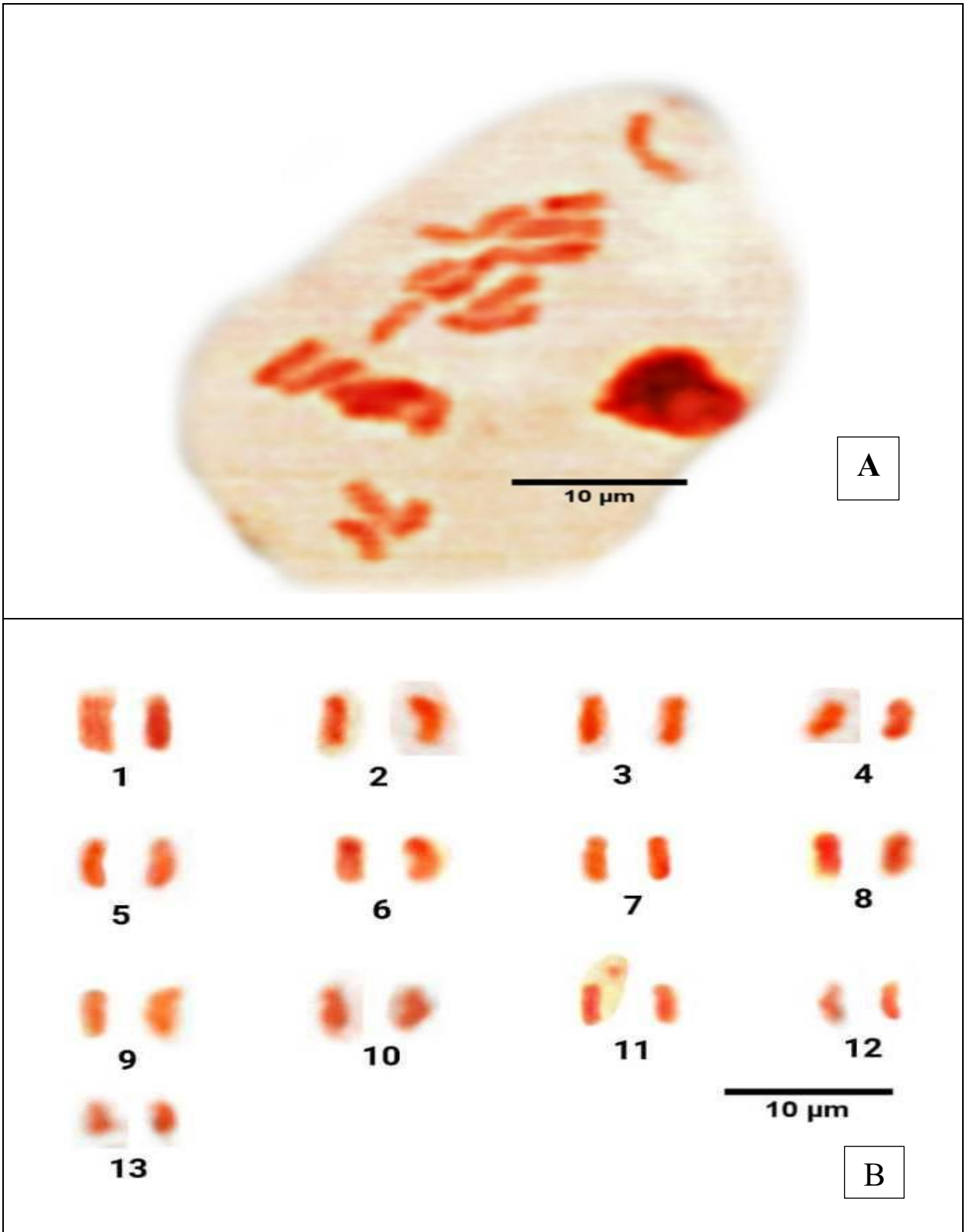


Figure 14. Metaphase chromosomes spread (A), and karyogram of *Arbutus pavarii* (B).

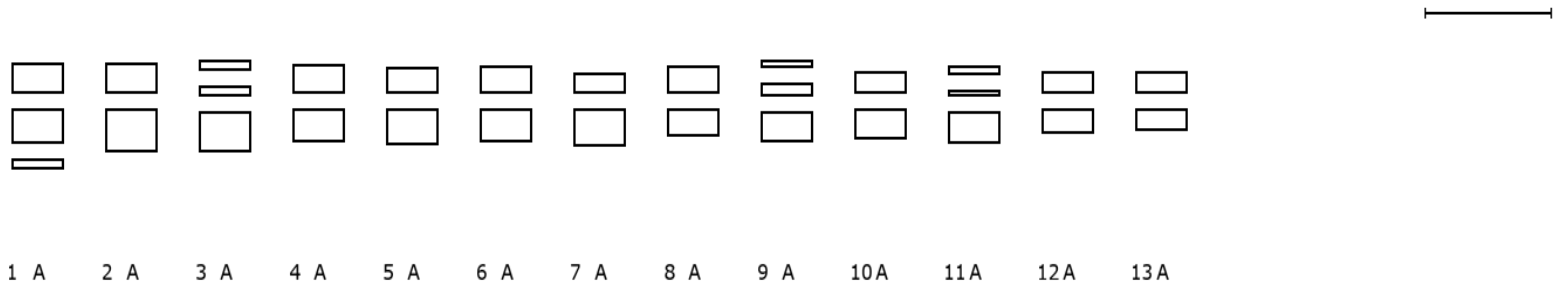


Figure 15. Ideogram of *Arbutus pavarii*

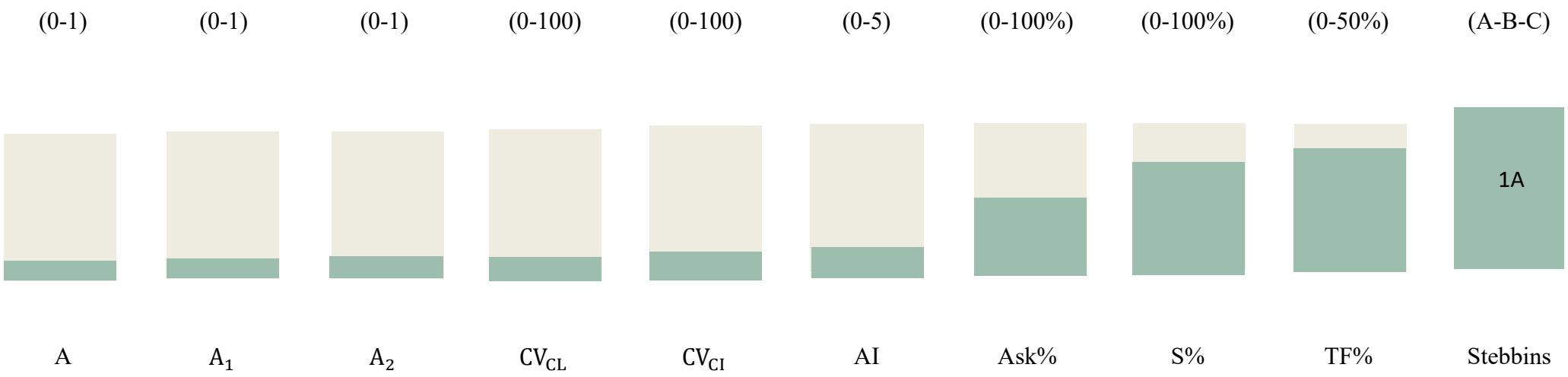


Figure 16. Karyotype parameters of *Arbutus pavarii*

Table 8. Estimators on a set of 13 chromosomes of *Arbutus pavarii*

Chromosome No.	S (µm)	L (µm)	(L+S)	S/L	(L-S)	S/(L+S)	L/(L+S)	(L-S)/L	(L-S)/(L+S)
1	1.36±.11	1.73±.11	3.09±.03	0.79±1.00	0.37±.00	0.44±3.67	0.56±3.67	0.21±.00	0.12±.00
2	1.36±.02	1.65±.04	3.01±.03	0.82±.5	0.29±.02	0.45±.67	0.55±1.33	0.18±.5	0.09±.67
3	1.02±.02	1.91±.02	2.92±.03	0.53±1.00	0.89±.00	0.35±.67	0.65±.67	0.47±.00	0.3±.00
4	1.29±.05	1.55±.05	2.84±.03	0.83±1.00	0.26±.00	0.45±1.67	0.55±1.67	0.17±.00	0.09±.00
5	1.13±.04	1.57±.04	2.70 ±.04	0.72±1.00	0.46±.00	0.42±1.00	0.58±1.00	0.29±.00	0.17±.00
6	1.19±.09	1.43±.10	2.62±.03	0.83±.9	0.24±.01	0.45±3.00	0.55±3.33	0.17±.1	0.09±.3
7	0.85±.05	1.71±.06	2.55±.05	0.5±.83	0.86±.01	0.33±1.00	0.67±1.2	0.5±.17	0.34±.2
8	1.24±.02	1.24±.02	2.48±.04	1.00±1.00	0.00±.00	0.5±.5	0.5±.5	0.00±.00	0.00±.00
9	1.02±.05	1.36±.05	2.38±.04	0.64±1.00	0.34±.00	0.43±1.25	0.57±1.25	0.25±.00	0.14±.00
10	0.95±.03	1.34±.03	2.29±.03	0.71±1.00	0.39±.00	0.41±1.00	0.59±1.00	0.29±.00	0.17±.00
11	0.76±.01	1.47±.03	2.23±.04	0.52±.3	0.71±.02	0.34±.25	0.66±.75	0.48±.67	0.32±.5
12	0.99±.03	1.12±.03	2.11±.04	0.88±1.00	0.13±.00	0.47±.75	0.53±.75	0.12±.00	0.06±.00
13	0.98±.03	1.00±.03	1.98±.03	0.98±3.00	0.02±.00	0.49±1.00	0.51±1.00	0.02±.00	0.01±.00
Average	1.08±.04	1.46±.05	2.55±.03	0.75±1.04	0.38±.00	0.42 ±1.26	0.57±1.39	0.24±.11	0.15±.13

Table9. Chromosome measurements of *Arbutus pavarii* (mean ± SD)

Chromosome No.	**Relative length%** **(CL/ ΣCL) *100**	**Arm ratio L/S**	**Centromere index%** **S/(S+L)*100**	**Chromosome type**
1	9.34 ±.41	1.26 ±.17	44.19 ±3.47	m
2	9.06 ±.06	1.21 ±.05	45.18 ±.91	m
3	8.81 ±.13	1.87 ±.06	34.93 ±.75	sm
4	8.55 ±.13	1.20 ±.08	45.42 ±1.68	m
5	8.07 ±.06	1.41 ±.09	41.85 ±1.69	m
6	7.89 ±.06	1.20 ±.19	45.42 ±3.73	m
7	7.71 ±.09	2.01 ±.18	33.2 ±1.87	sm
8	7.46 ±.11	1.00 ±.00	50.00 ±.00	M
9	7.17 ±.08	1.33 ±.09	42.86 ±1.72	m
10	6.91 ±.02	1.41 ±.08	41.48 ±1.31	m
11	6.72 ±.07	1.93 ±.03	34.08 ±.35	sm
12	6.36 ±.06	1.13 ±.04	46.92 ±.9	m
13	5.96 ±.07	1.02 ±.04	49.49 ±0.81	M
Average	7.69 ±.00	1.39 ±.07	42.69 ±.82	

4.3 *Origanum cyrenaicum* Bég. & Vacc.

In the current study, key chromosomal features were identified in the karyotype of *O. cyrenaicum* through detailed visual analysis using the "highlight" tool available in the PicsArt application. A secondary constriction was detected on the long arm of chromosome 1, while satellites were observed on the long arms of chromosomes 2. Chromosome 3 exhibited both a satellite on the short arm and a secondary constriction on the long arm. Additionally, the position of the centromere in chromosomes 4 and 5 was difficult to determine due to limited visibility (Fig. 17).

A diploid chromosome number ($2n$ = 30) was consistently observed in all cytological preparations of *O. cyrenaicum* examined in this study (Fig. 18), with a basic chromosome number of x = 15.

These findings are consistent with chromosome numbers previously reported in other *Origanum* species (Lepper, 1970; Von Bothmer, 1970; Gill, 1981; Fernandes & Leitão, 1984; Magulaev, 1984; Ayyangar & Vembu, 1985; Pastor *et al*., 1990; Khatoon & Ali, 1993; Bastida & Talavera, 1994; Markova & Goranova, 1995; Dobea *et al*., 1997; Kitiki, 1997; Balim & Kesercioğlu, 1998; Yildiz & Gücel, 2006; Bakha *et al*., 2017; Dirmenci *et al.*, 2018a, 2018b; Dirmenci *et al*., 2019; Martin *et al*., 2020).

The ideogram was generated automatically using the Ideokar program based on the basic chromosome number of x = 15 (Fig. 19). The karyotype of *O.cyrenaicum* consists of 11 M-m pairs and 4 sm pairs, with the KF=2n=2x=30= 6M+16m+8sm(3SAT) (Fig. 18).

The analysis of 15 chromosomes from *Origanum cyrenaicum* revealed significant variation in the arm lengths and their ratios. The average length of the short arm (S) was 1.12 µm, while the average length of the long arm (L) was 1.52 µm, resulting in an average total chromosome length of 2.64 µm. The S/L ratio, which indicates the degree of symmetry between the two arms, ranged from 0.51 (chromosome 4) to 0.98 (chromosomes 14 and 15), with an overall average of 0.76.

Further analysis using the (L − S)/(L + S) index, which reflects the relative difference between arm lengths, revealed a mean value of 0.14, reinforcing the presence of moderate arm length a symmetry in most chromosomes. The (L − S)/L index also averaged 0.02, which similarly supports this observation.

Out of the 15 chromosomes, three chromosomes (7, 14 and 15) showed perfect symmetry (S ≈ L), classifying them as metacentric(M). In contrast, several chromosomes, such as 4, 5, 9 and 11, had noticeably low S/L ratios (below 0.58), indicating submetacentric to telocentric configurations(Table 10).

The coefficient of variation of the centromere index (CV_{CI}) and the coefficient of variation of the chromosome length (CV_{CL}) values was (5.58 and 14.00), respectively. The intrachromosomal asymmetry index (A_1) = 0.25, interchromosomal asymmetry index (A_2) = 0.22, the degree of karyotype asymmetry (A) = 0.15, Arano asymmetry karyotype index (Ask%) = 57.51, asymmetry index (AI) = 1.25, symmetry index (S%) = 62.07, and the total form percentage of the homologous chromosome pairs (TF%) = 42.49. The karyotype of this species is classified as the 1A type in Stebbins' symmetry classification (Table 5) (Fig. 20).

The (IRL%) ranged from 5.01 to 8.06%, with an average value of 6.66%. The total haploid length of the entire set of chromosomes (chromosome complement) (THCL) was 39.56µm. The CI% ranged from 33.46% to 49.54%, with an average of 42.82%. The AR measured was 1.02 to 2.05, with an average of 1.39 (Table 11).

