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Chromosome counts of eight Iranian endemic species of *Nepeta* L. (Lamiaceae)

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Abstract. In this survey, the chromosome counts of eight *Nepeta* L. species were investigated and the karyotypic diversity among these species was studied. The examined species belong to *N. cephalotes* Boiss. species group, namely *N. eremokosmos* Rech.f., *N. gloeocephala* Rech. f., *cephalotes* Boiss., *N. pungens* (Bunge) Benth., *N. ispahanica* Boiss., *N. mahanensis* Jamzad & Simonds, *N. hormozganica* Jamzad and *N. denudata* Benth. collected from different habitats in Iran. The ploidy levels, karyotype formula, chromosome length range, total karyotype length, several karyotype asymmetries values and Stebbins classification were determined in this study. Results showed the same chromosome number, 2n = 2x= 18 for all studied species. The basic chromosome number for the above mentioned species are x = 9. Also, the smallest chromosome length is 1.02 µm in *N. mahanensis*. The largest chromosome length is 2.3 µm in *N. ispahanica*. The chromosomes of species were metacentric or submetacentric. According to the Stebbins classification, these species were located into three classes 1A, 2A and 3A. The chromosome numbers for six of studied species are reported here for the first time.

Keywords: chromosome number, cytotaxonomy, endemics, Lamiaceae, karyotype, Iran, *Nepeta* L.

INTRODUCTION

The family Lamiaceae consists of 7173 species in 236 genera worldwide. Many of its species have a great importance due to their economic values (Harley *et al*. 2004). *Nepeta* L. (catmint) is a genus belonging to the subfamily Nepetoideae (Cantino *et al*. 1992). It is one of the largest genera within Nepetoideae, growing as annual, herbaceous perennial and fruticose plants (Rechinger 1982; Jamzad 2003a, 2012; Kaya and Dirmenci 2008).

Endemic species, constituting valuable floristic elements are those which are confined to a particular geographic region. The narrow endemic species are not only scientifically interesting but also very important from conservation point of view. Therefore, the identification of endemic plants, their

conservation and genetic resources are interesting for the scientific community (Ghaffari *et a*l. 2005). In Iran, there are 165 endemic taxa of Lamiaceae including 42 endemic *Nepeta* species. Iran is one of the centers of diversity for the genus *Nepeta* (Jamzad *et al.* 2003b).

Most species of the Lamiaceae have medicinal values. There are numerous known species in the family that are used as analgesic drugs in traditional medicine (Uritu *et al*. 2018). Medicinal properties of the Lamiaceae species are often ascribed to their high content of volatile compounds (Khoury *et al*. 2016) and glandular hairs represent important sites for the synthesis of natural bioactive compounds (Giuliani *et al*. 2020). *Nepeta* is an important genus in Lamiaceae and is specified by terpenoid-type compounds and phenolic constituents, which exert several activities such as an antimicrobial, repellent against major pathogen vector mosquitoes, insecticide, larvicide against *Anopheles stephensi*, cytotoxic anticarcinogen, antioxidant, anticonvulsant, analgesic, anti-inflammatory agent, and antidepressant, disclosing its importance in medicinal and agricultural fields (Süntar *et al*. 2018).

Species of this genus have been studied in the fields of morphology-anatomy (Kaya and Dirmenci 2008; Acar *et al*. 2011), palynology (Jamzad *et al*. 2000; Celenk *et al*. 2008; Moon *et al*. 2008). Chemical composition (Baser *et al*. 2000; Asgarpanah *et al*. 2014) and molecular phylogeny (Jamzad *et al*. 2003b). The lack of sufficient data on the karyomorphology of the genus is probably due to the small size of its chromosomes (Esra *et al*. 2020).

Many karyological data concerning chromosome numbers of the genus have already been reported as x = 6, 7, 8, 9, 11, 12, 15, 13, 17, 18. (IPCN, http://www.tropicos.org/Project/IPCN, Darlington and Wylie, 1955; Goldblatt and Johnson 1979–2017; Chen *et al*. 2018) and there are a few reports from Iran (Aryavand 1977; Ghaffari and Kelich 2006; Kharazian *et al*. 2013; Payandeh *et al*. 2015; Akbarpur Mamagani *et al*. 2016; Hasaninejad *et al*. 2020). It should be admitted that the numerical variation in chromosome numbers within a genus is quite common.

