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# NEW CHROMOSOME COUNTS IN LAMIACEAE FROM FLORA OF IRAN - I

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**ABSTRACT.** The mitotic chromosome counts were presented for 11 species (12 populations) belonging to six genera of Lamiaceae mostly from south of Iran. Among them chromosome numbers of five species including *Lavandula sublepidota*, *Nepeta hormozganica*, *Salvia compressa*, *Salvia rhytidea* and *Satureja kermanica* are reported for the first time. The chromosome counts for *Lavandula sublepidota* is the confirmation of x=10 in *Lavandula* which supposed to be anomalous. Intra-specific variations of the chromosome number were found for *Nepeta dschuparensis* and *Thymus lancifolius*. The counts for *Cyclotrichium leucotrichum*, *Lavandula pubescens* and *Salvia mirzayanii* is the in accordance with the previous reports. While the chromosome number for *Nepeta depauperata* does not confirm the previous results.

**Keywords:** *Cyclotrichium, karyotype, Lavandula, Nepeta, Salvia, Satureja, Thymus.*

## INTRODUCTION

*Lamiaceae* is one of the largest families of the flowering plants. This family has a world-wide distribution and includes ca. 240 genera across more than 7200 species [10]. Many species of the family are aromatic plants and widely used in traditional medicine and flavor industries. Iran a part of the SW Asia is considered as one of the regions of high *Lamiaceae* diversity [11]. The flora of Iran comprises approximately 46 genera and 406 species of Lamiaceae [12, 24] including the species of *Nepeta* L., *Salvia* L., *Thymus* L., *Satureja* L., *Cyclotrichium* (Boiss.) Manden. & Scheng. and *Lavandula* L.

*Nepeta*, the largest genera of Lamiaceae in Iran, is represented by 80 species of which 60% is endemic. It comprises perennial, rarely annual, herbaceous and fruticose plants [12].

*Salvia* is the second largest genus including about 61 herbaceous, suffruticose or shrubby perennials, rarely biennials or annuals species in Iran. It is distinguished from other genera of Lamiaceae by the two posterior stamens aborted, and the connective separating the thecae of the two expressed stamens significantly elongated [12].

*Thymus* consists of 18 taxonomically problematic species of herbaceous, perennial and subshrubs or shrubs distributed over the Iranian flora [12].

*Satureja* species are annual or perennial semi-bushy aromatic plants mainly distributed in Mediterranean region but also extended to Irano-Turanian phytogeographical region. Seventeen species of *Satureja s.s.* are distributed in mainly west and north-west of Iran [24, 18, 12, 2, 3].

*Cyclotrichium* are perennial subshrubs contains five species in the Irano-Turanian phytogeographical region of Iran. They characterized by a dense annulus of hairs within the corolla tube and long exerted stamens and style [12].

*Lavandula* is native to the Old World although now widely grown in many parts of the world as both garden plants and field crops. The genus is characterized by the congested to lax terminal spikes borne on a distinct peduncle [31]. Three species of *Lavandula* recorded from the flora of Iran [24, 12, 4].

Chromosome number and ploidy level are beneficial to promote our understanding of the taxonomic delimitation and the evolutionary events among the taxa [29]. In this study, the chromosome numbers of 11 species belonging to six genera including *Nepeta*, *Salvia*, *Thymus*, *Satureja*, *Cyclotrichium* and *Lavandula* distributed in Iran are provided. The present work aimed to provide a basis for taxonomic, genetic and molecular studies.

## MATERIALS AND METHODS

### *Plant materials*

For mitotic chromosome counts, online databases, as well as related literatures, were used to assess the previous chromosomal works on the examined taxa. Seeds and herbarium specimens of studied materials were collected from natural habitats, and deposited in MIR herbarium (Table 1).

