

Original article

First Cytogenetic and Karyomorphological Analysis of Two Endemic Plant Species, *Arbutus pavarii* and *Origanum cyrenaicum*, from Al-Jabal Al-Akhdar, Libya

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Abstract

This study provides the first cytogenetic and karyomorphological analysis of two endemic Libyan plant species, *Arbutus pavarii* (Ericaceae) and *Origanum cyrenaicum* (Lamiaceae), collected from Tolmitha and Al-Gubba sites, respectively, in the Al-Jabal Al-Akhdar region, Libya. Chromosome number, karyotype structure, and a comprehensive set of karyomorphological parameters were investigated, including chromosome length, arm ratio, centromere index, symmetry indices, and intra- and interchromosomal asymmetry indices. The results showed that *Arbutus pavarii* is diploid with $2n = 2x = 26$ and a karyotype formula of $4M + 16m + 6sm$ (4SAT), whereas *Origanum cyrenaicum* has $2n = 2x = 30$ with a karyotype formula of $6M + 16m + 8sm$ (3SAT). Both species possess small-sized chromosomes, ranging from $3.09 \pm 0.03 \mu\text{m}$ in *Arbutus pavarii* to $1.98 \pm 0.02 \mu\text{m}$ in *Origanum cyrenaicum*. According to Stebbins' classification, the karyotypes of both species fall into type 1A, indicating a high degree of symmetry. Satellites were detected on four chromosome pairs in *Arbutus pavarii* and three pairs in *Origanum cyrenaicum*. These findings represent the first documented karyotypes of both species and provide baseline cytogenetic data that enhance their genetic characterization and support future taxonomic, evolutionary, and conservation studies.

Keywords. Chromosome Numbers, Karyotype, Endemic, *Arbutus Pavarii*, *Origanum Cyrenaicum*.

Introduction

Libya is one of the largest countries in North Africa, covering an area of approximately 1.7 million km². Most of its territory is dominated by desert and semi-desert ecosystems, while Mediterranean vegetation is largely confined to the narrow coastal region [1]. Consequently, the coastal strip and the Mediterranean mountain ranges represent the most important centers of plant diversity in the country [2]. Al-Jabal Al-Akhdar, located in northeastern Libya, is considered the most significant biodiversity hotspot in the country, despite occupying only about 1% of Libya's total land area [2]. The region is ecologically distinct and characterized by a Mediterranean climate [3], and it harbors approximately 70% of Libya's plant species, with more than 1,350 recorded taxa [4].

Arbutus pavarii (vernacular name: Shmari, (Figure 1)) is a plant species endemic to Libya [5] and belongs to the family Ericaceae, a large and morphologically diverse family of flowering plants that includes herbs, shrubs, and trees. In Libya, this family is represented by two genera, *Erica* and *Arbutus*, encompassing three species [6]. The genus *Arbutus* consists of about 12 species distributed across the Mediterranean region and the Americas [7]. The fruits of *Arbutus pavarii* are widely used in traditional medicine for the relief and prevention of several ailments, owing to their reported antiseptic, diuretic, and laxative properties. The leaves are also traditionally employed as remedies for urinary tract infections, constipation, and gastritis [8]. *Origanum cyrenaicum* (vernacular name: Martwosha; (Figure 20)) was first described as a Libyan endemic species by Béguinot and Vaccari [9]. *Origanum cyrenaicum* belongs to the family Lamiaceae, commonly known as the mint family, which is renowned for its aromatic oils with significant culinary, medicinal, and horticultural uses throughout history. Currently, the family comprises over 230 genera and more than 7,000 species worldwide [10], while the genus *Origanum* includes 43 species and 20 hybrids [11].

Chromosomal data are essential for differentiating between species, understanding the relationships between species through chromosomal similarities and variations, and for species identification [12]. All the reports concerning plant systematic studies need to know the genetic constitution of the taxon under study, and particularly its chromosome complement, together with its morphological and ecological description, to strengthen the uniqueness of the new entity [13]. 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 cytological datum is the number of chromosomes [14]. Also, at the species level, some chromosome characters, such as satellites, may be used to identify species in certain genus *Arbutus*. However, all species in the genus have the same chromosome numbers, which could not be used to identify species [15].