The chromosome numbers and karyotype studies are not only useful in predicting morphological similarities and diversity among species, but also, they are valuable sources of taxonomic and biosystematic information. Regarding to the complexities in taxonomy of the genus *Nepeta*, the phylogenetic relationships of species and the chromosomal evolutionary trend may elucidate the systematics, and lead to a comprehensive infrageneric classification of the genus. In this study, we aim to do a cytotaxonomic study of the genus, and follow up the process of chromosomal evolution and its use in the classification of this genus. Here we report part of our results on the chromosome counts of a natural species group, recognized previously as section *Capituliferae* Benth. p.p. (Bentham 1848) and group five (Jamzad *et al*. 2003b), with mostly Iranian endemic species.

MATERIALS AND METHODS

Seeds of 8 species were collected from different habitats of Iran are, as listed in Table 1. The voucher specimens of the examined species are preserved in the herbarium of the Research Institute of Forests and Rangelands of Iran (TARI).

For mitotic studies, the seeds were germinated at 25 °C on wet filter paper in petri dishes. After germination, roots of 0.5-1cm were selected for pretreatment. Root tips were pretreated for 1 h in α-monobromonaphthalene at 4 °C, washed and fixed in Carnoy solution (3:1 absolute ethanol glacial acetic acid) overnight. The root tips were hydrolyzed for 5-8 minutes in 1N HCl at room temperature, washed and stained in 2% Hematoxylin for 1 h.

Table 1. The voucher details of studied *Nepeta* species.

No	Species	Geographical Location
1	*N. cephalotes* Boiss.	Iran, Tehran, Jajroud highway towards Jajrood 1544 m, Golipour, 106883, TARI.
2	*N. denudata* Benth.	Iran, Hamedan, near Razan, 1889 m, Golipour, 106879, TARI.
3	*N. eremokosmos* Rech.f.	Iran, Semnan, Sorkhe, 1355 m, Golipour, 106880, TARI.
4	*N. gloeocephala* Rech. f.	Iran, Yazd, Taft, Nasr Abad, Gilok village in the river, 2800m, Mirhoseini, 95002, TARI.
5	*N. hormozganica* Jamzad	Iran, Hormozgan, N. Bandar Abbas, N. slop of M. Bokhon, 834 m, Ajani,105647, TARI.
6	*N. ispahanica* Boiss.	Iran, Kerman, Rayen to the first Garow, Fazlabad village road, 2618 m, Golipour, 106881, TARI.
7	*N. mahanensis* Jamzad & Simonds	Iran, Kerman East Silo Mahan-Before to Khaki-Asphalt Road, Hossein Abad 1980 m, Golipour, 106882, TARI.
8	*N. pungens* (Bunge) Benth.	Iran, Chaharmahal va Bakhtiari, Shahrekord, Babahidar, the first road to the village of Sepidaneh, 2340 m, Ajani & Hasaninejad, 107079, TARI.

OLYMPUS BH-2 photomicroscope provided the clearest mitotic metaphase among 5 cells and measured by Micro Measure software 3.3.

Karyotypes were prepared and chromosome pairs were classified according to Levan *et al*. (1964) and the metacentric and sub-metacentric chromosomes were symbolized using the letters m and sm, respectively. The chromosomes were arranged according to their lengths. The long arm (q), short arm (p), mean length of the chromosome (CL), and total chromosome length (TCL) were measured. Karyotype symmetry was determined according to Stebbins (1971) and total form percentage (TF, 100 × ΣS/C) (Huziwara 1962).

RESULTS

There was no difference between basic chromosome numbers of the eight studied species and they were x = 9. The details of each species are as follow:

Nepeta cephalotes is an Irano-Turanian endemic species and grows in central and northwest of Iran. This species showed a diploid chromosome number 2n = 2x = 18 (Figure 1A) and the basic chromosome number of x = 9. Karyotype consisted of 9 pairs of submetacentric chromosomes (Tables 2, 3; Figure 2A). The chromosome length ranged from 1.14 to 2.07μm. The chromosome number of this species is reported here for the first time.

