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Chromosome count and karyotype study of eleven *Nepeta* L. (Lamiaceae) species from Iran

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Abstract

Nepeta L. genus (Lamiaceae Family) with high morphological and chromosomal diversity is one of the large genus of Iran with important genetic resources in this country. In order to study the karyotype features in *Nepeta* species, 11 species and 31 accessions were collected from natural habitats of Zagros region. Their chromosome number and karyotype were studied using mitotic metaphase. The cluster analysis with Squared Euclidean Distance and Ward Methods were done by use of SPSS software ver.20 to display the chromosomal diversity. The results of this research showed that the studied taxa were diploid, tetraploid and hexaploid. Chromosome numbers were 2n=18, 22, 26, 32, 34, 36, 42, 54 and basic chromosome numbers were x=7, 8, 9, 11, 13, 17. Most of the numbers and all of the karyotypes were reported for Iran for the first time. The karyotypic results showed the diversity among the species as mostly displayed median point (M), median region (m) and sub median region (sm) and the chromosome lengths were in the range of 0.64-2 µm. From the clustering results, high chromosomal diversity was found in *N. glomerulosa, N. fissa, N. pungens, N. daenensis* and *N. schiraziana* accessions. It is concluded that Zagros region is one of the diversity centers in Iran and provide the evolutionary trends in this genus.

Key words: Karyotype, Chromosome number, *Nepeta*, Lamiaceae, Iran

1. Introduction

Nepeta L. (catmint) genus belonging to Lamiacaea Family and Nepetoideae subFamily (Cantino et al., 1992) is one of the largest and medicinal genera in this Family with 300 species growing as perennial, rarely annual, herbaceous and fruticose plants (Rechinger, 1982; Kaya and Dirmenci, 2008). *Nepeta* species are significantly distributed in Eurasia, North Africa, North and Central America and Canary Islands. The diversity and species richness are found in South West Asia and Himalayas (Jamzad et al., 2000; Celenk et al., 2008). This genus has 75 species in Iran, of which 39 are endemic (Rechinger, 1982; Jamzad et al., 2003).

Nepeta species being used in traditional medicine as antispasmodic, expectorant, diuretic and antiseptic activities are widely recommended by pharmaceutics and make them important among taxonomists (Celenk et al., 2008). The essential oils richness of species, not only enhances its medicinal value but also improves its acceptability beyond the domain of *Nepeta* genus (Kaya and Dirmenci, 2008).

Taxonomically, the classification of *Nepeta* has been contentious and debatable (Celenk et al., 2008). Bentham (1848) divided it into 8 sections and 109 species, Briquet (1896) documented 2 sections and 150 species. Additionally, Rechinger (1982) recognized 12 sections, Budantsev (1993) categorized 19 sections and 210 species, Dirmenci (2003 PhD thesis, 2005) acknowledged 11 sections for this genus, and Hassan et al. (2011) also reported 22 *Nepeta* species from Himalaya. Consequently, the above mentioned different classifications underscore the sharp and evolving disagreement among taxonomist concerning the subject including the research implication (Jamzad et al., 2003). Frequent hybridization and introgression, together with considerable habitat variation make *Nepeta* a particularly complex genus (Celenk et al., 2008).

Morphologically, the characters as leaf form, indumentum and margin, inflorescence type, calyx and corolla indumentum, nutlet form and color are appropriate morphological characters to determine the *Nepeta* species and display high variability, even among related species (Hedge and Lamond, 1982; Jamzad et al., 2003). These variations were first reported by Baden (1984) in leaves, bracteoles and calyx of *N. camphorata* Boiss. & Heldr. and *N. heldreichii*

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Hal. The Leaf morphology differences are noticeable even among the same species. Jamzad et al. (2000) and Kaya and Dirmenci (2008) argue that using nutlet micro morphology could help in classification of the *Nepeta* genus in the future. Furthermore, Jamzad et al. (2003) reported that the distribution of flavones in *Nepeta* genus provided some valuable data for the phylogenetic relationships. Moreover, Celenk et al. (2008) using palynological data in *Nepeta* genus argued that pollen features are appropriate markers for relationships in this genus.