Figure 1. *Arbutus pavarii* Pamp. is a small tree, 1.5-3m tall. The bark is reddish-brown, the leaves are lanceolate to ovate in shape, 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.

Karyotype symmetry is another important aspect in cytogenetic analysis. Symmetric karyotypes exhibit minimal differences in chromosome size, with a higher proportion of metacentric chromosomes, whereas asymmetric karyotypes show marked size differences between large and small chromosomes and contain fewer metacentric chromosomes. Differences in centromere position, chromosome size, and shape form the basis of these classifications [16]. Interchromosomal and intrachromosomal asymmetries are commonly used to quantify karyotype asymmetry. Studying these asymmetries is a widely applied, inexpensive, and effective method in comparative cytogenetics, particularly in botanical studies. Interchromosomal asymmetry is often assessed using the coefficient of variation of chromosome length, a powerful statistical parameter for evaluating differences among chromosomes within a set [7].



Figure 2. *Origanum cyrenaicum* Bég. & Vacc. 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.

The cytology of the Ericaceae family has received limited attention. Raven [18] proposed that the *Ericales* had a basic chromosome number of $x = 6$ (like Epacridaceae), from which Ericaceae evolved with a basic number of $x = 12$, frequently resulting in dysploid derivatives, particularly $x = 11$ and $x = 13$. Among Ericaceae, *Rhododendron* is the only large genus that has been thoroughly examined. Although the genus *Arbutus* includes only a few species in the Mediterranean and American regions, previous chromosome studies were limited to chromosome counts and lacked information on karyotype morphology [19-20]. These studies consistently reported a basic chromosome number of $x = 13$ with diploid species ($2n = 2x = 26$). In contrast, literature on *Origanum* shows a notable scarcity of karyotype analyses, likely due to the small size of its chromosomes. Recorded chromosome counts within the genus are $2n = 28, 30,$ and 32 [21-22].

Karyotype studies have yet to be conducted for many endemic plant species in Libya; 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, this study aims to apply karyotype analysis on *Arbutus pavarii* and *Origanum cyrenaicum* to determine the chromosome numbers, sizes, morphology, ploidy level, and the occurrence of satellites of these endemic species.

Methods

Sites Description

The natural habitats of the plant species used in the present study were: 1) the southern highlands of the Tolmitha region, and 2) the forested areas near the Al-Gubba region. Both sites are located within the Al-Jabal Al-Akhdar region of Cyrenaica, northeastern Libya (Figure 3).



Figure 3. 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)

Plant Materials

Mature fruits of *Arbutus pavarii* were collected from Wadi Emleka, located approximately 19 kilometers from the Tolmitha region, on 11 November 2021. In contrast, mature inflorescences of *Origanum cyrenaicum*, containing fully developed seeds, were gathered from Wadi Bouhalfaya, situated about 3 kilometers from the Al-Gubba region, on 6 November 2022. The seeds of *Arbutus pavarii* and *Origanum cyrenaicum* were extracted from the fruits and inflorescences, respectively, thoroughly washed with distilled water to remove any remaining fruit pulp. They were then soaked in distilled water for one day before being placed on wet filter paper in Petri dishes. Germination was conducted at 10-15°C during a period of seven days for *Arbutus pavarii* and three days for *Origanum cyrenaicum*.

Experimental method

Root tips of the germinated seeds ranging from 0.5 to 0.6cm for *Arbutus pavarii* and 0.3 to 0.4cm for *Origanum cyrenaicum* 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) in vials for 24 hour, and then stored in 70% ethanol at 4°C until use. The fixed roots were hydrolyzed in 1N HCl at 60°C for 30 min, followed by a 20-minute soak in cold distilled water. They were then stained in 2% aceto-orcein for 17-20 min. Squash preparations were made by gently pressing the root tips under a coverslip for microscopic observation. At least ten metaphase cells were used to determine chromosome numbers and karyological characteristics. Photo-micrographs were taken with a microscope at 1000 x magnification.

Karyotype analysis

Detected metaphase plates were recorded by computer, and then karyotypes were performed by cutting and arranging chromosomes with homologous pairs based on arm ratio and chromosome size using the ImageJ program [23] and Adobe Photoshop 2020 program.