### *Chromosome count investigations*

We used squash method according to Payandeh et al. [23] for the chromosomal studies. Root tips were obtained by germinating mature seeds on wet filter paper in Petri dishes at 25 °C. The root tips were pretreated for 2 h in α-monobromonaphthalene at 4 °C and washed and fixed in Carnoy I solution (3:1 absolute ethanol: glacial acetic acid) for 24 h. After being hydrolyzed in 1N HCl at 60 °C for 2 min, the root tips were stained in aceto-iron hematoxilin for 2 h at 30 °C. At least five mitotic metaphase cells were used to determine the somatic chromosome numbers. The roots were gently squashed in 45% acetic acid on a glass slide. Slides were photographed using an OPTIKA – OPTIKAM HDMI – 4083.13 microscope photomicrograph system.

***Table 1.*** *Voucher information and chromosomal data of Lamiaceae from flora of Iran examined in this study.*

| Taxon | Voucher information | Chromosome number |
|---|---|---|
| *Cyclotrichium leucotrichum* (Stapf ex Rech.f.) Leblebici | Iran: W, Ilam, 4 km SE of Eivan, near Imamzadeh Abdullah. N33°46'24.2", E46°16'22.0", 1345 m, 02.XI.2016. *Bordbar* 1990 (MIR) | 2n=16 |

***Table 1.*** *(continues)*

| Taxon | Voucher information | Chromosome number |
|---|---|---|
| *Lavandula pubescens* Decne. | Iran: S, Bushehr Province. Ahram, Ashi, 28°43'13.66"N, 51°31'09.51"E, 573 m, 03 Oct 2017, *Bordbar* 2903 (MIR) | 2n=36 |
| *Lavandula sublepidota* Rech. f. | Iran: S, Fars, Darab, Rostaq, Bezan pass, 28°22'30.35"N, 55°10'00.44"E,08 June 2018, *Bordbar* 2914 (MIR) | 2n=20 |
| *Nepeta depauperata* Benth. | Iran: S, Fars, Fasa, Mianjangal, Tang-e Ahru, 29°05'14"N, 53°26'05"E, 2044 m, 20.VIII.2018, *Bordbar* 2044 (MIR) | 2n=18 |
| *Nepeta dschuparensis* Bornm. | Iran: SE, E Kerman, Bondar-e Valiabad, 30°17'8.3"N, 57°19'12.8"E, 2500 m, *Mirtadzadini* 1986 (MIR) | 2n=18 |
| *Nepeta dschuparensis* Bornm. | Iran: SE, Baft to Rabor, N Esker, 19.VI.2015, *Bordbar* 2042 (MIR) | 2n=36 |
| *Nepeta hormozganica* Jamzad | Iran: SE, SW Kerman, Baft, Gijoo, 28°33'07"N, 56°30'30"E, 1470 m, *Mirtadzadini* 1946 (MIR) | 2n=18 |
| *Salvia compressa* Vent. | Iran: S, Fars, 13 km Fasa to Shiraz, 29°04'14.15"N, 53°37'52.19"E, 1490 m, *Bordbar* 1951 (MIR) | 2n=12 |
| *Salvia mirzayanii* Rech.f. & Esfand. | Iran: SE, Baft, Shadian, Godar-e Sabz, 28°51'50.14"N, 56°52'42.37"E, 2050 m, 14.VII.2019, *Bordbar* 2039 (MIR) | 2n=20 |
| *Salvia rhytidea* Benth. | Iran: SE, Kerman, N of Mt. Lalezar, 29°28'18"N, 56°48'29"E, 3100 m, *Mirtadzadini* 1959 (MIR) | 2n=22 |
| *Satureja kermanica* Payandeh, Bordbar & Mirtadz. | SE of Kerman, Sardu, Mt. Bahraseman, Hanzakuh, 2700 m, 16.X.2015, *Payandeh* 1996 (MIR) | 2n=30 |
| *Thymus lancifolius* Celak. | Iran: SE, Kerman, N of Mt. Lalezar, 29°27'13"N, 56°48'25"E, 3355 m, *Mirtadzadini* 2037 (MIR) | 2n=60 |

## RESULTS AND DISCUSSION

In the present study, the chromosome numbers of 12 populations representing of six genera of Lamiaceae mostly from south of Iran (Fig. 1) are determined, of which chromosome numbers of five species are studied for the first time. Chromosome numbers and polyploidy level for each genus are discussed below. The somatic chromosome numbers (2n) for the studied populations were presented in Table 1.