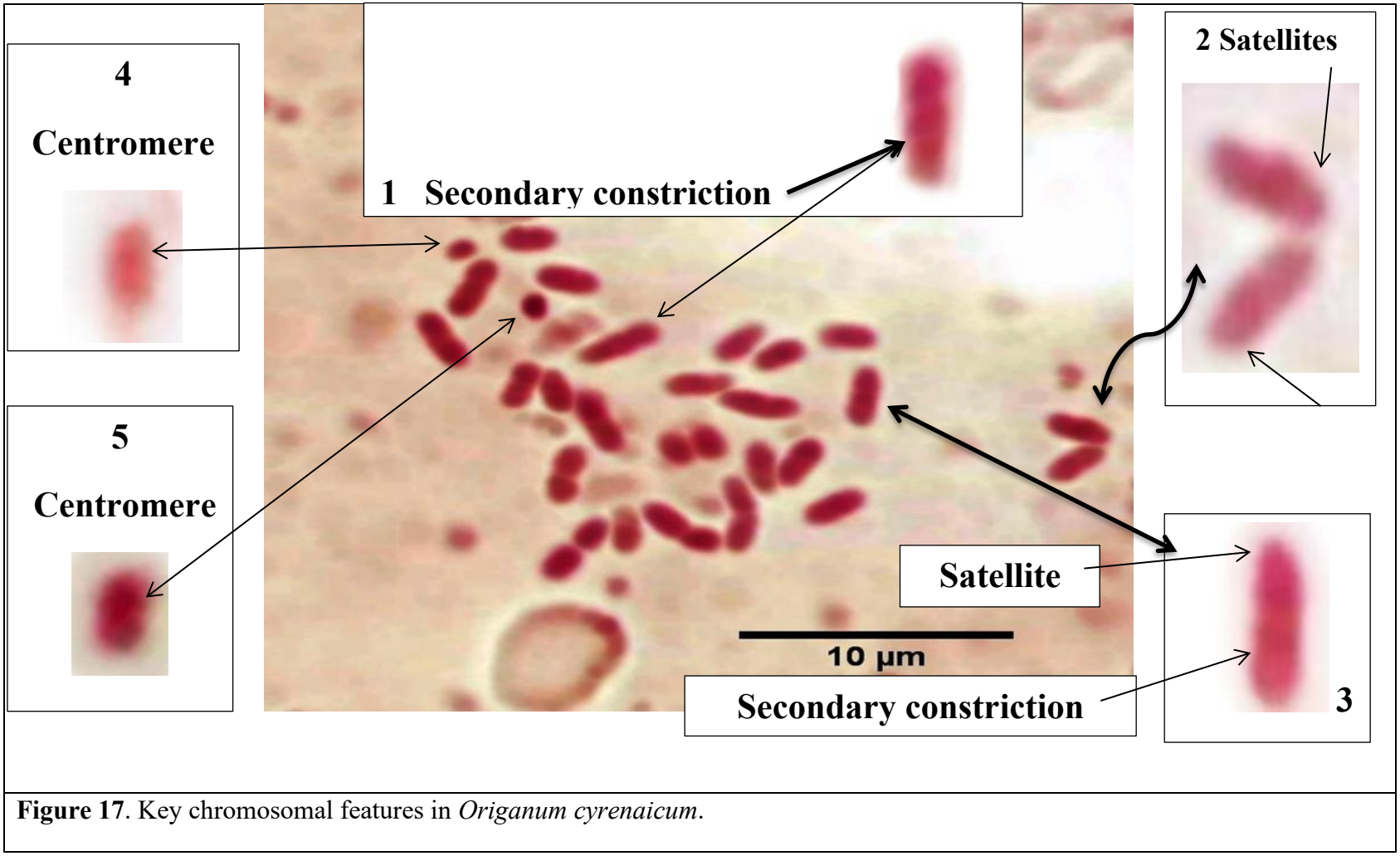
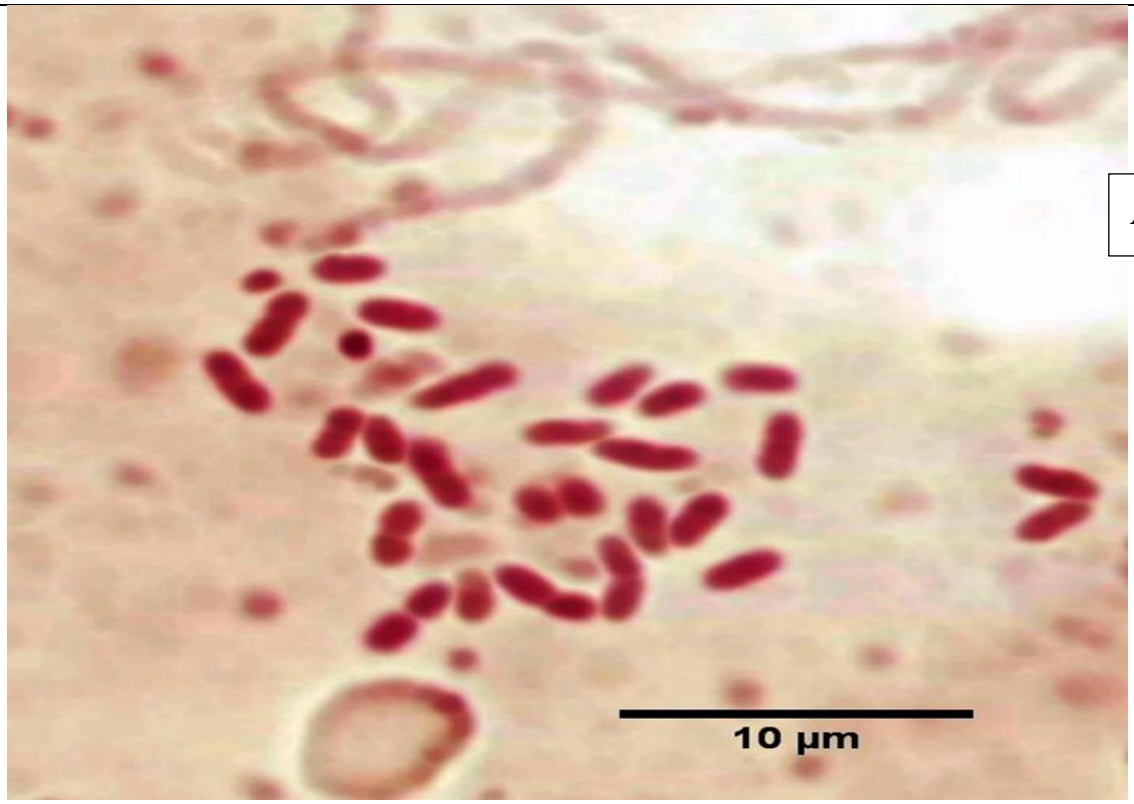


Figure 17. Key chromosomal features in *Origanum cyrenaicum*.



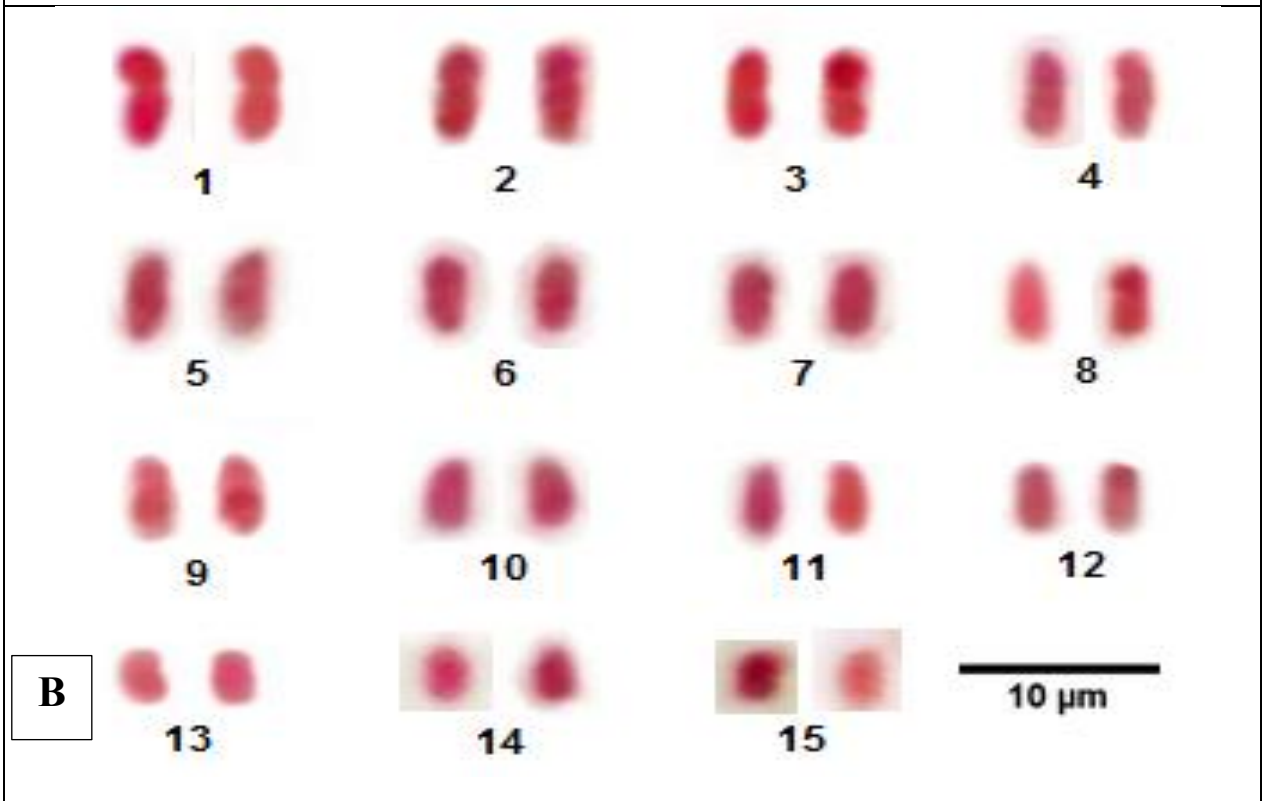


Figure 18. Metaphase chromosomes spread (A), and karyogram of *Origanum cyrenaicum* (B).