The chromosome type was determined using the centromere position and arm ratio and classified according to Levan et al. [24]. The degree of karyotype asymmetry was estimated with Stebbins's method [16]. The following chromosomal measurements were recorded: chromosome length (CL), arm ratio (AR = L/S), Index of relative length of chromosome (IRL% = (CL/ΣCL) × 100), centromere index (CI% = S/CL × 100). Also, the following karyotype parameters were documented: degree of karyotype asymmetry (A = Mean (L - S) / (L + S) [25], intrachromosomal asymmetry (A1 = 1 - Mean S / L), interchromosomal asymmetry (A2 = sCL/xCL) [26], coefficient of variation of chromosome length (CVcL = A2 × 100), coefficient of variation of centromere index (CVci = (sCI / x CI) × 100) [27], Arano index (Ask% = (ΣL/ΣCL) × 100) [28], symmetry index S% = (CLmin/CLmax) × 100 [25], total form percentage of homologous chromosome pairs TF% = (ΣS/ΣCL) × 100 [29] and Asym-metry index (AI = (CVcL × CVci) / 100) [27]. The Ideogram was drawn automatically by the Ideokar

program, and the mean and standard deviations were analyzed for all chromosomal and karyotype parameters using the SPSS (version 13.0) statistical program.

Results

The number, size, and asymmetry of chromosomes are important parameters to elucidate the phylogenetic relationships of species [30]. Chromosomal asymmetry indices have been widely used in interpreting and classifying plant karyotypes [31]. 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. The diploid chromosome number ($2n = 26$) (Figure 4) was observed in all the investigated populations of *Arbutus pavarii*, with the basic chromosome number of $x = 13$, and the ideogram generated automatically using the Ideokar program based on the basic chromosome number (Figure 6 A).

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) (Figure 6 A).

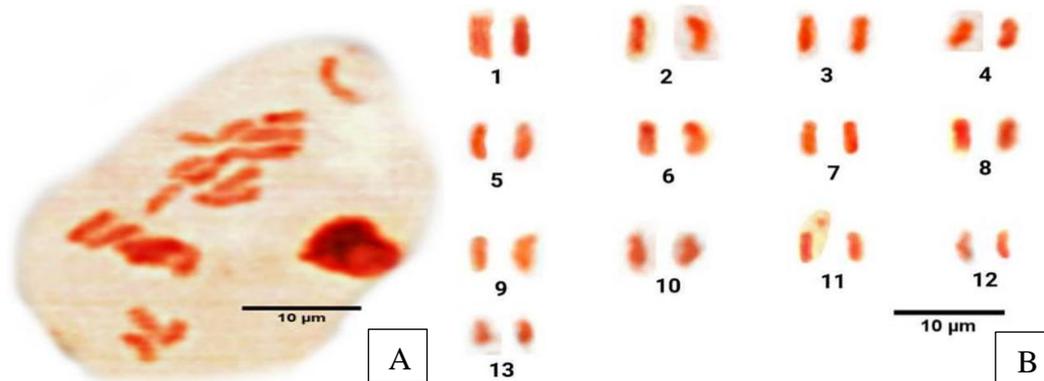


Figure 4. Metaphase chromosomes spread (A), and karyogram of *Arbutus pavarii* (B).

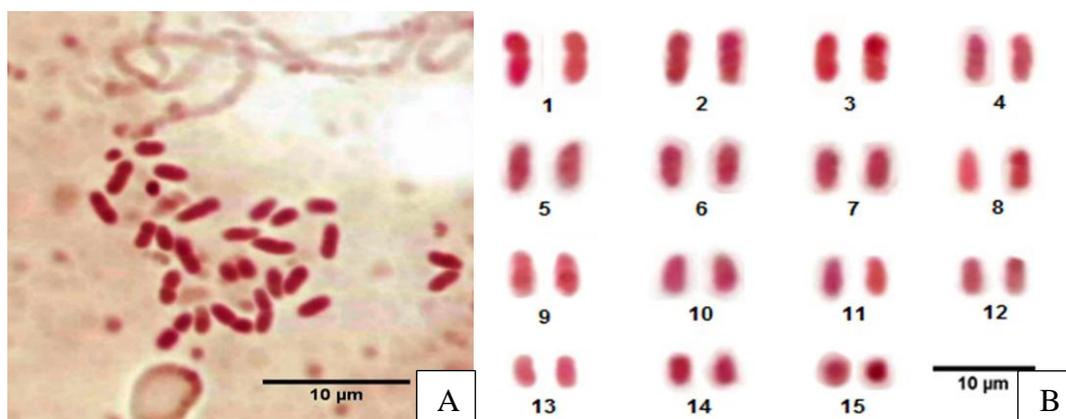


Figure 5. Metaphase chromosomes spread (A), and karyogram of *Origanum cyrenaicum* (B).