Consistent with the complex nature of the *Nepeta* genus, significant number of cytological reports are widely known. Based on chromosome studies in different *Nepeta* species, ranging from 2n=14, 16, 18, 32, 34, 36, 54 and basic chromosome numbers, x= 7, 8, 9, 13, 17, 18 (Aryavand, 1975, 1977; Gill, 1979; Marceno and Princiatto, 1980a; Gill, 1981; Ubera, 1983; Snogerup, 1985; Seidenbinder and Verlaque, 1985; Budantsev et al., 1992; Blatisberger and Huber, 1993; Khatoon and Ali, 1993; Trigas and Iatrou, 2006; Saggoo et al., 2011). The chromosome number 2n= 18 and 36 are common in this genus whereas basic chromosome number as x=9 and 17 and 2n=34 are few common (Baden, 1983). On the contrary, Saggoo et al. (2011) reported that x=8 and x=9 were common in this genus. Also, Baden (1983) was observed B chromosome in some of *Nepeta* species. The karyological and chromosome morphology reports of this genus are mainly limited. However, Baden (1983) reported the karyotype of *N. sibthorpii* Benth. with three different groups of chromosome.

The chromosome count and karyotype studies are not only useful in predicting morphological similarity and diversity among *Nepeta* species they are valuable sources of taxonomic and biosystematics information. The absence and sometimes limited research work on chromosome data on the *Nepeta* species in Iran and Zagros means that the chromosome counts and karyology were made on *Nepeta* chromosomes from Zagros region. The objectives of this study are 1) to present karyological information, particularly the differences among them and 2) to determine the chromosome number and basic chromosome number of these taxa. Some of the chromosome numbers and all of the karyotypic illustrations are first reported for Iran.

2. Materials and methods

2.1. *Plant materials*

11 species and 31 accessions collected from natural habitats of Zagros province are listed in Table 1. Voucher specimens of the taxa studied were deposited in the Herbarium of Shahrekord University. The chromosome counts and karyology were done on chromosome 11 species and 31 accessions from Zagros region including *N. bakhtiarica* Rech., *N. persica* Boiss., *N. kotschyi* Boiss, *N. Juncea* subsp. *destrorum* Bornm., *N. glomerulosa* subsp. *carmanica* (Bunge) Benth., *N. oxyodonta* Boiss., *N. sessilifolia* Bunge, *N. pungens* (Bunge) Benth., *N. schiraziana* Boiss., *N. daenensis* Boiss. and *N. fissa* C.A. Mey.

2.2. *Chromosomal studies*

The following procedures were followed, in carrying out the chromosomal studies. For mitotic studies, the seeds collected from various accessions were germinated in sterilized Petri dishes. Then root tips meristems were pre-treated with an ice bath at 4º C for 18 hours and then fixed in a mixture of ethanol: acetic acid (3:1, respectively) for 24 hours. The root tips were macerated in a 1N HCl solution at 60º C for about 5 minutes. A squash technique was used for cytological studies with 2% aceto-orcein solution (Ozkan, 2006). OLYMPUS BX50 photomicroscope provided the clearest mitotic metaphase among 25 cells. Ideograms prepared from mitotic metaphase. Chromosome measurements were based on five metaphase plates (Ozkan and Soy, 2007). From the point of view of chromosome morphology, the chromosome pairs were determined (Levan et al., 1964). In other to ensure the reliability and validity of the statistical estimates, the cluster analysis with Squared Euclidean Distance and Ward Methods were applied using SPSS software ver. 20 with eight cytological characters (L, S, L/S (AR), TL, %TF or the total form percentage [(ΣSA/ΣTL)*100], A1 or intra chromosomal asymmetry index [1- å(SA/LA)/n], A2 or intra chromosomal asymmetry index [Sd /X]; Sd is the average of standard deviation, and X is the mean chromosome length, DRL or difference of range relative length [MaxRL%-MinRL%]) (Huziwara, 1962; Romero-Zarko, 1986; Sheidaei and Jalilian, 2008; Kalvandi et al., 2012).

3. Results

Following the completion of the study, consistent with the chosen research design and methodology, the following results were recorded.

3.1. *N. schiraziana*

The results of this study showed that the chromosome number of *N. schiraziana* is 2n= 6x =54 (Figure 1 A, B), the basic chromosome number is x= 9 and hexaploid species. The above result was the first of its kind in Iran. The karyotype of this species showed Median point (M), median region (m) and sub median region (sm) (Table 2, Figure 3). The chromosome length ranged from 0.64-0.88 µm. The karyotype data were first reported for Iran.