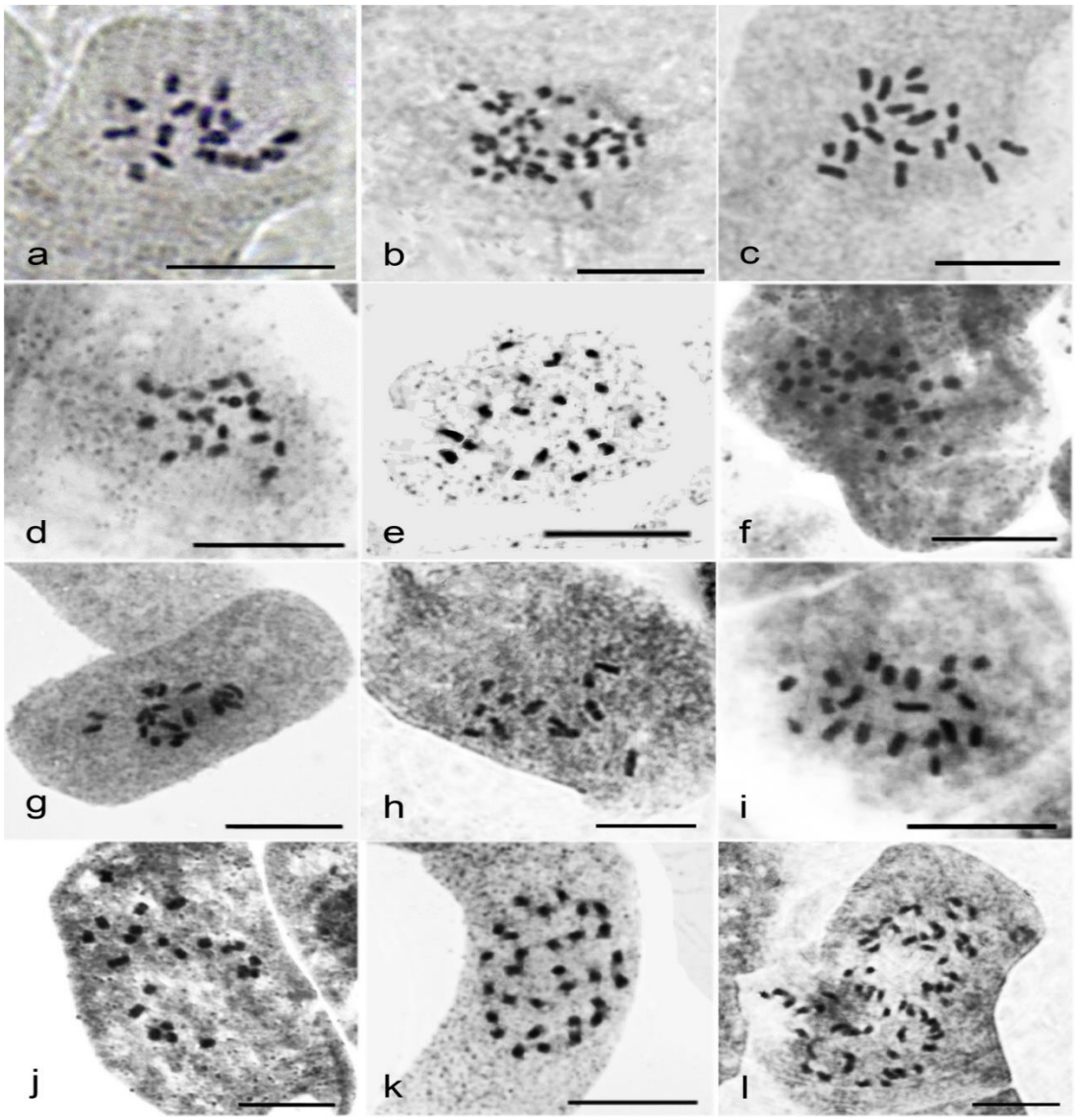



***Fig. 1.*** *Mitotic metaphase chromosomes of Lamiaceae from flora of Iran. (a) Cyclotrichium leucotrichum (Bordbar 1990), (b) Lavandula pubescens (Bordbar 2903), (c) Lavandula sublepidota (Bordbar 2914), (d) Nepeta depauperata (Bordbar 2044), (e) Nepeta dschuparensis (Mirtadzadini 1986), (f) Nepeta dschuparensis (Bordbar 2042), (g) Nepeta hormozganica (Mirtadzadini 1946), (h) Salvia compressa (Bordbar 1951), (i) Salvia mirzayanii (Bordbar 2039), (j) Salvia rhytidea (Mirtadzadini 1959), (k) Satureja kermanica (Payandeh 1996), (l) Thymus lancifolius (Mirtadzadini 2037). Scale bar = 10 μm.*

### *Cyclotrichium*

*Cyclotrichium* is a monophyletic genus [5, 7] consists of nine species distributed in Turkey, Lebanon, Iraq, and Iran among which five species can be found in Iran [12, 24].

Here, we report the chromosome number of *C. leucotrichum* (Stapf ex Rech.f.) Leblebici, to be 2n = 16 (Fig. 1A), which is in congruent with the result of Dirmenci et al. [7] on *C. leucotrichum* from flora of Turkey. They reported the numbers of six *Cyclotrichium* species, collected from different localities of Turkey. All the species investigated were diploid with chromosome number counted 2n = 16 and the basic chromosome number of x = 8.

### *Lavandula*

The genus comprises of over 40 species, 79 infraspecific taxa and hybrids, classified into three subgenera and eight sections [31, 22]. Two species *Lavandula coronopifolia* Poir. (syn. *L. stricta* Delile) and *L. sublepidota* Rech.f., had previously been recorded [12, 24], and *L. pubescens* Decne. newly reported by Bordbar and Upson [4] from the flora of Iran.