Nepeta denudata is an endemic perennial species, with a distribution range in central, northeast, and west of Iran. The results showed that this species is also diploid with chromosome number of 2n = 18 (Figure 1B). The karyotype was formed of eight pairs of submetacentric and one pair of metacentric chromosomes (Tables 2, 3; Figure 2B). The mean length of chromosome varied from 1.1 to 1.9μm. The chromosome number of this specie is reported here for the first time.

Nepeta eremokosmos is a narrow endemic species. It grows in a limited geographical area in central Iran. The studied specimens showed a diploid chromosome number of 2n = 2x = 18 in this taxon (Figure 1 C) and basic chromosome number of x = 9. Karyotype in this taxon consisted of 9 pairs of submetacentric chromosomes

Table 2. Karyotype formula according to Levan *et al*. (1964) of the studied *Nepeta* species: 2n– Chromosome number; x– Basic chromosome number; PL– Ploidy level; KF– Karyotype formula R– range; SC– The shortest chromosome length; LC– The longest chromosome length.

No	Species	2n	x	PL	KF	R (SC–LC) (μm)
1	*N. cephalotes*	18	9	2x	9sm	1.14-2.07
2	*N. denudata*	18	9	2x	8sm+m	1.17-1.90
3	*N. eremokosmos*	18	9	2x	9sm	1.31-1.99
4	*N. gloeocephala*	18	9	2x	6m+3sm	1.05-1.98
5	*N. hormozganica*	18	9	2x	5m+4sm	1.15-1.73
6	*N. ispahanica*	18	9	2x	7sm+2m	1.47-2.30
7	*N. mahanensis*	18	9	2x	9m	1.02-1.74
8	*N. pungens*	18	9	2x	7sm+2m	1.11-2.12

Table 3. Karyomorphological parameters of studied *Nepeta* species: AR– arm ratio; CI– Mean centromeric index; p– Mean length of the short arm; q– Mean length of the long arm; TCL– The total chromosome length of the haploid complement; CL– Mean length of the chromosome; TF– Total form percentage and Stebbins– Classification of Karyotypes in relation to their degree of asymmetry according to Stebbins (1971).

No	Species	AR (L/S) (μm)	CI (μm)	P mean (μm)	q mean (μm)	TCL	CLmean (μm)	TF(%)	Stebbins
1	*N. cephalotes*	2.18	0.32	0.47	1.10	14.47	1.61	31.41	3A
2	*N. denudata*	2.12	0.33	0.50	1.06	13.98	1.55	32.01	3A
3	*N. eremokosmos*	1.91	0.35	0.56	1.07	14.61	1.62	34.37	2A
4	*N. gloeocephala*	1.58	0.36	0.59	0.93	13.71	1.52	38.81	2A
5	*N.hormozganica*	1.67	0.33	0.52	0.87	12.58	1.40	37.47	1A
6	*N. ispahanica*	1.83	0.35	0.64	1.18	16.44	1.83	35.28	2A
7	*N. mahanensis*	1.34	0.43	0.58	0.78	12.29	1.37	42.79	1A
8	*N. pungens*	1.89	0.35	0.57	1.08	14.86	1.65	34.60	2A