Table 1. the vouchers details of studied *Nepeta* species from Iran

Species/accession	Locality	Altitude (m)
N. bakhtiarica	Chaharmahal va Bakhtiari- Naghan, dopolan	2114
N. persica 1	Chaharmahal va Bakhtiari- saman, Ben	1800
N. persica 2	Isfahan- damaneh	1750
N. persica 3	Kohkilouye va Boyer Ahmad- Yasouj	1800
N. glomerulosa subsp. *carmanica* 1	Chaharmahal va Bakhtiari- boroujen, Lordegan	1797
N. glomerulosa subsp. *carmanica* 2	Isfahan- Vanak Semirom	2000
N. glomerulosa subsp. *carmanica* 3	Kohkilouye va Boyer Ahmad- Sisakht	1900
N. glomerulosa subsp. *carmanica* 4	Chaharmahal va Bakhtiari- Lordegan, Vanak	2402
N. glomerulosa subsp. *carmanica* 5	Chaharmahal va Bakhtiari- Gandoman, Naghe	2440
N. glomerulosa subsp. *carmanica* 6	Chaharmahal va Bakhtiari- Gandoman	2470
N. oxyodonta 1	Chaharmahal va Bakhtiari- Naghan, Helen forest	1855
N. oxyodonta 2	Chaharmahal va Bakhtiari-Naghan, gardane Bare morde	1869
N. juncea subsp. *desertorum* 1	Chaharmahal va Bakhtiari- Boroujen, Sourak	2670
N. juncea subsp. *desertorum* 2	Chaharmahal va Bakhtiari- Boroujen	2610
N. juncea subsp. *desertorum* 3	Isfahan- Vanak Semorom	2150
N. sessilifollia	Chaharmahal va Bakhtiari- Ben, Sheikhe Shaban	2700
N. pungens 1	Chaharmahal va Bakhtiari- Chaleshtor	2000
N. pungens 2	Chaharmahal va Bakhtiari- Ben	2398
N. pungens 3	Isfahan- Damane	1850
N. pungens 4	Kohkilouye va Boyer Ahmad- Yasouj	1900
N. pungens 5	Isfahan- Semirom	1850
N. schiraziana 1	Chaharmahal va Bakhtiari- Naghan, kouh-e Kalar	2370
N. schiraziana 2	Chaharmahal va Bakhtiari- Nghan, Chahar Tagh	2400
N. schiraziana 3	Kohkilouye va Boyer Ahmad- toward Shiraz	1700
N. kotschyi	Chaharmahal va Bakhtiari- Naghan, Helen forest	1917
N. fissa 1	Isfahan- Fereydan	2200
N. fissa 2	Chaharmahal va Bakhtiari- Saman, Tiran	1941
N. fissa 3	Chaharmahal va Bakhtiari- Saman, Hore	2190
N. daenensis 1	Chaharmahal va Bakhtiari- Chahar tagh, kouh-e Kalar	2488
N. daenensis 2	Chaharmahal va Bakhtiari- Naghan, Chahar Tagh	2680
N. daenensis 3	Kohkilouye va Boyer Ahmad- Sisakht	2650

3.2. *N. pungens*

Cytological studies revealed that the chromosome number of 2n=2x=22 was observed in *N. pungens* (Figure 1 C, D), basic chromosome number is x=11 and diploid species. The karyotype is median point (M) and median region (m) (Table 2, Figure 3). The chromosome length varied from 0.69-1.1µm. The chromosome number and karyotype of this species were first reported for Iran.

3.3. *N. fissa*

The chromosome number of *N. fissa* was 2n=2x=22 (Figure 1 E, F), basic chromosome number is x= 11 and diploid species. Median point (M), median region (m) and sub median-region (sm) chromosomes were found in karyotype of this species (Table 2, Figure 3). The chromosome length varied from 0.82-1.47 µm. This is the first time that the karyotype of this species has been reported for Iran.

3.4. *N. juncea* subsp. *desertorum*

The chromosome number of *N. juncea* subsp. *desertorum* was 2n=2x=26 (Figure 1 G, H), the basic chromosome number is x= 13 and diploid species. The karyotype of this species is median point (M), median region (m) and sub-median region (sm) (Table 2, Figure 4). The chromosome length varied from 1.21-1.38 µm. The chromosome number and the karyotype of this species have been first accounted in Iran.

3.5. *N. glomerulosa* subsp. *carmanica*

The chromosome number of *N. glomerulosa* subsp. *carmanica* is 2n= 2x =18 (Figure 1 I, J), the basic chromosome number is x= 9 and diploid species. The karyotype of this species has median point (M), median region (m) and sub-median region (sm) (Table 2, Figure 4). The chromosome length ranged from 1.36-2 µm. The chromosome number and karyotype of this species were first reported in Iran.