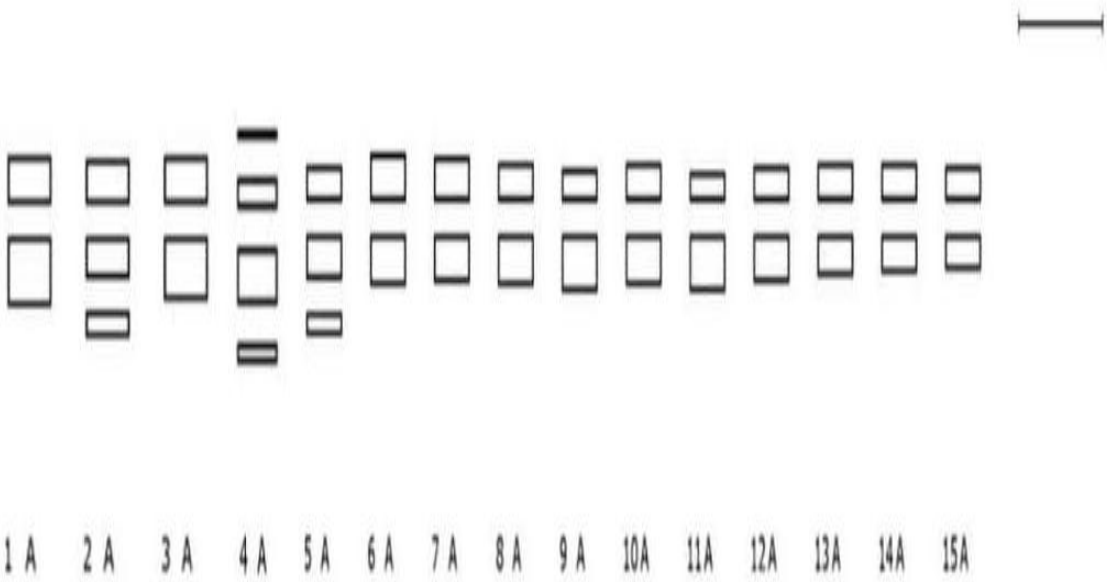


Figure 19. Ideogram of *Origanum cyrenaicum*

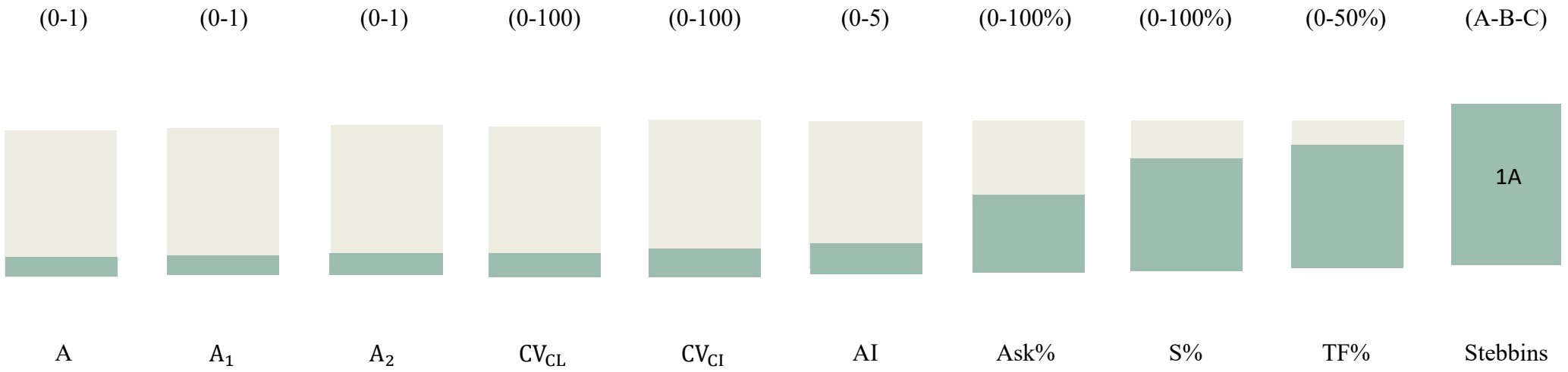


Figure 20. Karyotype parameters of *Origanum cyrenaicum*

Table 10. Estimators on a set of 15 chromosomes of *Origanum cyrenaicum*

Chromosome No.	S (µm)	L (µm)	(L+S)	S/L	(L-S)	S/(L+S)	L/(L+S)	(L-S)/L	(L-S)/(L+S)
1	1.34±.03	1.85±.04	3.19±.01	0.72±.75	0.51±.01	0.42±3.00	0.58±4.00	0.28±.25	0.16±1.00
2	1.27±.00	1.87±.01	3.14±.01	0.68±.00	0.6±.01	0.40±.00	0.59±1.00	0.32±1.00	0.19±1.00
3	1.29±.02	1.74±.02	3.03±.06	0.74±1.00	0.45±.00	0.43±.3	0.57±.3	0.26±.00	0.15±.00
4	0.99±.04	1.96±.05	2.95±.01	0.51±.8	0.97±.01	0.34±4.00	0.66±5.00	0.49±.2	0.3±1.00
5	1.01±.02	1.89±.03	2.90±.01	0.53±.67	0.88±.01	0.34±.67	0.65±3.00	0.47±.3	0.3±1.00
6	1.34±.03	1.43±.04	2.77±.07	0.94±.75	0.09±.01	0.48±.43	0.52±.57	0.06±.25	0.03±.14
7	1.32±.05	1.37±.06	2.69±.11	0.96±.83	0.05±.01	0.49±.45	0.51±.55	0.04±.17	0.02±.09
8	1.14±.04	1.50±.07	2.64±.10	0.76±.57	0.36±.03	0.43±.8	0.57±.7	0.24±.43	0.14±.3
9	0.94±.04	1.61±.05	2.55±.09	0.58±.8	0.67±.01	0.36±.4	0.63±.56	0.42±.2	0.26±.1
10	1.07±.00	1.43±.04	2.50±.06	0.75±.04	0.36±.04	0.42±.00	0.57±.67	0.03±1.00	0.14±.67
11	0.9±.02	1.56±.02	2.46±.06	0.58±1.00	0.66±.00	0.36±.3	0.63±.3	0.42±.00	0.27±.00
12	1.08±.03	1.29±.04	2.37±.03	0.84±.75	0.21±.01	0.45±1.00	0.54±1.3	0.16±.25	0.09±.3
13	1.09±.01	1.18±.01	2.27±.02	0.92±1.00	0.09±.00	0.48±.5	0.52±.5	0.08±.00	0.04±.00
14	1.05±.02	1.07±.04	2.12±.06	0.98±.5	0.02±.02	0.49±.3	0.5±.67	0.02±.5	0.00±.3
15	0.98±.02	1.00±.03	1.98±.02	0.98±.67	0.02±.01	0.49±1.00	0.51±1.5	0.02±.3	0.01±.5
Average	1.12±.02	1.52±.04	2.64±.05	0.76±.75	0.39±.01	0.42±.88	0.57±1.37	0.02±.32	0.14±.43

Table 11. Chromosome measurements of *Origanum cyrenaicum* (mean ± SD)

Chromosome No.	Relative length% (CL/ ΣCL) * 100	Arm ratio L/S	Centromere index% S/(S+L)*100	Chromosome type
1	8.06 ±.08	1.36 ±.08	42.07 ±1.02	m
2	7.95 ±.09	1.49 ±.04	40.44 ±.19	m
3	7.66 ±.17	1.31 ±.00	42.63 ±.05	m
4	7.44 ±.073	2.05 ±.13	33.46 ±1.52	sm
5	7.33 ±.08	1.92 ±.00	34.67 ±.93	sm
6	7.01 ±.12	1.07 ±.00	48.41 ±.08	m
7	6.79 ±.21	1.02 ±.02	49.16 ±.09	M
8	6.68 ±.20	1.32 ±.01	43.20 ±0.49	m
9	6.45 ±.19	1.71 ±.01	36.82 ±.30	sm
10	6.33 ±.09	1.34 ±.03	42.76 ±.54	m
11	6.21 ±.08	1.84 ±.06	36.40 ±.83	sm
12	5.99 ±.05	1.26 ±.08	45.28 ±1.21	m
13	5.75 ±.07	1.08 ±.01	48.06 ±.25	m
14	5.35 ±.19	1.02 ±.013	49.54 ±.46	M
15	5.01 ±.14	1.02 ±.02	49.50 ±.35	M
Average	6.67 ±.12	1.39 ±.03	42.82 ±0.55	

5. Discussion

This study reports the chromosome number and karyotype of three endemic plants from El-Jabal El-Akhdar (*Arum cyrenaicum*, *Arbutus pavarii* from the Tolmitha region, and *Origanum cyrenaicum* from the Al-Gubba region).

5.1 Chromosome number and morphology

Counting of chromosomes has been a very useful approach (particularly at the generic level) for researchers investigating evolutionary relationships (Levin & Wilson, 1976; Stuessy, 2009; Contreras & Ruter, 2011). Indeed, the chromosome numbers can affect inbreeding depression and the potential for introgression of traits through interspecific hybridization among other factors that can alter breeding strategy (Fehr, 1991; Contreras & Ruter, 2011).

5.1.1 *Arum cyrenaicum*

Arum has a basic number of x=14 and three ploidy levels have been reported (di-, tetra-, and hexaploid). *Arum orientale*, *Arum alpinum*, *A. hygrophilum* and *A. pictum* are diploid (2n=28), *Arum maculatum* and *Arum apulum* are tetraploid (2*n* = 56), while *Arum italicum* is hexaploid (2*n*=84) (D'Emerico *et al*., 1993; Bianco *et al*., 1994; Bedalov & Drenkovski, 1997; Turco *et al*., 2014). The tetraploid (2*n* = 4*x* = 56) observed here in *Arum cyrenaicum* is consistent with Boyce's (1989) findings but differs from that of Marchant (1973), who reported a somatic chromosome number of 2*n*=28. This difference in chromosomal number could be due to the difficulty of counting or the small number of individuals studied. In the aforementioned works, only chromosome numbers have been reported.

Arum cyrenaicum (2*n* = 56) shares its chromosome number with *A. apulum* (supporting its placement in subsection Dischroochiton; Boyce,1989) and *A. maculatum*. However, *Arum cyrenaicum* differs from both species in terms of its unique karyological features (e.g., high ratio of metacentric chromosomes and distinct chromosome size and morphology) may represent a unique adaption to its local environment in Libya. Additionally, its chromosome number and morphology is inconsistent with *A. hygrophilum*, *A. pictum* and *A. italicum*.

Polyploidy is a common phenomenon in plants that occurs naturally and spontaneously. It results in the increase in genome size caused by the presence of three or more chromosome sets (Otto & Whitton, 2000). Polyploidy, is an important mechanism regarding speciation and evolution of plants, occuring in two ways, which are autopolyploidy with genome duplication in only one species and allopolyploidy with genome duplication between species. D'Emerico *et al*. (1993); Bianco *et al*. (1994); and Turco *et al*. (2014) state that the tetraploid *Arum*'s chromosomes can be arranged in sets of two, and since it is impossible to arrange them in a series of four, it can be assume that the species is likely allopolyploid.

Polyploids frequently have a wider geographical range than their diploid parents (Schönswetter *et al.*, 2007; Whittemore & Olsen, 2011), probably because they are preadapted to habitats and resources off limits to their parents (Levin, 2003). They have a diversity of alleles that can confer a greater ecological niche than that of diploid progenitors (Pound *et al*., 2004). Polyploidy associated with structural changes in chromosomes is involved in bringing about further diversifications of karyotype morphology (Stebbins, 1971). Therefore, on this basis, it is suggested that *Arum cyrenaicum* is characterized by more rearrangement in its chromosome complement, these rearrangements may enhance the adaptive capacity of certain species.

This exceptional tetraploid population of *Arum cyrenaicum* could represent the starting point of evolutionary differentiation in this species. Findings such as these strongly demonstrate the need for extensive chromosome counts as a general procedure, even in taxa considered well-known, in which new cytotype can still be detected (e.g., *Melampodium* L.; Stuessy *et al.,* 2004).

An average centromere position and arm ratio indicate a predominantly median position for the centromeres and predominantly metacentric chromosomes in the karyotype of *A.cyrenaicum*. The karyotype formula of this species consisted of 22 pairs metacentric, 5 pairs submetacentric and 1 pair subtelocentric. Satellites were observed on the short arms of pairs 25 and 27 in *A.cyrenaicum*, the analyses show that the karyotype is similar to the previous reports for *Arum maculatum* (26m+24sm+6st; microsatellite on the short arm of pair 27) and *A. apulum* (40m+16sm; secondary constriction on the short arm and a microsatellite on the long arm of pair 27 (Turco *et al*., 2014). This is useful in characterizing a tetraploid *Arum* and studying the relationship

between them. In general, the Araceae family presents metacentric, submetacentric and subtelocentric chromosomes (Seansouk *et al*., 2022). In *A. cyrenaicum*, no B-chromosomes were observed during the study period.

5.1.2 *Arbutus pavarii*

Karyotype analysis of the genus *Arbutus* remains limited, and research focus mainly on determining chromosome number in only a few species, probably due to the small chromosome size.

In the current study, the findings revealed the chromosome number of the Libyan endemic *Arbutus pavarii* of $2n = 26$, with the basic chromosome number of $x = 13$. The results of the current study are consistent with the chromosome numbers of *A. unedo*, *A. menziesii*, *A. andrachne*, *A. canariensis*, and *A. xalapensis* (Darlington & Wylie, 1955; Sealy & Webb, 1950; Taylor & Taylor, 1977; Martins *et al*., 2022). According to Constantinidis *et al*. (2002) and Sun *et al*. (2016) consistency in chromosome number supports close relationship among species and their placement within the same genus. Chromosome number consistency has an important systematic function that supports the placement of *A. pavarii* in the genus *Arbutus*.

In most species, nucleolar organizing regions (NORs) are usually visible as secondary constrictions at metaphase as the arrays of genes active at the previous interphase remain decondensed (Heslop & Schwarzacher, 2011). In karyotype analysis, satellites and secondary constrictions are important features used to characterize chromosomes and to study phylogenetic relationships between species (Samaropoulou *et al*., 2019).

In the current study, karyotype formula of *A. pavarii* consisted of 10 metacentric and 3 submetacentric chromosome pairs. Through observation, the presence of satellites and secondary constrictions on some chromosome pairs of *A. pavarii* was noticed. This is a significant systematic method for characterizing this species. Chromosome pair 1 has a satellite on its long arm, while chromosome pairs 9 and 11 have a satellite on their short arms, and chromosome pair 3 has a secondary constriction on its short arm. No B-chromosomes were observed during the current investigation.

The centromere index (CI%) measures the position of the centromere, which holds the two sister chromatids of a chromosome together. The (CI%) of this species

indicate minimal variation in centromere position, with an average centromere position indicating a predominantly median position for the centromeres. The arm ratio (AR) compares the lengths of the long and short arms of a chromosome. A ratio of 1.00 to 1.04 indicates equal arm lengths, while a ratio greater than 1.04 indicates a longer long arm. The (AR) in *A.pavarii* indicates a small variation in the length of chromosome arms, with an average indicating predominantly metacentric chromosomes in the karyotype of *A.pavarii*.

The chromosome number and morphology of *A. pavarii* are described for the first time in this study. This work is, further, the first karyomorphological study of the genus *Arbutus*. Therefore, studying the karyomorphological data of *A. pavarii* can help characterize this endemic species of the El-Jabal El-Akhdar of Libya at the cytological level. As karyotype of *A. pavarii* is a powerful identification tool completed to the morphological characteristics and contributes to supporting the endemism of this species.

5.1.3 *Origanum cyrenaicum*

In *Origanum cyrenaicum*, the basic chromosome number of $x = 15$ dominates in *Origanum* taxa, but basic numbers of $x = 14$ and 16 characterize several taxa. Many taxa contain basic number variations possibly caused by the dysploidy mechanism. The dysploidy is likely to have occurred based on the fusion of metacentric chromosomes or reciprocal translocations in ancestral karyotypes, including dominant basic numbers. Basic number alterations are $x = 14$ in *O.sipyleum*, *O. rotundifolium*, and *O. vulgare* subsp. *vulgare*, $x = 16$ in *O. vulgare* (Martin *et al*., 2020).

The results indicate that *O. cyrenaicum* is diploid and have the same as the chromosome number of plants in the genus of *Origanum*. This finding supports the placement of this species in the genus *Origanum* (Lepper, 1970; Von Bothmer, 1970; Gill, 1981; Fernandes & Leitão, 1984; Magulaev,1984; Ayyangar & Vembu, 1985; Pastor *et al*., 1990; Khatoon & Ali, 1993; Bastida & Talavera, 1994; Markova & Goranova, 1995; Dobea *et al*., 1997; Kitiki, 1997; Balim & Kesercioğlu, 1998; Yildiz & Gücel, 2006; Bakha *et al*., 2017; Dirmenci *et al.*, 2018a, 2018b; Dirmenci *et al*., 2019; Martin *et al*., 2020). The karyotype analysis of the *Origanum* genus remains scarce and have primarily focused on chromosome number determination (Bakha *et al*., 2017). This is likely to have occurred due to the small size of the chromosomes.

Karyotype analysis revealed that the chromosomes of *O. cyrenaicum* are small in size, with predominantly median to sub-median centromeres. In the present study, the karyotype formula (KF) of *O. cyrenaicum* consisted of 11 metacentric and 4 submetacentric chromosome pairs, based on chromosome measurements. The karyotype formula, chromosome morphology, and size are powerful identifying tools of *O. cyrenaicum*. In the above-mentioned works, only chromosome numbers have been reported. So, there is not any information available on chromosome morphology of *Origanum*.

The morphological characteristics, as centromere position, number and position of satellites (SAT) and secondary constrictions (NORs) are useful taxonomical markers for distinguishing between related species or genera (Bareka *et al*., 2012; Uysal *et al*., 2015; Badr & El-Shazly, 2021). In *O.cyrenaicum*, chromosome pair 2 has a secondary constriction on its long arm, while chromosome pair 5 has a satellite on its long arm. Additionally, chromosome pair 4 has a satellite on its short arm and a secondary constriction on its long arm. No B-chromosomes were observed during the study period.

The average value of (IRL%) indicates that the chromosomes vary in length. The CI% show that most of the chromosomes have centromeres located in the median to sub-median positions. The AR reflects the relative length of the chromosome arms. An average centromere position and arm ratio indicate a predominantly median position for the centromeres and predominantly metacentric chromosomes in the karyotype of *O. cyrenaicum*.

The chromosome number and morphology of *Origanum cyrenaicum* are described for the first time in this study, and also this work is the first karyomorphological study of the genus *Origanum*. Therefore, studying the karyomorphological data of *O. cyrenaicum* can help characterize this endemic species of the El-Jabal El-Akhdar of Libya at the cytological level.

5.2 Chromosome Size

Chromosome length is useful for distinguishing individuals, samples, populations or species. It is also an indirect indicator of the total DNA content. Measurment of chromosome size correlates with evolutionary age, which provides an estimate genome size using the chromosomal data (Mehra & Bawa, 1972; Contreras & Ruter, 2011).