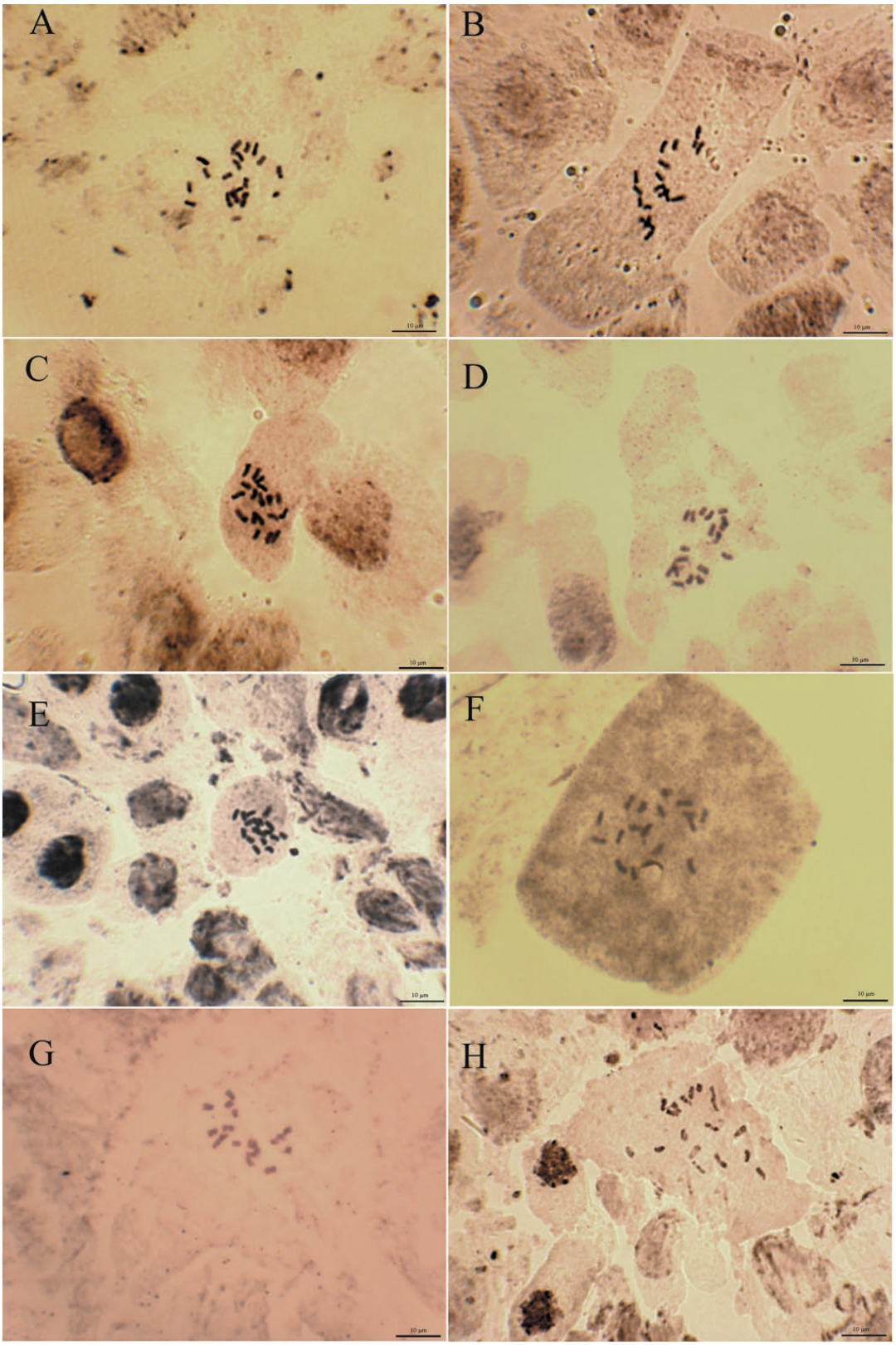

Figure 1A-H. Somatic chromosomes of *Nepeta* (A- *N. cephalotes*; B- *N. denudata*; C- *N. eremokosmos*; D- *N. gloeocephala*; E- *N. hormozganica*; F- *N. ispahanica*; G- *N. mahanensis*; H- *N. pungens*). Scale bars: 10 μm.