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. The species presented 33.2μm total haploid length of chromosome complement (THCL), 3.09μm largest chromosome to 1.98μm smallest chromosome ratio, indicating relatively small chromosomes in this species (Table1). 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%.

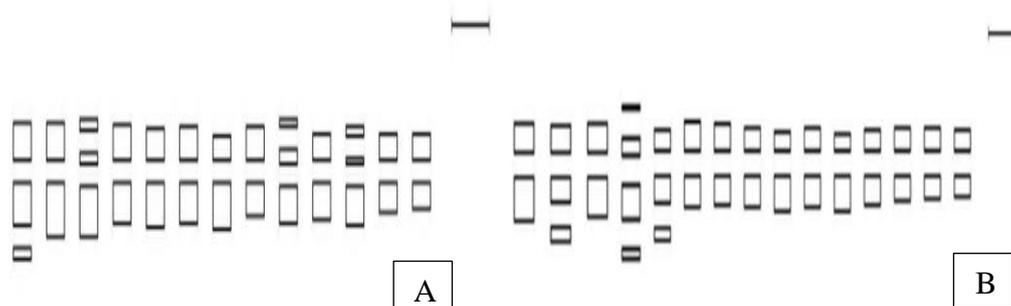


Figure 6. Ideogram of *Arbutus pavarii* (A), *Origanum cyrenaicum* (B)

Table 1. Chromosome measurements of *Arbutus pavarii* (mean \pm SD)

Chromosome Number	Short arm S (μ m)	Long arm L (μ m)	Total length (S+L)	Relative length% (CL/ Σ CL) *100	Arm ratio L/S	Centromere index% S/(S+L) *100	Chromosome type
1	1.36 \pm .11	1.73 \pm .11	3.09 \pm .03	9.34 \pm .41	1.26 \pm .17	44.19 \pm 3.47	m
2	1.36 \pm .02	1.65 \pm .04	3.01 \pm .03	9.06 \pm .06	1.21 \pm .05	45.18 \pm .91	m
3	1.02 \pm .02	1.91 \pm .02	2.92 \pm .03	8.81 \pm .13	1.87 \pm .06	34.93 \pm .75	sm
4	1.29 \pm .05	1.55 \pm .05	2.84 \pm .00	8.55 \pm .13	1.20 \pm .08	45.42 \pm 1.68	m
5	1.13 \pm .04	1.57 \pm .04	2.70 \pm .04	8.07 \pm .06	1.41 \pm .09	41.85 \pm 1.69	m
6	1.19 \pm .09	1.43 \pm .10	2.62 \pm .03	7.89 \pm .06	1.20 \pm .19	45.42 \pm 3.73	m
7	0.85 \pm .05	1.71 \pm .06	2.55 \pm .05	7.71 \pm .09	2.01 \pm .18	33.2 \pm 1.87	sm
8	1.24 \pm .02	1.24 \pm .02	2.48 \pm .04	7.46 \pm .11	1.00 \pm .00	50.00 \pm .00	M
9	1.02 \pm .05	1.36 \pm .05	2.38 \pm .04	7.17 \pm .08	1.33 \pm .09	42.86 \pm 1.72	m
10	0.95 \pm .03	1.34 \pm .03	2.29 \pm .03	6.91 \pm .02	1.41 \pm .08	41.48 \pm 1.31	m
11	0.76 \pm .01	1.47 \pm .03	2.23 \pm .04	6.72 \pm .07	1.93 \pm .03	34.08 \pm .35	sm
12	0.99 \pm .03	1.12 \pm .03	2.11 \pm .04	6.36 \pm .06	1.13 \pm .04	46.92 \pm .9	m
13	0.98 \pm .03	1.00 \pm .03	1.98 \pm .03	5.96 \pm .07	1.02 \pm .04	49.49 \pm 0.81	M
Average	1.09 \pm .04	1.46 \pm .05	2.55 \pm .04	7.69 \pm .00	1.39 \pm .07	42.69 \pm .82	