5.2.1. *Arum cyrenaicum*

Most species in the genus *Arum* possess small to medium-sized chromosomes, typically ranging from 2.65-5.40µm. In the current study, the chromosome size of *Arum cyrenaicum* (2.88-4.72µm) was comparable to that of *Arum alpinum* (2.95-4.80µm) and *Arum maculatum* (2.00-4.80µm) (D'Emerico *et al*., 1993). The total haploid length of the chromosome complement (THCL), a proxy for genome size (Carta & Peruzzi, 2016; Franzoni *et al*., 2024), is calculated as the sum of the lengths of all the chromosome in a metaphase plate, divided by the ploidy level (Peruzzi & Altinordu, 2014). The (THCL) of *A. cyrenaicum* (107.21µm) further confirms that this species has medium to small chromosomes.

5.2.2. *Arbutus pavarii*

In *Arbutus pavarii*, the chromosome size ranges between 1.98µm and 3.09µm. The total haploid length of the chromosome complement (THCL), which provides an estimate of the overall size of the chromosomes in *A. pavarii*, was 33.2µm. The total length of this species indicates relatively small chromosomes.

5.2.3. *Origanum cyrenaicum*

While most *Origanum* species exhibit small chromosomes (0.3 to 0.74µm; Martin *et al*., 2020). In the current study, *O. cyrenaicum* chromosomes are small in size, ranging from 1.98 to 3.19µm, and the total haploid length of the chromosome complement (THCL) (39.56µm) in this species indicates that they are relatively small in size.

5.3 Karyotype Asymmetry

Karyotype asymmetry is an important parameter in karyological studies (Eroğlu, 2015). It is further considered one of the most popular, inexpensive and widely used approaches, especially by botanists (Peruzzi & Eroğlu, 2013).

Karyotype symmetry has two components, one related to variation among chromosome size and the other to variation in centromere position (Peruzzi & Eroğlu, 2013). Most species of angiosperms are characterized by uniform symmetric karyotypes with mostly meta or submetacentric chromosomes (Weiss-Schneeweiss & Schneeweiss, 2012). It has been classically assumed that asymmetric karyotypes are derived from symmetric ones (Stebbins, 1971). Nevertheless, cytogenatics now believe that reversal situations may have occurred (Stace, 2000) and that karyotype asymmetry is a transitory state rather than an evolutionary endpoint (Lysák *et al.*, 2006).

5.3.1. *Arum cyrenaicum*

This study revealed that most chromosomes in *A. cyrenaicum* are either metacentric or submetacentric. The karyotype symmetry indices consistently exhibited low heterogeneity and variation values. The value of the intrachromosomal asymmetry index (A_1) plays a role in finding variations in chromosome types within a karyotype. The range of values for the intrachromosomal asymmetry index (A_1) is from zero to one (Muliawati *et al*., 2023). The low value of (A_1) for *A. cyrenaicum* signifies a value close to zero and this, in turn, indicates a predominantly metacentric chromosome in the karyotype of *A. cyrenaicum*. However, the value of the interchromosomal asymmetry index (A_2) is used to assess the deviation (dispersion) in chromosome length within a karyotype (Muliawati *et al*., 2023). The small A_2 value for *A. cyrenaicum* shows that the chromosomal length deviation in *A. cyrenaicum* is relatively small (Table 5).

Furthermore, the value of the coefficient of variation of chromosome length (CV_{CL}) and the value of coefficient of variation of the centromere index (CV_{CI}) indicates low variation in chromosome length and centromere index, respectively. According to Uysal *et al*. (2018), the larger the values of CV_{CL} and CV_{CI}, the greater the asymmetry in the karyotype. Therefore, the low values of both CV_{CL} and CV_{CI} in *A. cyrenaicum* indicate that its karyotype is predominantly composed of symmetric chromosomes.

The karyotype of this species is classified as a symmetry type 1A according to Stebbins classification, indicating that the karyotype of *A. cyrenaicum* is symmetrical.

The low values of the asymmetry index (AI), Arano asymmetry index (Ask%), and degree of karyotype asymmetry (A) indicate a relatively low level of asymmetry. Meanwhile, the high values of the total symmetry index (S%), total form percentage of homologous chromosome pairs (TF%), and centromere index (CI%) indicate a symmetrical structure of the karyotype.

These findings demonstrate that *A. cyrenaicum* exhibits a distinct, symmetrical chromosomal pattern. Combined with morphological and geographical evidence, these cytological results support Hruby's (1912) proposal that *A. cyrenaicum* warrants recognition as a distinct species. Although this species was recently recorded in 1992 in south-west Crete (Kakodikianos valley north of Paleohora as far as Kandanos), marking the first record of *A. cyrenaicum* outside Libya (Cretan Flora, 2025). The analysis offered by the current study reveals that its symmetric karyotype predominantly composed of metacentric chromosomes closely resembles that of *Arum apulum*. According to Stebbins (1971), a high proportion of metacentric chromosomes may indicate early evolutionary divergence in a species.

5.3.2. *Arbutus pavarii*

On the other hand, the karyotype of *A. pavarii* is classified as 1A type in Stebbins symmetry classes, indicating a relatively balanced, symmetrical arrangement of chromosomes. The (CV_{CL}) indicates low variation in chromosome length within the karyotype, suggesting that the karyotype of this species is mostly symmetric in terms of variation in the chromosome length, while a low (CV_{CI}) indicates that the centromeres are fairly stable and do not exhibit significant variation in their position within the chromosomes.

A higher S%, TF% and CI% values indicate a greater proportion of symmetric chromosomes in the karyotype of this species, while a low Ask%, A, A_1, A_2 and AI values (Table 5) indicate a relatively low level of asymmetry. These numerical indices indicate that the karyotype of *A. pavarii* is symmetrical, with a high percentage of symmetric chromosomes.

5.3.3. *Origanum cyrenaicum*

The information resulting from karyotype analysis of *O. cyrenaicum* indicated that the (CV_{CI}) value reflects a low variation in the position of the centromeres. The measurement of interchromosomal asymmetry (A_2), to determine how different the chromosome lengths of a complement are from each other, and the coefficient of variation of chromosome length (CV_{CL}) is perfectly suited for this (Paszko, 2006). *O. cyrenaicum* had a low (CV_{CL}) value indicating a low variation in the length of the chromosomes.

The AI, which has a high degree of precision and sensitivity to assess karyotype asymmetry, with higher values indicate higher levels of karyotypic heterogeneity (Paszko, 2006; Zhang *et al*., 2013), and the AI can also indicate an evolutionary trend. Hence, primitive species usually have a lower AI, whereas a plant with a higher AI value would indicate that it is more evolutionarily advanced (Deng *et al*., 2011; Gao *et al*., 2012; Zhang *et al*., 2013; Chen *et al*., 2023). In the current study, AI was used to assess karyotype asymmetry of *O. cyrenaicum*. Findings showed that it had a low value of heteroginity, indicating a low level of karyotype asymmetry.

The species showed (Table 5) low values for A_1 , A_2 , A, and Ask%, on the other hand, high values were observed for S%, TF%, and CI%, indicating a symmetrical karyotype in *O. cyrenaicum*. The karyotype of this species is classified as the 1A type in Stebbins' symmetry classification, indicating symmetrical karyotype of this species. Based on these findings, *Origanum cyrenaicum* is characterized by a symmetrical karyotype, with a predominance of metacentric chromosomes.

Stebbins (1971), Sheidai *et al.*(2000), and Vargas *et al.*(2007) found that an asymmetrical karyotype has evolved higher than a symmetrical karyotype. Stebbins (1950) suggested that the karyotype of organisms is related to the size and type of chromosomes, with organisms possessing predominantly metacentric and submetacentric chromosomes belonging to symmetrical karyotypes, while asymmetrical karyotypes contain a wide variety of chromosomes, including metacentric, submetacentric, subtelocentric, acrocentric, and telocentric chromosomes.

The predominance of metacentric chromosomes has demonstrated the consistency of a symmetrical karyotype across all the applied principles of *Arum*

cyrenaicum, *Arbutus pavarii*, and *Origanum cyrenaicum*. In addition, the somatic chromosome numbers, chromosome morphology, ploidy levels, karyotype formulas, karyograms, and numbers and sites of satellites, as well as chromosomal and karyotype parameters in this study, can be used as identifying tools for these endemic species.

6. Conclusion

In the present study, chromosome numbers and ploidy levels of three endemic species were examined: *Arum cyrenaicum* (Araceae), collected from the ancient ruins of Ptolemais, approximately 2 km from the Tolmitha region; *Arbutus pavarii* (Ericaceae), collected from wadi Emleka, about 19 km from the Tolmitha region; and *Origanum cyrenaicum* (Lamiaceae), collected from Wadi Bouhalfaya, 3 km from the Al-Gubba region in the El-Jabal El-Akhdar region. Additionally, the karyograms of these species were established .

The results of this study reveal two levels of ploidy: tetraploid in *Arum cyrenaicum*, diploid in *Arbutus pavarii,* and *Origanum cyrenaicum*. *Arum cyrenaicum* (2*n*=4*x*=56) with karyotype formula 6M+38m+10sm+2st (2SAT), *Arbutus pavarii* (2*n*=2*x*=26) with karyotype formula 4M+16m+6sm (4SAT), and *Origanum cyrenaicum* (2*n*=2*x*=30) with karyotype formula 6M+16m+8sm (3SAT). *Arum cyrenaicum*, *Arbutus pavarii* and *Origanum cyrenaicum* have symmetrical karyotypes.

Furthermore, satellites were observed on two chromosome pairs of *Arum cyrenaicum*, four chromosome pairs of *Arbutus pavarii*, and three chromosome pairs of *Origanum cyrenaicum*. No B-chromosome was observed among the treated endemic plant species.

Overall, the results obtained in this study contribute to characterizing these endemic species at the cytological level. Hence, this study has significantly increased the data on chromosome numbers and karyotype analysis of some endemic plant species at El-Jabal El-Akhdar region (now 20 out of 61).

7. References

Abdel-karim, M., Abdelshafeek, A. K., Saada, F. A., & Attafa, S. M. M. (2018). Isolation and characterization of some flavones from *Arum cyrenaicum* (Araceae). *World Journal of Pharmaceutical and Life Sciences*, *4*(2), 27-33.

Abdulrraziq, A. A., & Salih, S. M. (2020a). Morphological characterization of *Arum cyrenaicum* Hruby plant in Al-Jabal Al-Akhdar region, Libya. *Al-Mukhtar Journal of Sciences*, *35*(3), 246-254.

Abdulrraziq, A. A., & Salih, S. M. (2020b). Sensitivity test of some bacteria causing urinary tract infections to local *arbutus pavarii* extracts. *Bayan Scientific Journal*, *5*,102-114.

Adeigbe, O.O., Omoloye, A.A., Aliyu, O.M., Adewale, B.D., & Oyewole, S.O. (2013). Karyomorphotypic variation in *Eriospermum abyssinicum* Baker. *African Journal of Biotechnology*, *12*(10), 1010-1015.

Agiel, N., & Mericli, F. (2017). A survey on the aromatic plants of Libya. *Indian Journal Pharmaceutical Education and Research*, *51*(3),304-308.

Alberto, C.M., Sanso, A.M., & Xifreda, C.C. (2003). Chromosomal studies in species of *Salvia* (Lamiaceae) from Argentina. *Botanical Journal of the Linnean Society*, *141*(4),483-490.

Al Groshi, A., Nahar, L., Ismail, F.M.D., Evans, A.R., & Sarker, S. D. (2022). Dichloromethane extract of the leaves of *Arbutus pavarii* Pamp. Exhibits cytotoxicity against the prostate cancer cell line PC3: A bioassay-guided isolation and identification of Arbutin and Betulinic Acid Methyl Ester. *Journal of Natural Products Discovery*, *1*(3),9-9.

Ali, S.I., & Jafri, S.M.H. (Eds.). (1976-1989). *Flora of Libya* (Vol. 1-147). Department of Botany, Al-Faateh University, Tripoli, Libya.

Ali, E. S. M. (2024). *Biodiversity of Al-Jabal Al-Khdar: Origin and Evolution*. Media Platform of Omar Al-Mukhtar University. https://omu.edu.ly

Al-Sodany, Y. M., Shehata, M.N., & Shaltout, K.H. (2003). Vegetation along an elevation gradient in El-Jabal El-Akhdar, Libya. *Ecologia Mediterranea*, 2,125-138.

Al-Traboulsi, M., & Alaib, M. A. (2021). A Survey of medicinal plants of Wadi Al-Kouf in Al-Jabal Al-Akhdar, Libya. *Natura Croatica: Periodicum Musei Historiae Naturalis Croatici*, 30(2), 389-404.

Antunes, P. (2010). *Indução de plantas tetraplóides através de tratamentocom agentes c-mitóticos no tamarilho (Cyphomandra betacea) e no medronheiro (Arbutus unedo)*. [Master's thesis, Universidade de Coimbra]. Universidade de Coimbra.

Arano, H. (1963). Cytological studies in subfamily Carduoideae (Compositae) of Japan IX. The karyotype analysis and phylogenic considerations on *Pertya* and *Ainsliaea* (2), *Botanic Magazine*, *76*(895), 32-39.

Astuti, G., Roma-Marzio, F., & Peruzzi, L. (2017). Traditional cytotaxonomic studies: can they still provide a solid basis in plant systematics? *Flora Mediterranea*, *27*,91-98.

Ayyangar, K. R., & Vembu, B. (1985). Karyo-specific and karyo-generic affiliations amongst *Mentha arvensis* Benth., *M. piperita* L. and *Origanum vulgare* L. *Proceedings of the Indian Science Congress Association*,*72*(3-VI),127.

Badr, A., & El-Shazly. H.H. (2021). Chromosomes as sources of taxonomic information for plant systematics and evolution. *Taeckholmia*, *41*,70-90.

Bakha, M., Faiz, C.A., Daoud, M., Mtili, N.E., Aboukhalid, K., Khiraoui, A., Machon, N., & Siljak-Yakovlev, S. (2017). Genome size and chromosome number for six taxa of *Origanum* genus from Morocco. *Botany Letters*, *164*(4),361-370.

Balim, A. G., & Kesercioğlu, T. (1998). Doğu akdeniz bölgesinde yayılış gösteren bazı *Origanum* L. türleri üzerinde sitotaksonomik araştımalar. XIV. In *XIV. Ulusal Biyoloji Kongresi* , (Vol. 1, pp. 277-282).

Bareka, P., Siljak-Yakovlev, S., & Kamari, G. (2012). Molecular cytogenetics of *Bellevalia* (Hyacinthaceae) species occurring in Greece. *Plant Systematics Evolution*, *298*, 421-430.

Bartolo, G., Brullo, S., Pavone, P., & Terrasi, M.C. (1984). Cytotaxonomical notes on some "Liliaceae" of N. Cyrenaica. *Webbia*, *38*(1), 601-622.

Bastida, F., & Talavera, S. (1994). Nŭmeros cromosomăticos de plantas occidentales, 688-695. *Anales del Jardín Botánico de Madrid*, *51*(2), 279-280.

Bedalov, M. (1973). IOPB chromosome number reports:40. *Taxon*, *22*(2-3),285.