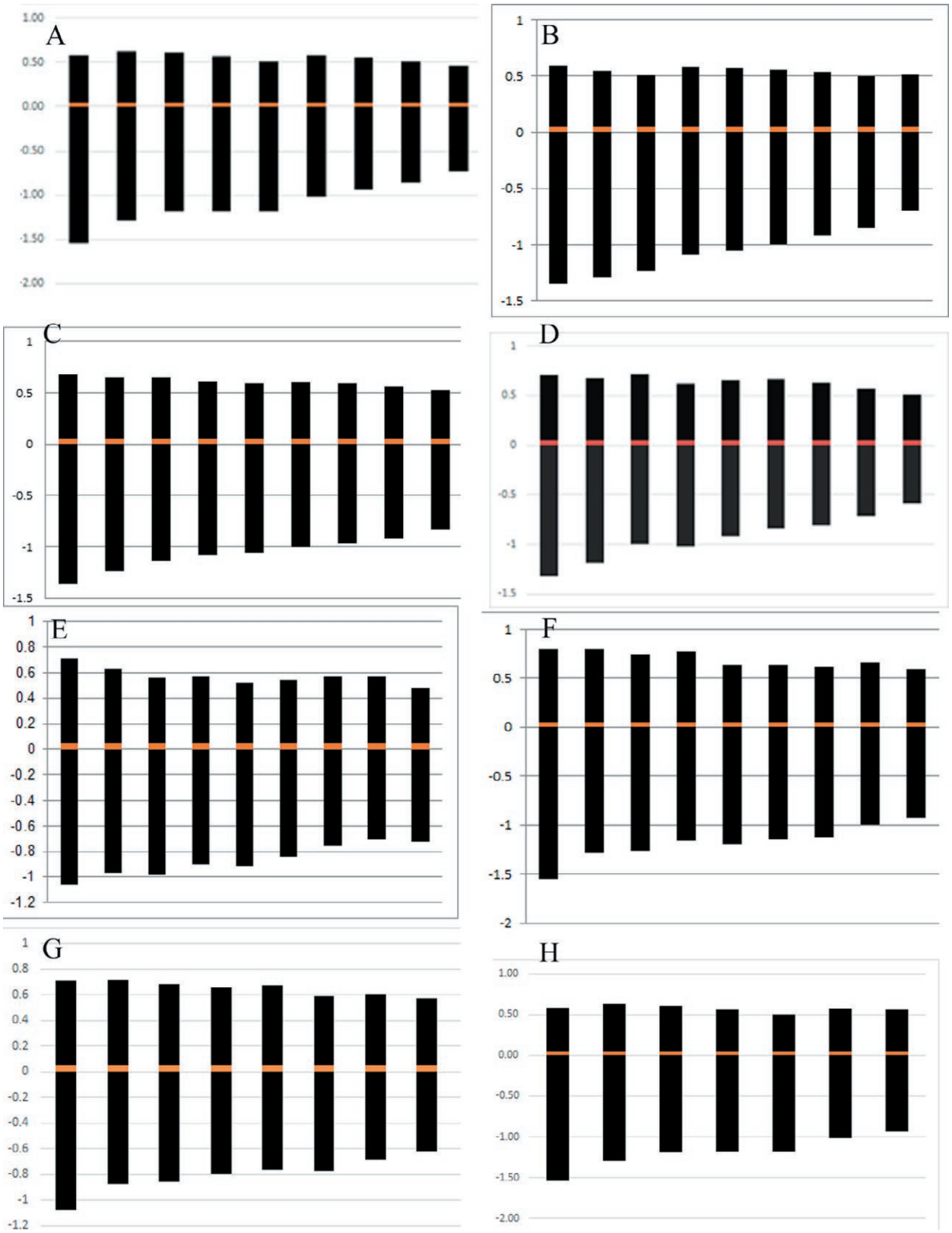

Figure 2A-H. Idiograms of the karyotypes of *Nepeta* (A- *N. cephalotes*; B- *N. denudata*; C- *N. eremokosmos*; D- *N. gloeocephala*; E- *N. hormozganica*; F- *N. ispahanica*; G- *N. mahanensis*; H- *N. pungens*). Scale bars: 10 μm.

(Tables 2, 3; Figure 2C). The chromosome length is 1.3 to 1.9 µm. This is the first chromosome count for this species.

Nepeta gloeocephala is an endemic species found in few localities in central Iran. Chromosome number of 2n = 2x = 18 and somatic chromosome count in this species showed an x = 9 (Figure 1D). Karyotype was included 6 pairs of metacentric and 3 pairs of submetacentric chromosomes in this specie (Tables 2, 3; Figure 2D). The chromosome length varied from 1.05 to 1.98 µm. This is the first chromosome number reported for this taxon.

Nepeta hormozganica is an annual species from Saharo-Sindian region, growing in south Iran. The diploid chromosome number of 2n = 18 was counted in this species (Figure 1E). Five chromosome pairs were metacentric and four pairs were submetacentric (Tables 2, 3; Figure 2E). The chromosome length was in the range of 1.1 to 1.7 µm. The chromosome number of this species is reported here for the first time.