Polyploidy events have an important role in evolutionary process in *Lavandula*. It has a highly diverse chromosome number ranging from 2n=18 to 75. Here, we report the chromosome number of *L. pubescens*, to be 2n = 36 and x=9 (Fig. 1B), which is in congruent with the previous result for this species [31]. The chromosome number of *L. sublepidota* (2n=20) is reported here for the first time (Fig. 1C). This is the confirmation for the counts of n=10 for *L. mairei* Humbert and 2n=20 for *L. pinnata* Lundmark, both from sect. *Pterostoechas* and was supposed to be anomalous [31]. *L. sublepidota* previously placed in sect. *Hasikenses* but transferred into sect. *Subnudae* based on the recently published data of Bordbar and Upson [4]. The same chromosome numbers occur in different sections in *Lavandula* [31].

### *Nepeta*

The genus *Nepeta* has a large variation of chromosome number that ranges from 12 to 54 with the most common primary basic chromosomes of x=8 and x=9 [25, 15, 23, 1, 9]. The other basic chromosome numbers are considered to be secondarily evolved [30]. *Nepeta dschuparensis* Bornm. and *N. depauperata* Benth. are endemic species for the flora of Iran. *Nepeta dschuparensis* is restricted to Province of Kerman, south-east of Iran, and *N. depauperata* is mostly distributed in south of Iran, Fars and Hormozgan Province [12].