Bedalov, M. (1975a). Cytotaxonomical and phytogeographical investigation of the species *A. italicum* Miller in Jugoslavia. *Acta Botanica Croatica*, *34*,143-50.

Bedalov, M. (1975b). Taxonomic problems and distribution of the species *A. nigrum* Schott in the Balkan Flora. *In Problems of the Balkan Flora and vegetation*. Sofia.

Bedalov, M. (1981). Cytotaxonomy of the genus *Arum* (Araceae) in the Balkan and Aegean area. *Botanische Jahrbücher für Systematik, Pflanengeschichte und Pflanzengeographie*, *102*(1-4),183-200.

Béguinot, A., & Vaccari, A. (1913). Terzo contributo alla Flora della Libia. *Annali di Botanica (Roma)*, *12*, 117.

Bedalov, M. (1983). Distribution of the species *A. alpinum* Schott et Kotschy in the west Mediterranean. *Rapports et Procés-Verbaux des Réunions de la Commission Internationale pour l 'Exploration Scientifique de la Mer Méditerranée*, *28*,107-9.

Bedalov, M. (2006). Investigations in the genus *Arum* L. in Istrai (Croatia). *Webbia*, *61*(2), 209-215.

Bedalov, M., & Drenkovski, R. (1997). The genus *Arum* (Araceae) in the F.Y.R. Makedonija. *Bocconea*, *5*(2),781-785.

Bedalov, M., & Fischer, M.A. (1995). *Arum alpinum* (Araceae) and its distribution in the Eastern Mediterranean. *Phyton (Austria)*, *35*(1), 103-113.

Ben Ramadan, L., Zwawi, A., Salem, M., Auzi, A., & El marghani, A. (2021). Antibacterial activity of *Arum cyrenaicum* Hruby corms. *Libyan Journal of Basic Sciences*,*14*(1), 91-100.

Bianco, P., D'Emerico, S., Medagli, P., & Bedalov, M. (1994). Indagini sistematiche su *Arum apulum* (Careno) Bedalov (Araceae), entita endemic delle Murge Pugliesi. *Journal of Plant Taxonomy and Geography*, *49*(1),43-49.

Boyce, P.C.(1989). A new classification of *Arum* with keys to the infra generic taxa. *Kew Bulletin*, *44*(3),383-395.

Boyce, P.C. (1993). *The genus Arum*. Kew Magazine Monograph, Royal Botanic Gardens, Kew.

Brullo, S., & Furnari, F. (1979). Researches on the genus (*Amaracus*) Gled. (Labiatae) in Cyrenaica: I1 genere (*Amaracus*) Gled. (Labiatae) in Cyrenaica. *Journal of Plant Taxonomy and Geography*, *34*(1), 439-449.

Brullo, S., Guglielmo, A., Pavone, P., & Terrasi, M. N. (1990). Chromosome counts of flowering plants from N. Cyrenaica. *Candollea*, *45*(1), 65-74.

Carta, A., & Peruzzi, L. (2016). Testing the large genome constraint hypothesis: plant traits, habitat and climate seasonality in Liliaceae. *New Phytologist*, *210*(2), 709-716.

Chanderbali, A.S., Jin, L., Xu, Q. (2022). *Buxus* and *Tetracentron* genomes help resolve eudicot genome history. *Nature Communications*, *13*, Article 643.

Chen, L., Chen, Q., Fan, Y., Xu, Y., Wu, B., Xu, L., da Silva, J., & Yu, X. (2023). Ploidy and karyotype analysis of different populations of *Paeonia mairei*, an endemic species to China. *Cytologia*, *88*(1), 55-59.

Chiarugi, A. (1949). Saggio di una revision cito-sistematica della flora Italiana. I:II tetraploidismo della *Bellevalia webbiana* Parl. e il suo diritto di cittadinana nella flora Italiana. *Caryologia*, *1*(3), 362–377.

Choi, B., Kim, H., Byun, H., Gang, G.H., Lee, Y., Myeong, H., So, S., & Jang, T.S. (2022). A study of the chromosome number and genome size of the rare species *Rhododendron keiskei* var. *hypoglaucum* in korea. *Korean Journal of Plant Taxonomy*, *52*(2), 102-107.

Christenhüsz, M. J. M., & Byng, J. W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa*, *261*(3), 201-217.

Christou, E., Bareka, P., & Kamari, G. (2008). Karyosystematic study of selected plant taxa from Cyprus. *Botanika Chronika*, *19*,13-20.

Coelho, N., Gonçalves, S., & Romano, A. (2020). Endemic plant species conservation: Biotechnological Approaches. *Plants*, *9*, Article 345.

Constantinidis, T., Bareka, E. P., & Kamari, G. (2002). Karyotaxonomy of Greek serpentine angiosperms. *Botanical Journal of the Linnean Society*, *139*(1),109-124.

Contreras, R.N., & Ruter, J.M. (2011). Genome size estimates and chromosome numbers of *Callicarpa* L. (Lamiaceae). *Horticultural Science*, *46*(4), 567-570.

Corlett, R.T., & Bigger, A. (2017). Toolbox: Biotechnology in biodiversity conservation. *Trends in Biotechnology*, *35*,55-65.

Cosson, E. (1875). *Plantae in Cyrenaica et agro Tripolitano notae*. *Bulletin de la Société Botaniqué de France*, *22*,45-51.

Crawford, D.J., Mort, M.E., & Archibald, J.K. (2005). Biosystematics, chromosomes and molecular data: melting the old and the new. *Taxon*, *54*, 285–289.

Cretan Flora. (n.d.). *Arum cyrenaicum*. Retrieved April 9, 2025, from http://www.cretanflora.com/

Cuccuini, P., Nepi, C., Abuhadra, M.N., Cecchi, L., Freitag, H., Luccioli, E., Maier-Stolte, M., Marcucci, R., Peruzzi, L., Pignotti, L., Stinca, A., Wallnofer, B., & Wood. J. (2015). The Libyan collections in FI (Herbarium Centrale Italicum and Webb Herbarium) and studies on the Libyan flora by R. Pampanini - Part 1. *Bocconea*, *27*(2), 3-132.

Cui, L., Liu, Z., Yin, Y., Zou, Y., Faizan, M., Alam, P., & Yu, F. (2023). Research progress of chromosome doubling and 2*n* gametes of ornamental plants. *Horticulturae*, 9, Article 752.

Darlington, D.C. (1937). Mitosis:the constancy of the chromosomes. In *Recent advances in cytology* (pp.36-37). Blackiston's Son and Co., Inc. Philadelphia.

Darlington, C.D., & Wylie, A.P. (1955). Chromosome atlas of flowering plants (2nd ed., p. 216). Allen and Unwin. London, UK.

Deng, T., Meng, Y., Sun, H., & Nie, Z.L. (2011). Chromosome counts and karyotypes in *Chaetoseris* and *Stenoseris* (Asteracesae-Cichorieae) from the Hengduan Mountains of SW China. *Journal of Systematics and Evolution*, *49*(4),339-346.

Devadas, R., Medhi, R.P., & Das, S.P. (2010). Interspecific Hybrid Developed in *Epidendrum Orchid* from the Cross *E. radicans* Pav.Ex. Lindl. Xe. *Xanthinum* Lindl. *Journal of Horticultural Sciences*, *5*(2),144-147.

D'Emerico, S., Bianco, P., & Medagli, P. (1993). Chromosome numbers and karyotypes in *Arum* (Araceae). *Caryologia*, *46*(2-3), 161-170.

Dhar, U. (2002). Conservation implications of plant endemism in high-altitude Himalaya. *Current Science*, *82*(2),141-148.

Dirmenci, T., Yazıcı, T., Özcan, T., Çelenk, S., & Martin, E. (2018a). A new species and a new natural hybrid of *Origanum* L. (Lamiaceae) from the west of Turkey. *Turkish Journal of Botany*, *42*(1), 73-90.

Dirmenci, T., Özcan, T., Yazıcı, T., Arabacı, T., & Martin E. (2018b). Morphological, cytological, palynological and molecular evidence on two new hybrids: An example of homoploid hybridization in *Origanum* (Lamiaceae). *Phytotaxa*, *371*(3), 145-167.

Dirmenci, T., Özcan, T., Açar, M., Arabacı, T., Yazıcı, T., & Martin, E. (2019). A rearranged homoploid hybrid species of *Origanum* (Lamiaceae): *O.*×*munzurense* Kit Tan & Sorger. *Botany Letters* , *166*(2),153-162.

Dobea, C., Hahn, B., & Morawetz, W. (1997). Chromosomenzahlen zur gefässpflanzen-flora österreichs. *Linzer Biologische Beiträge*, *29*(1), 5-43.

Dobignard, A., & Chatelain, C. (2010-2013). *Index Synonymique de la Flore D'Afrique du Nord* (Vols. 1-5). Éditions Des Conservatoire et Jardin Botaniques.

Dobigny, G., Ducro, J.F., Robinson, T.J., & Volobouev, V. (2004). Cytogenetics and cladistics. *Systematic Biology*, 53,470-484.

Durand, E., & Barratte, G. (1910). *Florae Libycae Prodromus*. Geneva.

Elabbar, F.A., EL-Barasi ,Y.M., & ALawamy, M.W.M. (2014). Seasonal variation study of the volatile oils of *Origanum cyrenaicum* in Libya. *Advances in Applied Science Research*, *5*(1),39-42.

El-Darier, S. M., & El-Mogaspi, F. M. (2009). Ethnobotany and relative importance of some endemic plant species at El-Jabal El-Akhdar region (Libya). *World Journal of Agricultural Sciences*, *5*(3), 353-360.

El-Mokasabi, F.M. (2010). *Ecology, ethnobotany and floristic composition of the medicinal plants at sallum, Egypt and Gebel Akhder, Libya* (Ph.D. thesis). Alexandria University, Egypt.

El-Mokasabi, F. M. (2014). Floristic composition and traditional uses of plant species at Wadi Alkuf, Al-Jabal Al-Akhder, Libya. *American Eurasian Journal Agriculture and Environment Sciences*, *14*(8), 685-697.

El Rabiae, G., Eltira, F. R., & Elmogasapi, A.M. (2024). Morphological and anatomical study of *Origanum cyrenaicum* endemic to Libya. *GPH-International Journal of Applied Science*, *7*(4).

Elshatshat, S. (2009). Biological conservation of the endemic *Arbutus pavarii* Pamp: Seed germination as attempt. *International Journal of Human Geography and Environmental Studies*, *1*(1), 20-22.

Elshatshat, S., & Elshibani, F. (2020). Characteristics, nutritive value and antioxidant content of the Libyan endemic (*Arbutus pavarii* Pamp.) strawberry tree fruits. *EPRA International Journal of Research and Development*, *5*(9), 255-260.

El-Zwaam, S.M. (1995). *El-Jabal El-Akhdar* (In Arabic). Garyounis University, Benghazi, Libya.

Eroğlu, H.E., Simsek, N., Koc, M., & Hamzaoğlu, E. (2013). Karyotype analysis of some *Minuartia* L. (Caryophyllaceae) taxa. *Plant Systematic Evolution*. *299*,67-73.

Eroğlu, H.E. (2015). Which chromosomes are subtelocentric or acrocentric? A new karyotype symmetry/asymmetry index. *Caryologia*, *68*(3), 239-245.

Eroğlu, H. E. (2024). Karyotype symmetry/asymmetry index (S/AI) in Bovidae Taxa. *Journal of Engineering and Natural Sciences*, *6*(1), 28-37.

FAO. (2019). *The state of the world's biodiversity for food and agriculture*. (J. Bélanger & D. Pilling, Eds.). FAO.

Favarger, C., & Contandriopoulus, J. (1961). Essai sur L'endémisme. *Bulletin de la Société Botanique Suisse*, *71*, 384-408.

Fehr, W.R. (1991). In principles of cultivar development: Theory and technique, polyploidy (Vol. 1: 59-65). Macmillan.

Fernandes, A., & Leitão, M. T. (1984). Contribution à l'étude cytotaxinomique des Spermatophyta du Portugal XVIII - Lamiaceae. *Memorias da Sociedade Broteriana*, *27*, 27-5.

Ferreira, P.M.A., & Boldrini, I.I. (2011). Potential reflection of distinct ecological units in plant endemism categories. *Conservation Biology*, *25*(4), 672-679.

Foggi, B., Viciani, D., Baldini, R.M., Carta, A., & Guidi, T. (2014). Conservation assessment of the endemic plants of the Tuscan Archipelago, Italy. *Oryx*, *49*, 118–126.

Franzoni, J., Astuti, G., Bacchetta, G., Barone, G., Bartolucci, F., Bernardo, L., Carta, A., Counti, F., Domina, G., Frajman, B., Giusso del Galdo, G., Iamonico, D., Iberite, M., Minuto, L., Sarigu, M., Terlevic, A., Turini, A., Varaldo, L.,Volgger, D., & Peruzzi, L. (2024). A cytosystematic study of the *Dianthus virgineus* complex (Caryophyllaceae) in the Central Mediterranean. *Journal of Systematics and Evolution*, *62*(4), 589-602.

Fukui, K. (1998). Smallness: Gain and loss in chromosome research. *Cytogenetics and Cell Genetics*, *81*, 105.

Gao, Y.D., Zhou, S.D., He, X.J., & Wan, J. (2012). Chromosome diversity and evolution in tribe Lilieae (Liliaceae) with emphasis on Chinese species. *Journal of Plant Research*, *125*,55-69.

Garbari, F. (1975). The genus *Allium* in Italy. V. *Allium* subgen. *Chamaeprason* (F. Hermann), status novus. *Taxon*, *24*, 541-542.

GBIF. (2025). *The Global Biodiversity Information Facility*. Retrieved February 17, 2025, from http://www.gbif.org

GEA (General Environment Authority). (2010). *Fourth Report on the Implementation of the Convention on Biological Diversity*. Tripoli: General Environment Authority Publications.

Ghaffari, M., Hejazi, A., & Pourahmad, A. (2005). New chromosome counts in nine endemic species. *Folia Geobotanica*, *40*(4),435-440.

Gianfranco, V., Ravalli, C., & Cremonini, R. (2008). The karyotype as a tool to identify plant species: *Vicia* species belonging to *Vicia* subgenus. *Caryologia*, *61*(3), 300-319.

Gill, L. S. (1981). Biosystematics of the tribe Satureineae (Labiatae) in Canada I. *Cytologia*, *46*, 45-55.

González-Elizondo, M. S., González-Elizondo, M., & Sørensen, P. D. (2012). *Arbutus bicolor* (Ericaceae, Arbuteae), a new species from Mexico. *Acta Botánica Mexicana*, *99*(1), 55-72.

Graphodatsky, A.S, Trifonov, V.A., & Stanyon, R. (2011). The genome diversity and karyotype evolution of mammals. *Molecular Cytogenetics*, *4*, 22.

Greilhuber, J., & Speta, F. (1976). C-banded karyotypes in the *Scilla hohenackeri* Group, *S. persica* and *Puschkinia* (Liliaceae). *Plant Systematics and Evolution*,*126*, 149-188.