Table 2. Karyomorphometric data for the studied plant taxa

Plant Taxa	CVci	Interchromosomal index		Intrachromosomal index				Symmetry Index S%	Asymmetry Index AI
		CVci	A2	TF%	AsK%	A	A1		
<i>Arbutus pavarii</i>	4.53 \pm .32	17.36 \pm .09	0.17 \pm .00	42.59 \pm 1.27	57.46 \pm 1.41	0.15 \pm .95	0.25 \pm .03	64.38 \pm .80	0.79 \pm .05
<i>Origanum cyrenaicum</i>	5.58 \pm .04	22.48 \pm .00	0.22 \pm .00	42.48 \pm .51	57.51 \pm .76	0.15 \pm .01	0.25 \pm .03	62.07 \pm .90	1.25 \pm .01

The arm ratio (AR) ranged in *Arbutus pavarii* from 1.00 to 2.01, with an average of 1.39 (Table 1). The karyotype of this species is classified as 1A type in Stebbins' symmetry classes, 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 2). In *Origanum cyrenaicum*, the diploid chromosome number ($2n = 30$) (Figure 5) was observed in all the investigated populations, with the basic chromosome number of $x = 15$. The ideogram was generated automatically using the Ideokar program based on the basic chromosome number of $x = 15$ (Figure 6 B). The karyotype of *Origanum cyrenaicum* is shown in (Figures 5-6B) and (Tables 2-3). It consists of 11 M-m pairs and 4 sm pairs, with the $KF = 2n = 2x = 30 = 6M + 16m + 8sm$ (3SAT). Karyotype analysis revealed that the chromosomes of *Origanum cyrenaicum* are small, with predominantly median to submedian centromeres.

Table 3. Measurements of *Origanum cyrenaicum* (mean \pm SD).

Chromosome Number	Short arm S (μ m)	Long arm L (μ m)	Total length (S+L)	Relative length% (CL/ Σ CL) * 100	Arm ratio L/S	Centromere index% S/(S+L)*100	Chromosome type
1	1.34 \pm .03	1.85 \pm .04	3.19 \pm .01	8.06 \pm .08	1.36 \pm .08	42.07 \pm 1.02	m
2	1.27 \pm .00	1.87 \pm .01	3.14 \pm .01	7.95 \pm .09	1.49 \pm .04	40.44 \pm .19	m
3	1.29 \pm .02	1.74 \pm .02	3.03 \pm .06	7.66 \pm .17	1.31 \pm .00	42.63 \pm .05	m
4	0.99 \pm .04	1.96 \pm .05	2.95 \pm .01	7.44 \pm .073	2.05 \pm .13	33.46 \pm 1.52	sm
5	1.01 \pm .02	1.89 \pm .03	2.90 \pm .01	7.33 \pm .08	1.92 \pm .00	34.67 \pm .93	sm
6	1.34 \pm .03	1.43 \pm .04	2.77 \pm .07	7.01 \pm .12	1.07 \pm .00	48.41 \pm .08	m
7	1.32 \pm .05	1.37 \pm .06	2.69 \pm .11	6.79 \pm .21	1.02 \pm .02	49.16 \pm .09	M
8	1.14 \pm .04	1.50 \pm .07	2.64 \pm .10	6.68 \pm .20	1.32 \pm .01	43.20 \pm 0.49	m
9	0.94 \pm .04	1.61 \pm .05	2.55 \pm .09	6.45 \pm .19	1.71 \pm .01	36.82 \pm .30	sm
10	1.07 \pm .00	1.43 \pm .04	2.50 \pm .06	6.33 \pm .09	1.34 \pm .03	42.76 \pm .54	m
11	0.9 \pm .02	1.56 \pm .02	2.46 \pm .06	6.21 \pm .08	1.84 \pm .06	36.40 \pm .83	sm
12	1.08 \pm .03	1.29 \pm .04	2.37 \pm .03	5.99 \pm .05	1.26 \pm .08	45.28 \pm 1.21	m
13	1.09 \pm .01	1.18 \pm .01	2.27 \pm .02	5.75 \pm .07	1.08 \pm .01	48.06 \pm .25	m
14	1.05 \pm .02	1.07 \pm .04	2.12 \pm .06	5.35 \pm .19	1.02 \pm .01	49.54 \pm .46	M
15	0.98 \pm .02	1.00 \pm .03	1.98 \pm .02	5.01 \pm .14	1.02 \pm .02	49.50 \pm .35	M
Average	1.12 \pm .02	1.52 \pm .04	2.64 \pm .05	6.67 \pm .12	1.39 \pm .03	42.82 \pm 0.55	