Two populations of *N. dschuparensis* both from Kerman Province are included in present study. The population *Mirtadzadini* 1986 (MIR) has 2n = 18 chromosomes (Fig. 1E), which is consistent with the previous studies of Hasaninejad et al. [9], while the chromosome number of the population *Bordbar* 2042 (MIR) is 2n = 36 (Fig. 1F), indicating diploid and tetraploid cytotypes for this species.

*Nepeta depauperata* were recently reported to have 2n = 34 chromosomes and x=17 based on the population collected from Hormozgan Province, Mt. Bukhun [9]. Since *N. dschuparensis* and *N. depauperata* are two closely related taxa, x=17 seems erroneous. On the other hand, the result of this study revealed that the population collected from Fars Province, Fasa has the chromosome number of 2n=18 and x=9 (Fig. 1D). The small size of the chromosomes in Lamiaceae can often result in undercounting [28]. In another

scenario, it can be an aneuploidy for this species. However, further chromosome analysis on more populations of this species is required to clarify the situation.

*Nepeta hormozganica* Jamzad is an endemic species distributed in south of Iran mostly in Hormozgan Province. It has 2n = 18 chromosomes (Fig. G), indicating it to be a diploid with a basic number of x = 9. This is the first report of the chromosomes number in this species.

### *Salvia*

*Salvia* is a worldwide genus with diverse chromosome numbers ranging from 2n=12, 14, 16, 18, 20, 22, 32, 44, 66 [21, 14, 27, 8]. Here, we investigate the chromosome numbers of *Salvia compressa* Vent., *S. mirzayanii* Rech.f. & Esfand. and *S. rhytidea* Benth.

*Salvia compressa* is distributed in Iraq, Afghanistan as well as Iran [24]. This species is widely distributed in center, west, south-west and south of Iran [12]. The species has 2n = 12 chromosomes (Fig. 1H). The chromosome number of this taxon is presented here for the first time.

Our results showed the chromosome number of 2n=20 for *Salvia mirzayanii* (Fig. 1I) which is in congruent with the result of Mortazavi et al. [20]. This species is distributed in south of Iran as well as Oman [24].

The chromosome number for *Salvia rhytidea* is reported here for the first time. It possess 2n=22 chromosomes (Fig. 1J). This species in Iran is restricted to south, Kerman and Baluchistan however it is mostly distributed in Afghanistan [24].

### *Satureja*

*Satureja kermanica* Payandeh, Bordbar & Mirtadz. is a newly reported and narrow endemic species from south of Iran, Kerman Province [3]. The results of this study revealed 2n=30 for this species (Fig. 1K). It seems that 2n=30 is the most frequent chromosome number in *Satureja* [17, 26]. This is the first chromosome number report for this species.

### *Thymus*

Here, we reported the chromosome number of *Thymus lancifolius* Celak. distributed in Iraq as well as Iran [24]. This taxon is treated as a subspecies under the name *Thymus daenensis* Celak. [24] or as a separate species [12]. Cytogenetic works in this genus have revealed species with diversified chromosome numbers, 2n=24, 26, 28, 30, 32, 42, 48, 50, 52, 54, 56, 58, 60, 84 and 90, corresponding to the diploid, tetraploid and hexaploid levels, and the most frequent numbers of 2n=28, 30, 56 and 60. Aneuploidy has been found in the genus and is responsible for the other numbers. Our study showed that *T. lancifolius* collected from SE Iran, Kerman Province, Mt. Lalezar is a tetraploid (2n = 60) (Fig. 1L). However, Daftaril and Safarnejad [6] found that populations of *T. lancifolius* from NE Iran, Khorasan Province had 2n = 30 chromosomes. Therefore, this is the first report of 2n=60 for this species. The intraspecific variation of the chromosome number is also found in *T. daenensis* [16, 13], *T. kotschyanus* Boiss. & Hohen. [13] and *T. trasncaspicus* Klokov [19].

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