Greilhuber, J., & Ehrendorfer, F. (1988). Karyological approaches to plant taxonomy. In A. M. Grimwade (Ed.), *Atlas of Science: Animal and Plant Science* (Vol. 1, pp. 289-297). ISI.

Greilhuber, J. (1995). Chromosomes of the monocotyledons (general aspects). In P. J. Rudall, P. J. Cribb, D. F. Cutler, & C.J.Humphries (Eds.). Monocotyledons: *Ssystematics and evolution* (pp. 379-414). Royal Botanic Gardens, Kew.

Guerra, M. (2008). Chromosome numbers in plant cytotaxonomy: Concepts and implications. *Cytogenetic and Genome Research*, *120*(3-4), 339-50.

Guerra, M. (2012). Cytotaxonomy: The end of childhood. *Plant Biosystems*, *146*, 703-710.

Guittonneau, G., & Le Houérou, H. N. (1968). Deux nouvelles espéces du genre Erodium L'Hérit. Découvertes en Libye. *Bulletin de la Société Botanique de France*, *115*(7-8), 591-599.

Hand, R.(Ed.). (2015). Supplementary notes to the flora of Cyprus VIII. *Willdenowia*, *45*(2), 245-259.

Heslop, J. S. H., & Schwarzacher, T. (2011). Organisation of the plant genome in chromosomes. *The Plant Journal*, *66*(1), 18-33.

Hörandl, E., Paun, O., Johansson, J.T., Lehnebach, C., Armstrong, T., Chen, L., & Lockhart, P. (2005). Phylogenetic relationships and evolutionary traits in *Ranunculus* L. (Ranunculaceae) inferred from ITS sequence analysis. *Molecular Phylogenetic Evolution*, *36*(2), 305-327.

Hruby, J. (1912). Le genre *Arum*. *Bulletin de la Société Botaniqué de Genéve*, *2*(4), 159.

Hunt, C., McClung, L.C., Edwards, L., & el-Rishi, H. (2024). Pollen-vegetation-rainfall relationships in the Gebel al-Akhdar, Northeast Libya. *Review of Palaeobotany and Palynology*, *334*(6), 105272.

Huziwara, Y. (1962). Karyotype analysis in some genera of Compositae VIII. Further studies on the chromosomes of *Aster*. *American Journal of Botany*, *49*(2), 116-119.

Ietswaart, J.H. (1975). A new species of *Origanum* (Labiatae) from Libya. *Acta Botanica Neerlandica*, *24*(3-4), 285-287.

Iijima, K., Kakeda, K., & Fukui, K. (1991). Identification and characterization of somatic rice chromosomes by imaging methods. *Theoretical Applied Genetics*, *81*, 597-605.

IPCN. (2024). *Index to Plant Chromosome Numbers*. Missouri Botanical Garden. Retrieved October 26, 2024, from http://www.tropicos.org/Project/IPCN/

Isik, K. (2011). Rare and endemic species: Why are they prone to extinction? *Turkish Journal of Botany*, *35*, 411-417.

IUCN.(2019). *The IUCN Red List of Threatened Species* (Version 2019-2). http://www.iucnredlist.org

Jafri, S.M.H., & EL-Gadi, A.A. (1977). *Flora of Libya: Araceae* (Vol. 41, pp. 1-9). Al-Faateh University, Tripoli, Libya.

Jafri, S.M.H., & EL-Gadi, A.A. (1978). *Flora of Libya: Ericaceae* (Vol. 54, pp. 1-8). Al Faateh University, Tripoli, Libya.

Jafri, S.M. H., & EL-Gadi, A. A. (1984). *Flora of Libya: Lamiaceae* (Vol. 118, pp. 1-116). Al Faateh University, Tripoli, Libya.

Jones, K., & Brighton, C. (1972). Chromosome numbers of tropical Rhododendrons. *Kew Bulletin*, *26*(3), 559-561.

Khatoon, S., & Ali, S.I. (1993). *Chromosome atlas of the angiosperms of Pakistan*. Department of Botany, University of Karachi, Karachi.

Kıtıkı, A. (1997). Status of cultivation and use of oregano in Turkey. In S. Padulosi (Ed.). *Oregano*. *Proceeding of the IPGRI International Workshop on Oregano*, *8-12 May 1996*, *CIHEAM*, *Valenzano (Bari)*, *Italy* (Vol. 14, pp. 122-132). *International Plant Genetic Resources Institute*.

Ladle, R.J., & Whittaker, R.J. (2011). *Conservation biogeography*. Wiley-Blackwell.

Lavania, U. C., & Srivastava, S. (1992). A simple parameter of dispersion index that serves as adjunct to karyotype asymmetry. *Journal Biosciences*, *17*,179-182.

Legro, R. A. H.(1959). The cytological background of *Cyclamen* breeding. *Mededelingen van de Landbouwhogeschool te Wageningen*, Netherland, *59*(8), 1-51.

Lepper, L. (1970). Beiträge zu einer Flora des Orientes. *Linnaea*, *21*, 639-663.

Leuenberger, R. (1965). *Water resources and water utilization in northern Cyrenaica. Libya*. Trust Fund 94, FAO.

Levan, A., Fredga, K., & Sandberg, A. A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas*, *52*(2), 201-220.

Levin, D.A. (2002). *The role of chromosome change in plant evolution*. Oxford University Press.

Levin, D. A. (2003). The ecological transition in speciation. *New Phytologist*, *161*,91-96.

Levitzky, G.A.(1931). The karyotype in systematics. *Bulletin Applied Botany Genetics Plant Breeding*, *27*(1), 220-240.

Lysák, M.A., Berr, A., Pecinka, A., Schmidt, R., McBreen, K., & Schubert, I. (2006). Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. *Proceedings of the National Academy of Sciences of the United States of North America*, *103*, 5224-5229.

Maire, R.(1958). *Flore de I'Afrique du Nord* (Vol. 5, pp. 284-298). Paris.

Mahklouf, H.M., & Etayeb, S.K. (2018). Biodiversity in Libya: Selected countries in Africa. *Global Biodiversity*, *3*(5), 113-132.

Magulaev, A. V. (1984). Cytotaxonomic study in some flowering plants of the north Caucasus. *Botanicheskii Zhurnal*, *69* (4), 511-517.

Marchant, C.J. (1973). Chromosome variation in Araceae: V. Acoreae to Lasieae. *Kew Bulletin*, *28* (2), 199-210.

Markova, M., & Goranova, V. (1995). Mediterranean chromosome number reports 5 (435-473). *Flora Mediterranea*, *5*, 289-317.

Martin, E., Altinordu, F., Celep, F., Kahraman, A., & Doğan, M. (2015). Karyomorphological studies in seven taxa of the genus *Salvia* (Lamiaceae) in Turkey. *Caryologia*, *68*(1), 13-18.

Martin,E., Dirmenci,T., Arabaci, T., Yazici, T., & Özcan, T. (2020). Karyotype studies on the genus *Origanum* L.(Lamiaceae) species and some hybrids defining homoploidy. *Caryologia*, *73*(2), 127-143.

Martin,E., Bozkurt, H., Kahraman, A., & Dirmenci, T. (2022). New chromosomal data and karyological relationships in *Geranium*: Basic number alterations, dysploidy, polyploidy, and karyotype asymmetry. *Brazilian Archives of Biology and Technology*, *65*(1).

Martins, J., & Canhoto, J. (2014). Biotecnologia do medronheiro (*Arbutus unedo* L.): Ensaios de cultura *in vitro* e hibridação. *Actas Portuguesas de Horticultura*, *24*, 494-499.

Martins, J., Correia, S., Pinto, G., & Canhoto, J. (2022). Cloning adult trees of *Arbutus unedo* L. through somatic embryogenesis. *Plant Cell Tissue and Organ Culture*, *150*(3), 611-626.

Mckelvey, D. S., & Sax, K. (1933). Taxonomic and cytological relationships of *Yucca* and *Agave*. *Journal of the Arnold Arboretum*, *14*(1), 76-81.

Mehra, P.N., & Bawa, K.S.(1972). Cytogenetical evolution of hardwoods. *Nucleus*, *15*, 64-83.

Meusel, H., Jaeger, E., & Weinert, E. (1965).Vergleichende Chorologie der zentra-leuropäischen Flora. *Flora*, 1.

Middleton, D.J., & Wilcock, C.C. (1990). Chromosome counts in *Gaultheria* and related genera. *Edinburgh Journal of Botany*, *47*(3), 303-313.

Mirzaghaderi, G., & Marzangi, K. (2015). IdeoKar: An ideogram constructing and karyotype analyzing software. *Caryologia*, *68*(1), 31-35.

Mossa, L., & Scrugli, A.E. (1970). Osservazioni cariologiche in *Allium chamaemoly* L. *Morisia*, *2*, 53-62.

Morrone, J.J. (2008). Endemism. *Evolutionary Ecology*, *2*(5), 1254-1259.

Mugnier, C., & Siljak-Yakovlev, S. (1987). Karyological study in some Yugoslavian populations of Hypochoeris(Compositae). *Caryologia*, *40*, 319–325.

Muliawati,E.S., Hartati,S., Parjanto,P., Sukaya,S., Nandariyah,N., Yuniastuti,E., Manurung, I.R., & Purmiyoto, C.W.W. (2023). Karyotype of *Phaius tankervilleae* and *Phaius amboinensis* orchid. *E3S Web Conferences*, *373*, 03029.

NASA/POWER SRB/FLASH Flux/MERRA2/GEOS 5.12.4. (n.d.). *FP-IT (0.5x 0.5) degree daily average data*. Retrieved June 12, 2025, from http://power.larc.nasa.gov/

Nelson, E.C., & Oliver, E.G.H. (2005). Chromosome numbers in Erica-An updated checklist. *Heathers*, *2*, 57-58.

Pampanini, R. (1931). *Prodromo della Flora Cirenaica*. Forli.

Pampanini, R. (1933). La vegetazione spontanea della Cirenaica. *Rendiconti del Seminario Scientifico della Reale Universitá di Cagliari*, *3*, 157-158.

Pampanini, R. (1936). Aggiunte e correzioni al "Prodromo della Flora Cirenaica". *Archivio Botanico*, *12*, 17-53.

Pastor, J., Diosdado, J. C., Bárbara, C. S., Vique, J., & Pérez, E. (1990). Números cromosómicos para la flora Española, 556-591. *Lagascalia*, *15*, 269-282.

Paszko, B. (2006). A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution*, *258*(1), 39-48.

Peruzzi, L., Leitch, I.J., & Caparelli ,K.F. (2009). Chromosome diversity and evolution in Liliaceae. *Annals of Botany*, *103*, 459–475.

Peruzzi, L., & Eroğlu, H.E. (2013). Karyotype asymmetry: Again, how to measure and what to measure?. *Comparative Cytogenetics*, *7*(1), 1-9.

Peruzzi, L., & Altinordu, F. (2014). A proposal for a multivariate quantitative approach to infer karyological relationships among taxa. *Comparative Cytogenetic*, *8*(4), 337-349.

Peruzzi, L., Franzoni, J., Tiburtini, M., Abidi, M., Alu, E., Barone, G., Bianchi, E., Cataudella, C., Di Iorio, E., Guerrina, M., Mondello, F., Paino, L., Pentassuglia, M., Porrovecchio, M., & Rivieccio, G. (2024). Different observes introduce not negligible biases in comparative karyomorphological studies. *Comparative Cytogenetics*, *18*,175-182.

Petersen, G. (1993). Chromosome numbers of the genera Araceae. *Aroideana*, *16*(1), 37-46.

The Plant List.(2024). *The Plant List*. Facilitated by the Royal Botanic Gardens, Kew, and Missouri Botanical Garden. Retrieved November 5, 2024, from https://theplantlist.org/

Pound, G. E., Cox, S. J., & Doncaster, C. P. (2004). The accumulation of deleterious mutations within the frozen niche variation hypothesis. *Journal of Evolutionary Biology*, *17*(3), 651-662.

POWO. (2024). *Plants of the World Online | Kew Science*. Facilitated by the Royal Botanic Gardens, Kew. Retrieved October26,2024, from https://powo.science.kew.org/

Preston, R.J. (2014). Chromosome aberrations. In *Encyclopedia of Toxicology* (3rd ed., pp. 955-958). Elsevier.

Prime, C. T. (1980). *Arum* L. In T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D. H.Valentine, S.M.Walters, & D.A.Webb (Eds.), *Flora Europaea* (Vol. 5, pp.269-271). Cambridge University Press.

Otto, S. P., & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, *34*,401-437.

Qaiser, M., & El-Gadi, A. (1984). A critical analysis of the flora of Libya. *Libyan Science Journal*, *13*, 31-40.

Radford, E.A., Catullo, G., & de Montmollin, B. (Eds.). (2011). *Important plant areas of the south and east Mediterranean region: priority sites for conservation*. IUCN, Gland, Switzerland and Malaga, Spain.

Rasband, W.S. (2012). Image processing and analysis in Java. *Astrophysics Source Code Library*, ascl:1206.013.

Raven, P.H. (1975). The bases of angiosperm phylogeny: Cytology. *Annals of the Missouri Botanical Garden*, *62*, 724-764.

Reed, B.M., Sarasan, V., Kane, M., Bunn, E., & Pence, V.C. (2011). Biodiversity conservation and conservation biotechnology tools. *In Vitro Cellular and Developmental Biology-Plant*, *47*, 1-4.

Rice,A., Glick, L., Abadi,S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O., & Mayrose, I. (2014). The Chromosome Counts Database (CCDB)-A community resource of plant chromosome numbers. *New Phytologist*, *206*(1), 19-26.

Rivero, R., Sessa, E.B., & Ferguson, R.Z. (2019). EyeChrom and CCDBcurator: Visualizing chromosome count data from plants. *Applications in Plant Sciences*, *7*(1):1-5.

Rice,A., Glick, L., Abadi,S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O., & Mayrose, I. (2014). The Chromosome Counts Database (CCDB)-A community resource of plant chromosome numbers. *New Phytologist*, *206*(1), 19-26.

Saaed, M.W.B., El-Barasi, Y.M., & EL-Shaikhy, A. (2022). Noteworthy records of the lichens associated with *Juniperus phoenicea* L. die-off in El-Jabal El-Akhdar region, NE Libya. *Ecologia Mediterranea*, *47*(2), 41-49.

Saensouk,S., Saensouk, P., & Senavongse, R. (2019). Karyological study of three Thailand species *Colocasia* (Araceae). *Cytologia*, *84*(2), 179-182.

Saensouk, P., Saensouk, S., & Senavongse, R. (2022). Cytogenetic studies of six species in family Araceae from Thailand. *Caryologia*, *75*(2), 5-13.

Samaropoulou, S., Bareka, P., & Kamari, G. (2019). Hybridization and karyotype variability of three endemic *Fritillaria* L. (Liliaceae) in Argolis Peninsula (Greece). *Plant Biosystems*, *154*(3), 1-13.