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. The length measurements of metaphase chromosomes ranged from the largest at 3.19 μ m to the smallest at 1.98 μ m, and the total haploid length of the chromosome complement was 39.56. The (IRL%) ranged from 5.01 to 8.06%, with an average value of 6.66%, indicating that the chromosomes vary in length. The total haploid length of the entire set of chromosomes (chromosome complement) (THCL) was measured to be 39.56 μ m, indicating that they are relatively small. The CI% ranged from 33.46% to 49.53%, indicating that most of the chromosomes have centromeres located in the median to sub-median positions, with an average of 42.82%.

The AR measured was 1.02 to 2.05, reflecting the relative length of the chromosome arms, with an average of 1.39. An average centromere position and arm ratio indicate a predominantly median position for the centromeres and predominantly metacentric chromosomes in the karyotype of *Origanum cyrenaicum* (Table 2). The information resulting from karyotype analysis of *Origanum cyrenaicum* indicated that the (CV_{ci}=5.58) value reflects a low variation in the position of the centromeres. The measurement of interchromosomal asymmetry (A₂), 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 [27]. *Origanum cyrenaicum* had a low (CV_{cl}=14.00) value, indicating a low variation in the length of the chromosomes. The species showed (Table 2) low values for A₁ (0.25), A₂ (0.22), and A (0.15), Ask% (57.51), and AI (1.25), indicating a low level of karyotype asymmetry in *Origanum cyrenaicum*. On the other hand, high values were observed for S% (62.07), TF% (42.49), indicating a relatively symmetrical karyotype. The karyotype of this species is classified as the 1A type in Stebbins' symmetry classes. Based on these findings, *Origanum cyrenaicum* is characterized by a symmetrical karyotype, with a predominance of metacentric chromosomes. The karyotype of this species is classified as the 1A type in Stebbins' symmetry classification.

Discussion

This study reports the chromosome number and karyotype of two endemic plants from El-Jabal El-Akhdar (*Arbutus pavarii* and *Origanum cyrenaicum*).

Chromosome number and morphology

Counting of chromosomes has been a very useful approach (particularly at the generic level) for researchers investigating evolutionary relationships [32; 33]. 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 [34; 35].

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

In the current study, was revealed on chromosome number of the Libyan endemic *Arbutus pavarii* was revealed as 2n = 26, with the basic chromosome number of x = 13. Our results are consistent with the chromosome numbers of *Arbutus unedo*, *Arbutus menziesii*, *Arbutus andrachne*, *Arbutus canariensis*, and *Arbutus xalapensis* [19-20]. According to Constantinidis et al. [36] Sun et al. [37] Consistency in chromosome numbers supports a close relationship among species and their placement within the same genus. Chromosome number consistency has an important systematic function that supports the placement of *Arbutus 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 [38]. In karyotype analysis, satellites and secondary constrictions are important features used to characterize chromosomes and to study phylogenetic relationships between species [39].

In the current study, the karyotype formula of *Arbutus pavarii* consisted of 10 metacentric and 3 submetacentric chromosome pairs. 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 observed presence of satellites and secondary constrictions on some chromosome pairs of *Arbutus pavarii* is an important systematic tool to characterize this species.

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 indicates 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 *Arbutus pavarii* indicates a small variation in the length of chromosome arms, with an average indicating predominantly metacentric chromosomes in the karyotype of *Arbutus pavarii*.

In *Origanum cyrenaicum*, the basic chromosome number of x = 15 dominates in *Origanum* taxa, but basic numbers of x = 14 and 16 characterize several tax *Arbutus* Many taxes contain basic number variations, possibly caused by the dysploidy mechanism. Dysploidy is likely to have happened depending on the fusion of metacentric chromosomes or reciprocal translocations in ancestral karyotypes, including dominant basic

numbers. Basic number alterations are $x = 14$ in *Origanum sipyleum*, *Origanum rotundifolium*, and *Origanum vulgare* subsp. $x = 16$ [11].