Nepeta ispahanica is a regional endemic annual species growing in west, northeast, central, south, and southeast of Iran. It is also distributed in Afghanistan. The studied specimens showed a diploid chromosome number of 2n = 2x = 18 (Figure 1F) and basic chromosome number of x = 9. *N. ispahanica* had 7 pairs of submetacentric and 2 pairs of metacentric chromosomes (Tables 2, 3; Figure 2F). The chromosome length ranged from 1.4 to 2.2 µm. This is the first chromosome count for this species.

Nepeta mahanensis is a narrow endemic annual species. This species grows in a limited geographical area in Kerman province. Chromosome number in this species was 2n = 18 (Figure 1G). The karyotype was formed of 9 pairs of metacentric chromosomes (Table 2, 3; Figure 2G). The chromosome length varied from 1.02 to 1.74 µm. This is the second report on the chromosome numbers of this species. The result of this study is in agreement with the previous report conducted by Payandeh *et al*. (2015) for *N. mahanensis* (x = 9; 2n = 18).

Nepeta pungens is a regional endemic species with wide distribution in central, northwest, west, northeast and southwest of Iran, Afghanistan, Turkmenistan and Central Asia. *Nepeta pungens* (2n = 2x = 18) had 7 pairs of submetacentric and 2 pair of metacentric chromosomes (Figure 1 H). The chromosome length was in the range of 1.1 to 2.1µm. This is the second report on the chromosome numbers of this species (Table 2, 3; Figure 2 H). However, the result of this study was not in agreement with the previous report conducted by Kharazian *et al*. (2013) for the *N. pungens* (x = 11; 2n = 22).

DISCUSSION

According to the Index to Plant Chromosome Numbers (IPCN, http://www.tropicos.org/Project/IPCN) (Goldblatt and Johnson 1979-2017), in Lamiaceae, the chromosome numbers vary from 2n = 10 to 2n = 240 in different genera and species. Alloplipoid and autoploid changes can be an important reason for this diversity. Extensive cytological studies of the different genera, including *Thymus* L., *Ajuga* L., *Lamium* L., *Salvia* L., *Scutellaria* L. and *Elsholtzia* Willd. had revealed the presence of diploid, tetraploid, hexaploid and octaploid species in the family Lamiaceae (Rather *et al*. 2018).

The chromosome numbers together with other factors can alter breeding strategy in plants (Fehr 1991; Contreras and Ruter 2011). Genome size can be estimated by measuring chromosomal data. Therefore, chromosome size is directly related to evolution (Mehra and Bawa 1972; Contreras and Ruter 2011; Esra *et al*. 2020).

The results of our study show that the examined species have 2n = 18 chromosome numbers and the basic chromosome numbers are x = 9.

Different researchers have suggested x = 8, 9 and 17 as the most common primary and secondary base numbers for the genus *Nepeta* (Gill 1972, 1979; Aryavand 1977; Saggoo 1983; Bir and Saggoo, 1984; Hasaninejad *et al*. 2020). The previous studies support the results of our study (Kaczmarek 1957; Gill 1979, 1984; Ghaffari and Kelich 2006; Saggoo *et al*. 2011; Kharazian *et a*l. 2013; Payandeh *et al*. 2015; Akbarpur Mamagani *et al*. 2016; Hasaninejad *et al*. 2020), reporting the base chromosome number, x = 9 for *Nepeta* as a common number.

The studied species in this research had small chromosomes according to the classification of Lima-De-Faria (1980), with mean chromosome lengths (CLm) ranging from 1.37 to 1.83 µm (Table 3). Whereas Baden (1983) argued that the karyotype details studies are difficult because of the small size of chromosomes.

Although the chromosome number of all studied *Nepeta* species was the same (2n = 18), their karyotype formulas were different, 9 sm of *N. cephalotes* and *N. eremokosmos* and 9m of *N. mahanensis* and 8sm+m, 6m+3sm, 5m+4sm, 7sm+2m and 7sm+2m of *N. denudate, N. gloeocephala, N. hormozganica, N. ispahanica* and *N. pungens,* respectively.