Schönswetter, P., Lachmayer, M., Lettner, C., Prehsler, D., Rechnitzer, S., Reich, D. S., & Trávníček, P. (2007). Sympatric diploid and hexaploid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. *Journal of Plant Research*, *120*(6), 721-725.

Sealy, J.R., & Webb, D.A. (1950). *Arbutus unedo* L. *Journal of Ecology*, *38*(1), 223-236.

Senavongse, R., Saensouk, S., & Saensouk, P. (2018). Comparative karyotype analysis in five strains of *Colocasia esculenta* Schott in Thailand. *Cytologia*, *83*(2), 169-173.

Sharaf, A.T.(1971). *Geography of Libya*. Monshaat Al-Maaref, Alexandria, Egypt.

Sheidai, M., Nasirzadeh, A., & Kheradnam, M. (2000). Karyotypic study of *Echinops* (Asteraceae) in Fars province. *Botanical Journal of the Linnean Society*, *134*, 453-463.

Singh, R., Kaur, R., & Tanisha, A. (2023). Chromosome number of some medicinal angiosperms: A review article. *International Journal of Botany Studies*, *8*(7), 22-25.

Siljak-Yakovlev, S., & Peruzzi, L. (2012). Cytogenetic characterization of endemics: Past and future. *Plant Biosystems*, *146*(3), 694-702.

Soltis, P.S., Marchant, D.B., Van de Peer, Y., & Soltis, D.E. (2015). Polyploidy and genome evolution in plants. *Current Opinion in Genetics and Development*, *35*,119-125.

Stace, C. A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. *Taxon*, *49*, 451-477.

Stebbins, G.L. (1950). *Variation and evolution in plants*. Columbia University Press, New York, USA.

Stebbins, G. L., & Major, J. (1965). Endemism and speciation in the California flora. *Ecological Monographs*, *35*(1), 1-35.

Stebbins, G. L. (1971). *Chromosomal evolution in higher plants*. Edward Arnold (Publishers) Ltd., London, UK.

Strasburger, E.(1910). Chromosomenzahl. *Flora*, *100*(3), 398-446.

Stuessy, T.F., Weiss-Schneeweiss, H., & Keil, D.J. (2004). Diploid and polyploid cytotype distribution in *Melampodium cinereum* and *M. leucanthum* (Asteraceae, Heliantheae). *American Journal of Botany*, *91*, 889-898.

Stuessy, T.F. (2009). *Plant taxonomy*: The systematic evaluation of comparative data. Columbia University Press, New York.

Sun, W. G., Zhang, Y. Z., Peng, D. L., Zhang, Y. H., Zhang, J. W., & Li, Z. M. (2016). Karyotype of nine endemic species from alpine subnival belt in the Hengduan Mountains, SW China. *The Journal of Japanese Botany*, *91*(4), 242-249.

Sun, W.G., Sun, H., & Li, Z.M. (2019). Chromosome data mining and its application in plant diversity research. *Plant Science Journal*, *37*(2), 260-269.

Taylor, R. L., & Taylor, S. (1977). Chromosome numbers of vascular plants of British Columbia. *Syesis*, *10*, 125-138.

Terpó, A. (1973). Kritische Revision der *Arum*-Arten des Karpatenbeckens. *Acta Botanica Academiae Scientiarum Hungaricae*, *18*, 216-255.

Thor, G., & Nascimbene, J. (2010). An annotated checklist and bibliography of lichens and lichenicolous fungi of Libya. *Cryptogamie Mycologie*, *31*(1), 67-95.

Torres, J. A., Valle, F., Pinto, C., Garcia-Fuentes, A., Salazar, C., & Cano, E. (2002). *Arbutus unedo* L. Communities in Southern Iberian Peninsula Mountains. *Plant Ecology*,*160*(2),207-223.

Turco, A., Medagli, P., Albano, A., & D'Emerico, S. (2014). Karyomorphometry on three polyploid species of *Arum* L. (Araceae, Aroideae). *Comparative Cytogenetics*, *8*(1),71-80.

Uysal, T., Bozkurt, M., Sezer, E.N.S., Ertugrul, K., & Tugay, O. (2015). Karyological studies of four endemic *Centaurea* L. species. *Caryologia*, *68*(4), 339-346.

Uysal, T., Tekkanat, B.S., Sezer, E.N.S., Ada, R., & Bozkurt, M. (2018). Karyotype analysis of some lines and varieties belonging to *Carthamus tinctorius* L. species. *Anatolian Journal of Botany*, *2*(1), 1-9.

Vargas, S.M., Torres, G.A., Sobrinho, F.S., Pereira, A.V., & Davide,L.C. (2007). Karyotypic studies of *Cratylia argentea* (Desv.), *C.kuntze* and *C.mollis* Mart. Ex Benth. (Fabaceae-Papilionoideae). *Genetic Molecular Research*, *6*(3), 707-712.

Venkatesh, K.H., Dinesh, B., Venu, N., & Munirajappa. (2019). Chromosome numbers and karyotype studies of few members of *Malvales*. *American Journal of Phytomedicine and Clinical Therapeutics*, *3*(2), 178-184.

Venora, G., Blangiforti, S., Castiglione, M.R., Pignone, D., Losavio, F., & Cremonini, R. (2002). Chromatin organisation and computer aided karyotyping of *Triticum durum* Desf. cv *Timilia*. *Caryologia*, *55*, 91–98.

Villa, S., Montagna, M., & Pierce, S. (2022). Endemism in recently diverged angiosperms is associated with polyploidy. *Plant Ecology*, *223*(4), 479-492.

Von Bothmer, R. (1970). Studies in the Aegean flora XV. Chromosome numbers in Labiatae. *Botaniska Notiser*, *123*, 52-60.

Watanabe, K., Yahara, T., Denda, T., & Kosuge, K. (1999). Chromosomal evolution in the genus *Brachyscome* (Asteraceae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. *Journal of Plant Research*, *112*(2), 145-161.

Weiss-Schneeweiss, H., & Schneeweiss, G.M. (2012). Karyotype diversity and evolutionary trends in angiosperms. Genome size and the phenotype. In: I.J.Leitch, J. Greilhuber, J. Doležel, & J.Wendel (Eds.), *Plant Genome diversity* (Vol. 2, pp. 209-230). Springer-Verlag, Vienna.

Whittemore, A. T., & Olsen, R. T. (2011). *Ulmus americana* (Ulmaceae) is a polyploid complex. *American Journal of Botany*, *98*(4), 754-760.

WCMC. (1992). *World Conservation Monitoring Centre*: *Global biodiversity status of the earth's living resources* (p. 585). Chapman and Hall, London.

Yildiz, K., & Gücel, S. (2006). Chromosome numbers of 16 endemic plant taxa from northern Cyprus. *Turkish Journal of Botany*, *30*(3), 181-192.

Yu, X. F., Zhang, H. Q., Yuan, M., & Zhou, Y. H. (2009). Karyotype studies on ten *Iris* species (Iridaceae) from Sichuan China. *Caryologia*, *62*(3), 253-260.

Zhang, Y., Zhu, M.L., & Dai, S.L. (2013). Analysis of karyotype diversity of 40 Chinese *Chrysanthemum* cultivars. *Journal of Systematics and Evolution*, *51*, 335-352.

Zhao, F., Chen, Y.P., Salmaki, Y., Drew, B.T., Wilson, T.C., Scheen, A.C., Celep, F., Christian, B., Mika, B., Wang, Q., Min, D.Z., Peng, H., Olmstead, R.G., Li,

B., & Xiang, C.L. (2021). An updated tribal classification of Lamiaceae based on plastome phylogenomics. *BMC Biology*, 19(1): 1-27.

Zuo,L., & Yuan, Q. (2011). The difference between the heterogeneity of the centromeric index and intrachromosomal asymmetry. *Systematics and Evolution*, *297*(1),141-145.

Appendix 1. Check list of the plants endemic to the El-Jabal El-Akhdar region.

No.	Scientific name	Accepted name	Family	New family	Distribution
1	*Stachys rosea* (Desf.) Boiss.		Lamiaceae		Susa, Wadi El-Kouf, Wadi Derna
2	*Ballota andreuzziana* Pamp.		Lamiaceae		Shahat, Susa, Qaser Libya, Derna
3	*Micromeria guicharddii* (Quez. & Zaff.) Brullo & Furnari		Lamiaceae		Al-Marj
4	*Micromeria Juliana* var. *conferta* Coss. & Daveau	*Micromeria conferta* (Coss. & Daveau) Stefani	Lamiaceae		Al-Marj, Al-Gubba, Derna, Ras Al-hilal
5	*Nepeta cyrenaica* Quézel & Zaffran		Lamiaceae		Qaser Libya, Al-Marj
6	*Nepeta vivianii* (Coss.) Bég. & Vacc.		Lamiaceae		Qaser Libya, Al-Bayda
7	*Origanum cyrenaicum* Bég. & Vacc.		Lamiaceae		Wadi El-Kouf, Al-Gubba, Shahat,Derna
8	*Teucrium apollinis* Maire & Wailler		Lamiaceae		Al-Abiar, Wadi Derna
9	*Teucrium barbeyanum* Asch. & Taub.		Lamiaceae		Tolmitha, Tocra, Al-Marj, Messa
10	*Teucrium davaeanum* Coss.		Lamiaceae		Al-Benieh, Derna
11	*Teucrium zanonii* Pamp.		Lamiaceae		Benghazi
12	*Thymbra linearifolia* (Brullo & Furnari) Brauchler		Lamiaceae		Bersis, Wadi zaza
13	*Cicerbita haimanniana* Beauverd	*Lactuca haimanniana Asch.*	Asteraceae		Shahat, Al-Mansora, Wadi El-Kouf
14	*Tolpis vurgata* subsp. *apolloniae* Brullo & Furnari		Asteraceae		Shahat, Derna

Appendix 1. (continued). Check list of the plants endemic to the El-Jabal El-Akhdar region.

No.	Scientific name	Accepted name	Family	New family	Distribution
15	*Anthemis toubertii* Barratte & E.A.Durand		Asteraceae		Al-Bakur, Zawiat El-Qsor, Taknes
16	*Anthemis cyrenaica* var. *radiate* Pamp.	*Anthemis cyrenaica*	Asteraceae		Wadi Derna, Taknes, Susa
17	*Centaurea cyrenaica* Bég. & Vacc.		Asteraceae		Tolmitha, Labrag, Lamlouda
18	*Pallenis cyrenaica* Alavi		Asteraceae		Taknes, Zawiat El-Qsor, Shahat
19	*Echinops cyrenaicus* E.A.Durand & Barratte		Asteraceae		Al-Abiar, Al-Bakur, Wadi El-Kouf, Derna
20	*Onopordum cyrenaicum* Maire & Weiller		Asteraceae		Al-Abiar, Zawiat El-Qsor, El-Hamamah, Wadi El-Kouf
21	*Picris mauginiana* Pamp.		Asteraceae		Al-Marj, Wadi El-Kouf
22	*Senecio trilobus* L.		Asteraceae		Daryana
23	*Sedum mirum* Pamp.	*Umbilicus mirus* (Pamp.) Greuter	Crassulaceae		Al-Bayda
24	*Sedum cyrenaicum* Brullo & Furnari	*Sedum creticum* subsp.*cyrenaicum* (Brullo & Furnari) Afferni	Crassulaceae		Al-Bayda
25	*Sedum bracteatum* Viv.		Crassulaceae		Jardas, Slonta, Lamlouda, Al-Marj, Daryana

Appendix 1.(continued). Check list of the plants endemic to the El-Jabal El-Akhdar region.

NO.	Scientific name	Accepted name	Family	New family	Distribution
26	*Limonium cyrenaicum* (Rouy) Brullo.		Plumbaginaceae		Susa, Derna, Ras Al-hilal
27	*Limonium subrotundifolium*		Plumbaginaceae		Derna
28	*Limonium teuchirae* Brullo		Plumbaginaceae		Benghazi
29	*Pachyctenium mirabile* Maire & Pamp.	*Daucus mirabilis* (Maire & Pamp.) Reduron, Banasiak & Spalik	Umbiliferae	Apiaceae	Labrag, Shahat
30	*Athamanta della-cellae Asch. & Barbey ex E.A.Durand & Barratte*	*Daucus della-cellae (Asch. & Barbey ex E.A.Durand & Barratte) Spalik, Banasiak & Reduron*	Apiaceae		Wadi El-Kouf, Wadi Derna, Susa
31	*Libyella cyrenaica* (E.A.Durand & Barratte) Pamp.	*Poa cyrenaica* E.A.Durand & Barratte	Gramineae	Poaceae	Benghazi
32	*Poa pentapolitana* H.Scholz		Poaceae		Labrag, Shahat
33	*Silene cyrenaica* Maire & Weiler		Caryophyllaceae		Al-Bayda, Al-Mansora, Wadi Zaza
34	*Petrohagia rupestris* Brullo & Furnari		Caryophyllaceae		Wadi El-Kouf
35	*Petrohagia cyrenaica* (E.A.Durand & Barrate) Ball.		Caryophyllaceae		Al-Marj, El-Hania, Lamlouda
36	*Bellevalia cyrenaica* Maire & Weiller		Liliaceae	Asparagaceae	Bata, Wadi El-Kouf
37	*Scilla cyrenaica* (Pamp.) Bartolo, Brullo, Pavone & Terrasi	*Prospero cyrenaica* (Pamp.) Speta	Liliaceae	Asparagaceae	Tocra, Wadi El-Kouf, Derna, Ras Al-hilal
38	*Gagea trinervia* (Viv.) Greuter		Liliaceae		(Sidi Rafa) Al-Bayda

Appendix 1.(continued). Check list of the plants endemic to the El-Jabal El-Akhdar region.

No.	Scientific name	Accepted name	Family	New family	Distribution
39	*Crocus boulosii* Greuter		Iridaceae		Marauah, Wadi El-Kouf
40	*Romulea cyrenaica* Bég.		Iridaceae		Tolmitha, Al-Abiar, Martoba, Labrag
41	*Orchis cyrenaica* E.A.Durand & Barratte	*Anacamptis cyrenaica* (E.A.Durand & Barratte) H.Kretzschmar, Eccarius & H.Dietr	Orcidaceae		Slonta, Shahat, Al-Gubba
42	*Euphorbia pseudoapios* Maire & Weiller		Euphorbiaceae		Qayqab
43	*Hypericum decaisneanum* Coss.& Daveau		Guttiferae	Hypericaceae	Al-Gubba, Al-Bayda,Derna
44	*Orobanche cyrenaica* Beck ex E.A.Durand & Barratte		Orobanchaceae		Shahat, Susa, Al-Gubba
45	*Plantago lagopus* subsp. *ptolemaidis* Brullo & Furnari		Plantaginaceae		Tolmitha
46	*Plantago cyrenaica* E.A.Durand & Barratte		Plantaginaceae		El-Auelia, Wadi Bilkaf, Qaser Libya, Jarjar-oma
47	*Allium ruhmerianum* Asch. ex E.A.Durand & Barratte		Alliaceae	Amaryllidaceae	Tolmitha, Wadi El-Kouf
48	*Arum cyrenaicum* Hruby.		Araceae		Tolmitha, Tocra, Bata, Qaser Libya, Slonta
49	*Convolvulus maireanus* Pamp.		Convolvulaceae		Tolmitha, Bata, Ras Al-hilal
50	*Erodium tocranum* Guitt. & Le Houér.	*Erodium salzmannii* subsp.*tocranum* (Guitt. & Le Houér.) Guitt.	Geraniaceae		Tocra
51	*Erodium hirtum* var. *cyrenaicum* Pamp.	*Erodium cyrenaicum* (Pamp.) Guitt.	Geraniaceae		Derna

Appendix 1.(continued). Check list of the plants endemic to the El-Jabal El-Akhdar region.