The results indicate that *Origanum cyrenaicum* is diploid and have the same as the chromosome number of plants in the genus *Origanum*, and this finding supports the placement of this species in the genus *Origanum* [11;21;22;40;41; 42]. The karyotype analysis of the *Origanum* genus remains scarce and has primarily focused on chromosome number determination [41], likely due to the small size of the chromosomes.

Karyotype analysis revealed that the chromosomes of *Origanum cyrenaicum* are small, with predominantly median to sub-median centromeres. In the present study, the karyotype formula (KF) of *Origanum cyrenaicum* consisted of 11 metacentric and 4 submetacentric chromosome pairs, based on chromosome measurements. In the above-mentioned works, only chromosome numbers have been reported. So, no information is available on the chromosome morphology of *Origanum*.

Origanum 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, and no B-chromosomes were observed during the study period.

The average value of (IRL%) indicates that the chromosomes vary in length. The CI% 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 *Origanum cyrenaicum* (Table 3).

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

Chromosome size

Chromosome length is useful for distinguishing individuals, samples, populations, or species, and is also an indirect indicator of the total DNA content. Measuring chromosome size correlated with evolutionary age provides a means to estimate genome size using the chromosomal data [33; 43].

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

In *Origanum cyrenaicum*, most *Origanum* genes have small-sized chromosomes, mostly within the 0.33 and 0.74 μ m range [11]. In this study, *Origanum cyrenaicum* chromosomes are small, ranging from 1.98 to 3.19 μ m, and the total haploid length of the chromosome complement (THCL) (39.56 μ m) in this species indicates that they are relatively small in size.

Karyotype asymmetry

Karyotype asymmetry is an important parameter in karyological studies [44]. And one of the most popular, inexpensive, and widely used approaches, especially by botanists [17].

Karyotype symmetry has two components, one related to variation among chromosome size and the other to variation in centromere position [17]. Most species of angiosperms are characterized by uniform symmetric karyotypes with mostly meta or submetacentric chromosomes [45], and it has been classically assumed that asymmetric karyotypes are derived from symmetric ones [16]. Nevertheless, cytogenetics now believe that reversal situations may have occurred [46], and that karyotype asymmetry is a transitory state rather than an evolutionary endpoint [47].

The karyotype of *Arbutus 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, meaning 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 2) indicate a relatively low level of asymmetry. These numerical indices indicate that the karyotype of *Arbutus pavarii* is symmetrical, with a high percentage of symmetric chromosomes.

The information resulting from karyotype analysis of *Origanum 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 [31]. *Origanum 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 considered to indicate higher levels of karyotypic heterogeneity [27;48], and the AI can also indicate an evolutionary trend, so primitive species usually have a lower AI, whereas a plant with a higher AI value

would indicate that it is more evolutionarily advanced [49;50]. In this study, AI was used to assess karyotype asymmetry of *Origanum cyrenaicum* and a low value indicate to low level of karyotype asymmetry.

The species showed (Table 2) 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 *Origanum cyrenaicum*. The karyotype of this species is classified as the 1A type in Stebbins' symmetry classification, indicating the symmetrical karyotype of this species. Based on these findings, *Origanum cyrenaicum* is characterized by a symmetrical karyotype, with a predominance of metacentric chromosomes.

Stebbins [16], Sheidai et al. [51], and Vargas et al. [52] found that an asymmetrical karyotype has evolved higher than a symmetrical karyotype. Stebbins [53] 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 *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.

Conclusion

In the present study, we revealed the chromosome numbers and ploidy levels of two endemic species, *Arbutus pavarii* (Ericaceae), which were collected from Wadi Emleka, and *Origanum cyrenaicum* (Lamiaceae), specimens were collected from Wadi Bouhalfaya in the El-Jabal El-Akhdar region, and established the karyograms of these species. The results of this study reveal one level of ploidy: diploid in *Arbutus pavarii* ($2n=2x=26$) and *Origanum cyrenaicum* ($2n=2x=30$), which have symmetrical karyotypes. Satellites were observed on four chromosome pairs of *Arbutus pavarii* and three chromosome pairs of *Origanum cyrenaicum*. No B-chromosome was observed among the treated endemic plant species.

Conflict of interest. Nil

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