Baden (1983) reported the metacentric and submetacentric karyotype formula for *N. sibthorpii* Benth. and Kharazian *et al*. (2013), suggested the metacentric, sub-metacentric and metacentric point karyotype formula, which confirms our results.

N. cephalotes is distinguished by having the highest AR and the lowest CI values, and *N. mahanensis* by

having lowest AR, TCL and CL values, *N. isphanica* by having the highest TCL and CL values; *N. denudata* by having the lowest AR value (Table 2).

It was found that all studied *Nepeta* species are in classes 1A, 2A and 3A based on Stebbins classification. 3A species are more asymmetric or more advanced than class 1A species. Thus, *N. cephalotes* and *N. denudate* are more symmetric and *N. hormozganica* and *N. mahanensis* are more asymmetric. This study suggested that TF% varied from 31.41 to 42.79. *N. mahaensis* was distinguished by having the highest TF%, *N. cephalotes* by having the lowest TF% (Table 3).

Kharazian *et al*. (2013) reported 2n = 22 for *N. pungens,* which is in line with the previously reported base numbers (Chen *et al*. 2018). In our study, the chromosome number of *N. pungens* was counted 2n = 18, which is contrary to the previous reports. In this case of variability, Gill (1979) reported the intra-specific races for some of *Nepeta* species, or the case may be incorrect identification of the studied specimen.

Moreover, *N. mahanensis* was reported with 2n = 18 by Payandeh *et al*. (2015). In our report, the basic chromosome number is x = 9, which is fully in agreement with the results of our study for this species.

All studied species are either Iranian or regional endemics and showed chromosome numbers of x = 9. Srivastava (2012) believed that, there is a probability of base number x = 9 at the phylogenetic root of the *Nepeta*, but annual species are considered to be the most evolved species in the genus (Jamzad *et al*. 2003b). As it is shown here for four annual *Nepeta* species (*N. ispahanica*, *N. mahanensis* and *N. hormozganica*), the base number is x = 9, which does not support Sirvastava's idea. Previous literatures indicate that the genus *Nepeta* has a heterogeneous set of chromosome numbers. Considering the close phylogenetic relationship among the studied species (Jamzad *et al.* 2003b), it may be inferred that the similar chromosome numbers approve their close phylogenetic relationships. Future comprehensive cytotaxonomic studies and inferring the results on the *Nepeta* phylogenetic tree may elucidate the evolutionary trends in the genus and lead us to better understanding of the evolutionary values of chromosome numbers.

Most frequent count of the base chromosome number in *Nepeta* is x = 9. Whereas, in most species of Lamiaceae, the base chromosome numbers are different. The chromosome number as 2n = 30 is typical in some genera including *Origanum*, *Clinopodium* L., *Micromeria* Benth., *Satureja* L., *Thymus* etc. (Esra *et al*. 2020). In genus *Caryopteris* Bunge the chromosome number was reported as 2n = 26 and x = 16 in genus *Chelonopsis* Miq. was (Chen *et al*. 2018). Huang *et al.* (1996) reported that the basic chromosome number was x = 8 in *Eriophyton* Benth.. *Phlomoides* Moench is known to have a base chromosome number of x = 11, which is distinct from the base number x = 10 in *Phlomis* L. (Fang *et al*. 2007). *Scutellaria* is one of the largest genera within Lamiaceae that also has a complex chromosomal variation as at least 14 different chromosome numbers have been found for the genus 2n = 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 44, 60, 88. The basic chromosome number x = 13 in East and Southeast Asia, x = 12 in America and x = 11 in North Africa and Eurasia are predominating (Ranjbar and Mahmoudi 2013).

Whereas all studied species were homoploid, but according to previous studies (Gill 1972; Bir and Saggoo 1979, 1984; Saggoo 1983; Chen *et al*. 2018; Hasaninejad *et al*. 2020), aneuploidy and dysploidy changes had role in taxa evolution. Variation in the chromosome numbers is one of the important factors in the process of evolution (Srivastava 2012). However, all these species were not affected by chromosome number variation.

The results of this study provided a considerable contribution to the cytotaxonomic data of the genus *Nepeta*.

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