No.	Scientific name	Accepted name	Family	New Family	Distribution
52	*Lonicera nummulariifolia* subsp. *occidentalis* (Pamp.) Brullo & Furnari		Caprifoliaceae		Wadi El-Kouf
53	*Scabiosa libyca* Alavi		Dipsacaceae	Caprifoliaceae	Al-Marj, Taknes, Zawiat El-Qsor, Shahat
54	*Arbutus pavarii* Pamp.		Ericaceae		between El-Garib and Tolmitha, Wadi El-Fahaga
55	*Polygala aschersoniana* Chodat.		Polygalaceae		Taknes, Sidi Al-Hamri
56	*Cyclamen rohlfsianum* Asch.		Primulaceae		Tocra to Derna
57	*Asperula hirsuta* var. *cyrenaica* (E.A.Durand & Barrate)	*Hexaphylla cyrenaica* (E.A.Durand & Barrate) P. Caputo & Del Guacchio	Rubiaceae		EL-Jabal EL-Akhdar
58	*Ranunculus cyclocarpus* Pamp.		Ranunculaceae		Al-Abiar, Wadi El-Kouf
59	*Rhamnus pendula* Pamp.	*Rhamnus alaternus* subsp. *pendula* (Pamp.) Jafri	Rhamnaceae		Shahat, Ras Al- hilal
60	*Thesium erythronicum* Pamp.		Santalaceae		Al-Bayda
61	*Fumaria macrocarpa* Parlatore	*Fumaria macrocarpa* subsp.*cyrenaica* Liden	Fumariaceae	Papaveraceae	Shahat, Al-Gubba, Derna, Al-Bayda

Appendix 2. karyotype of some endemic plants in the El-Jabal El-Akhdar region according to IPCN.

Endemic plant species	Basic chromosome number (*x*)	Chromosome numbers (2*n*) and ploidy levels	Karyotype Formula (KF)	Karyograms, Chromosome measurements, and Karyotype indices	References
Cyclamen rohlfsainum	*x*= **?**	2*n* = 96	**?**	**?**	(Legro,1959)
Erodium salzmannii subsp. *tocranum*	*x*= **?**	2*n* = 6*x* = 60	**?**	**?**	(Guittonneau & Le Houérou,1968)
Arum cyrenaicum	*x*= 7	2*n* = 28	**?**	**?**	(Marchant,1973)
Arum cyrenaicum	*x*= 14	2*n* = 4*x* = 56	**?**	**?**	(Boyce,1989)
Allium ruhmerianum	*x*= 11	2*n* = 3*x* = 33	33m	**?**	(Bartolo *et al*.,1984)
Bellevalia cyrenaica	*x*= 4	2*n* = 2*x* = 8	2m+4sm+2st	**?**	(Bartolo *et al*.,1984)
Prospero cyrenaicum	*x*= 7	2*n* = 2*x* = 14	6m+4sm+2st+2t	**?**	(Bartolo *et al*.,1984)
Lactuca haimanniana	*x*= **?**	2*n* = 2*x* = 16	**?**	**?**	(Brullo *et al*.,1990)
Anthemis taubertii	*x*= 9	2*n* = 2*x* = 18	6m+2sm+6st+4t	**?**	(Brullo *et al*.,1990)
Anthemis cyrenaica	*x*= 9	2*n* = 2*x* = 18	8m+4sm+4st+2t	**?**	(Brullo *et al*.,1990)

Appendix 2. (continued). karyotype of some endemic plants in the El-Jabal El-Akhdar region according to IPCN.

Endemic plant species	Basic chromosome number (*x*)	Chromosome numbers (2*n*) and ploidy levels	Karyotype Formula (KF)	Karyograms, Chromosome measurements, and Karyotype indices	References
Limonium teuchirae	*x*= 9	2*n* = 3*x* = 27	**?**	**?**	(Brullo *et al*.,1990)
Limonium subrotundifolium	*x*= 9	2*n* = 4*x* = 32	**?**	**?**	(Brullo *et al*.,1990)
Limonium cyrenaicum	*x*= 9	2*n* = 6*x* = 54	**?**	**?**	(Brullo *et al*.,1990)
Centaurea cyrenaica	*x*= **?**	2*n* = 2*x* = 18	**?**	**?**	(Brullo *et al*.,1990)
Picris mauginiana	*x*= 5	2*n* = 2*x* = 10	2m+4sm+4st	**?**	(Brullo *et al*.,1990)
Tolpis virgata subsp. *apolloniae*	*x*=**?**	2*n* = 6*x* = 54	**?**	**?**	(Brullo *et al*.,1990)
Senecio trilobus	*x*=**?**	2*n* = 20	**?**	**?**	(Brullo *et al*.,1990)
Ranunculus cyclocarpus	*x* =**?**	2*n* = 16	**?**	**?**	(Brullo *et al*.,1990)

Appendix 3. Chromosome numbers of some species belong to *Arum* genus according to IPCN.

Species	Chromosome numbers(2*n*)	Reference
A.byzantinum	2*n*=28	(Boyce,1989)
A.creticum	2*n*=28	(Boyce,1989)
A.cyrenaicum	2*n*=28,56	(Marchant,1973; Boyce,1989)
A.orientale	2*n*=28	(D'Emerico *et al*.,1993; Bedalov & Drenkovski,1997)
A.alpinum	2*n*=28	(D'Emerico *et al*.,1993; Bedalov & Drenkovski,1997)
A.nigrum	2*n*=28	(D'Emerico *et al*.,1993)
A.pictum	2*n*=28	(D'Emerico *et al*.,1993)
A.cylindraceum	2*n*=28	(Bedalov *et al*.,2006)
A.dioscoridis	2*n*=28	(Christou *et al*.,2008)
A.maculatum	2*n*=56	(D'Emerico *et al*.,1993; Bedalov & Drenkovski,1997; Turco *et al*.,2014)
A.apulum	2*n*=56	(Bianco *et al*., 1994; Turco *et al*.,2014)
A.italicum	2*n*=84	(Turco *et al*., 2014)
A.sintenisii	2*n*=28	(Hand,2015)
A.hygrophilum	2*n*=28	(Hand,2015)

Appendix 4. Chromosome numbers of some species belong to *Arbutus* genus according to IPCN.

Species	**Chromosome numbers(2*n*)**	**Reference**
A.unedo	2*n*= 26	(Sealy & Webb,1950; Martins *et al*.,2022)
A.andrachne	2*n*= 26	(Darlington & Wylie,1955)
A.canariensis	2*n*= 26	(Darlington & Wylie,1955)
A.xalapensis	2*n*= 26	(Darlington & Wylie,1955)
A.menziesii	2*n*= 26	(Taylor & Taylor,1977)

Appendix 5. Chromosome numbers of some species belong to *Origanum* genus according to IPCN.

Species	Chromosome numbers (2*n*)	Reference
O. calcaratum	2*n* = 30	(Von Bothmer,1970)
O. dictamnus L.	2*n* = 30	(Lepper,1970)
O.syriacum subsp. *bevanii*	2*n* = 30	(Balim & Kesercioğlu,1998; Yildiz & Gücel, 2006; Martin *et al*.,2020)
O.vulgare subsp. *virens*	2*n* = 30	(Fernandes & Leitão, 1984; Pastor *et al*. 1990, Bakha *et al*.,2017)
O.vulgare subsp. *hirtum*	2*n* = 30	(Markova & Goranova,1995; Dirmenci *et al*.,2018b; Martin *et al*.,2020)
O.vulgare subsp. *viride*	2*n* = 30	(Martin *et al*.,2020)
O.vulgare subsp. *gracile*	2*n* = 30	(Dirmenci *et al*., 2019; Martin *et al*.,2020)
O.vulgare subsp. *viridulum*	2*n* = 30	(Martin *et al*.,2020)
O.vulgare subsp. *vulgare*	2*n* = 30	(Gill,1981; Khatoon & Ali, 1993; Dobea *et al*., 1997; Martin *et al*.,2020)
O.vulgare L.	2*n* = 28	(Magulaev,1984)
O.vulgare L.	2*n* = 32	(Ayyangar & Vembu,1985)
O.ayliniae	2*n* = 30	(Dirmenci *et al*.,2018a; Martin *et al*.,2020)
O.boissieri	2*n* = 30	(Kitiki,1997; Dirmenci *et al*.,2018b; Martin *et al*.,2020)
O.saccatum	2*n* = 30	(Kitiki,1997; Martin *et al*.,2020)
O.onites	2*n* = 30	(Von Bothmer,1970; Bakha *et al*., 2017; Martin *et al.,*2020)
O.elongatum	2*n* = 30	(Bastida & Talavera,1994; Bakha *et al*.,2017)

Appendix 5.(continued). Chromosome numbers of some species belong to *Origanum* genus according to IPCN.

Species	Chromosome numbers (2*n*)	Reference
O.grosii	2*n* = 30	(Bakha *et al*.,2017)
O.compactum	2*n* = 30	(Bakha *et al*.,2017)
O.laevigatum	2*n* = 30	(Balim & Kesercioğlu,1998; Martin *et al*.,2020)
O.majorana	2*n* = 30	(Fernandes & Leitão,1984; Balim & Kesercioğlu,1998; Martin *et al*.,2020)
O.acutidens	2*n* = 30	(Dirmenci *et al*., 2019; Martin *et al*.,2020)
O.leptocladum	2*n* = 30	(Martin *et al*.,2020)
O.minutiflorum	2*n* = 30	(Martin *et al*.,2020)
O.brevidens	2*n* = 30	(Martin *et al*.,2020)
O.husnucan-baseri	2*n* = 30	(Martin *et al*.,2020)
O.haussknechtii	2*n* = 30	(Martin *et al*.,2020)
O.amanum	2*n* = 30	(Lepper,1970; Martin *et al*.,2020)
O.hypericifolium	2*n* = 30	(Martin *et al*.,2020)
O.bilgeri	2*n* = 30	(Martin *et al*.,2020)
O.vogelii	2*n* = 30	(Martin *et al*.,2020)
O.rotundifolium	2*n* = 28	(Martin *et al*.,2020)
O.sipyleum	2*n* = 28,30	(Martin *et al*.,2020)

المستخلص

حددت هذه الدراسة عدد الصبغيات و الهيئة الصبغية لثلاثة أنواع متوطنة، و هي : الرينش *Arum cyrenaicum* (القلقاسية), الشماري *Arbutus pavarii* (الخلنجية) ,اللتان جمعتا من موقع طلميثة, بالإضافة إلى المرتوشة *Origanum cyrenaicum* (الشفوية) التي تم جمعها من موقع القبة في منطقة الجبل الاخضر. تتميز هذه النباتات بقيمة غذائية و طبية هامة , حيث تقدم دراسة وتعريف عدد الصبغيات و الهيئة الصبغية معلومات وراثية مهمه لهذه النباتات. تم تحليل نطاق طول الصبغيات (CL) , نسبة الأذرع الصبغية (AR), مؤشر النسبة المئوية للجزيء المركزي (CI%), مؤشر التجانس (S%), مؤشر درجة عدم تجانس النمط النووي (A) , مؤشر أرانو لعدم تجانس النمط النووي (Ask%), مؤشر اجمالي النسبة المئوية للأزواج الصبغية الشقيقة (TF%), مؤشر عدم التجانس ضمن الصبغيات (A_1), مؤشر عدم التجانس بين الصبغيات (A_2), معامل التباين في أطوال الصبغيات (CV_{CL}), معامل التباين في موقع الجزيء المركزي (CV_{CI}) , و مؤشر عدم تجانس النمط النووي (AI) لجميع الانواع تحت الدراسة. اظهرت النتائج أن عدد الصبغيات و صيغة النمط النووي (KF) لكل من *Arum cyrenaicum* الرينش *Arbutus pavarii* , $2n = 4x = 56$ (6M+38m+10sm+2st(2SAT) الشماري $2n = 2x = 26$ (4M+16m+6sm(4SAT) و *Origanum cyrenaicum* المرتوشة $2n=2x=30$(6M+16m+8sm(3SAT). علاوة على ذلك، كان حجم الصبغيات في نبات *Arum cyrenaicum* متوسطا الى صغير, حيث يتراوح طول الصبغيات من الاكبر 4.72 ±.21 ميكرون إلى الاصغر 2.88 ±.01 ميكرون, في حين أن نبات *Origanum cyrenaicum* و نبات *Arbutus pavarii* لهما صبغيات صغيرة الحجم بأطوال تتراوح من3.19±.01 ميكرون إلى 1.98±.02 ميكرون و من 3.09±.03 ميكرون إلى 1.98±.03 ميكرون ,على التوالي. وفقا لتصنيف Stebbins لتقييم تجانس و عدم تجانس النمط النووي, فإن الهيئة الصبغية للأنواع *Arum cyrenaicum* , *Arbutus pavarii* , و *Origanum cyrenaicum* تنتمي للنوع 1A. لكل من *Arum cyrenaicum* , *Arbutus pavarii* و *Origanum cyrenaicum* هيئة صبغية متجانسة. تم ملاحظة وجود التوابع على زوجين صبغيين لنبات *Arum cyrenaicum* و على اربعة ازواج صبغية لنبات *Arbutus pavarii* وعلى ثلاثة ازواج صبغية لنبات *Origanum cyrenaicum*. تم تسجيل الهيئة الصبغية لهذه الأنواع الثلاثة لأول مرة , حيث تساهم هذه النتائج في الفهم العلمي لهذه النباتات من منظور وراثي.

الكلمات المفتاحية: عدد الصبغيات, الهيئة الصبغية, الرينش, الشماري, المرتوشة, الجبل الاخضر.

جامعة بنغازي

كلية الآداب والعلوم- المرج

قسم علم النبات

تحليل الهيئة الصبغية لبعض النباتات المتوطنة في منطقة الجبل الأخضر

مقدمة من

حليمة جبريل ادم يونس

تحت اشراف

د. جميلة عبدالحفيظ علي بشاشة

استاذ مساعد

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الاجازة العالية (الماجستير) في علم النبات

بتاريخ: 17\مايو\2025