3.6. *N. persica*

The chromosome number of *N. persica* was 2n=4x=36 (Figure 2 A, B), basic chromosome number is x=9 and tetraploid species. The karyotype of this species is median point (M), median region (m) and sub-median region (sm) (Table 2, Figure 4). The chromosome length ranged from 1.02-1.22 µm. The karyotype of this species was first reported for Iran.

3.7. *N. oxyodonta*

The chromosome number of *N. oxyodonta* is 2n=6x=42 (Figure 2 C, D), the basic chromosome number is x= 7 and hexaploid species. The karyotype of this species displayed median point (M) and median region (m) (Table 2, Figure 4). The chromosome length varied from 0.83-1.25 µm. Chromosome record of this species was first reported for Iran.

3.8. *N. Daenensis*

Cytological studies showed that the chromosome count of *N. daensisi* is 2n=4x=32 (Figure 2 E, F), the basic chromosome number is x=8 and tetraploid species. Median point (M), median region (m) and sub-median region (sm) chromosomes were observed in karyotype (Table 2, Figure 5). The chromosome length was in range of 0.92-1.27 µm. Chromosome data were first accounted in Iran.

3.9. *N. sessilifolia*

Cytological studies showed that the chromosome number of *N. sessilifolia* is 2n=2x=26 (Figure 2 G), basic chromosome number is x=13 and diploid species. The karyotype of this species is median point (M) and median region (m) (Table 2, Figure 5). The chromosome length ranged from 1.08-2.25 µm. The chromosome number and karyotype of this species were first reported in Iran.

3.10. *N. kotschyi*

The chromosome number of 2n=2x=34 is reported for *N. kotschyi* (Figure 2 H), the basic chromosome number is x= 17 and diploid species. Median point (M), median region (m) and sub-median region (sm) were observed in karyotype of this species (Table 2, Figure 5). The chromosome length ranged from 1.09-2.5 µm. Chromosome data of this species were first recorded in Iran.

3.11. *N. bakhtiarica*

N. bakhtiarica is one of the Zagros endemic species which displayed the chromosome number of 2n=2x=18 (Figure 2 I), the basic chromosome number is x=9 and diploid species. The karyotype of this species showed median point (M) and median region (m) (Table 2, Figure 5). The chromosome ranged from 1.06-2.21 µm. The chromosome counts and karyotype of this Iranian endemic species were recorded for the first time.

The ploidy levels of *Nepeta* species were diploid, tatraploid and hexaploid, and basic chromosome numbers were as x=7, 8, 9, 11, 13, 17 which eight of chromosome counts were first reported for Iran. The highest symmetrical karyotype was observed in *N. schiraziana* (TF= 49%), *N. oxyodonta* (TF= 48%), *N. daenensis* (TF=48%) and *N. persica* (TF=48%) (Table 3). Moreover, the highest cytological variations were observed in A1, DRL, TL, L and A2 characters (Table 3). The highest DRL displays the structural variation in chromosome which is observed in *N. glomerulosa* (DRL=4.99). The highest C.V. was found in *N. daenensis* (C.V.=80.7; A1) and the lowest was found in *N. persia* (C.V.=0.02; TF%). The highest chromosome arm was observed in *S. glomerulosa* (L=1.13) and the lowest was in *S. schiraziana* (L=0.31) (Table 3).

The results of cytological characters and cluster analysis showed two groups, 1) this group comprised two subgroups a) *N. glomerulosa* subsp. *carmanica* (diploid), b) *N. glomerulosa* subsp. *carmanica* (diploid), *N. juncea* subsp. *desertorum* (diploid), *N. bakhtiarica* (diploid), *N. fissa* (diploid), *N. pungens* (diploid) and 2) this group contained two subgroups c) *N. sessilifolia* (diploid), *N. oxyodonta* (hexaploid), *N. daenensis* (tetraploid), *N. schiraziana* (hexaploid) d) *N. daenensis* (tetraploid), *N. schiraziana* (hexaploid), *N. pungens* (diploid), *N. fissa* (diploid), *N. persica* (tetraploid), *N. kotschyi* (diploid) (Figure 6). High chromosomal diversity was found in *N. glomerulosa*, *N. fissa*, *N. pungens*, *N. daenensis* and *N. schiraziana* accessions.

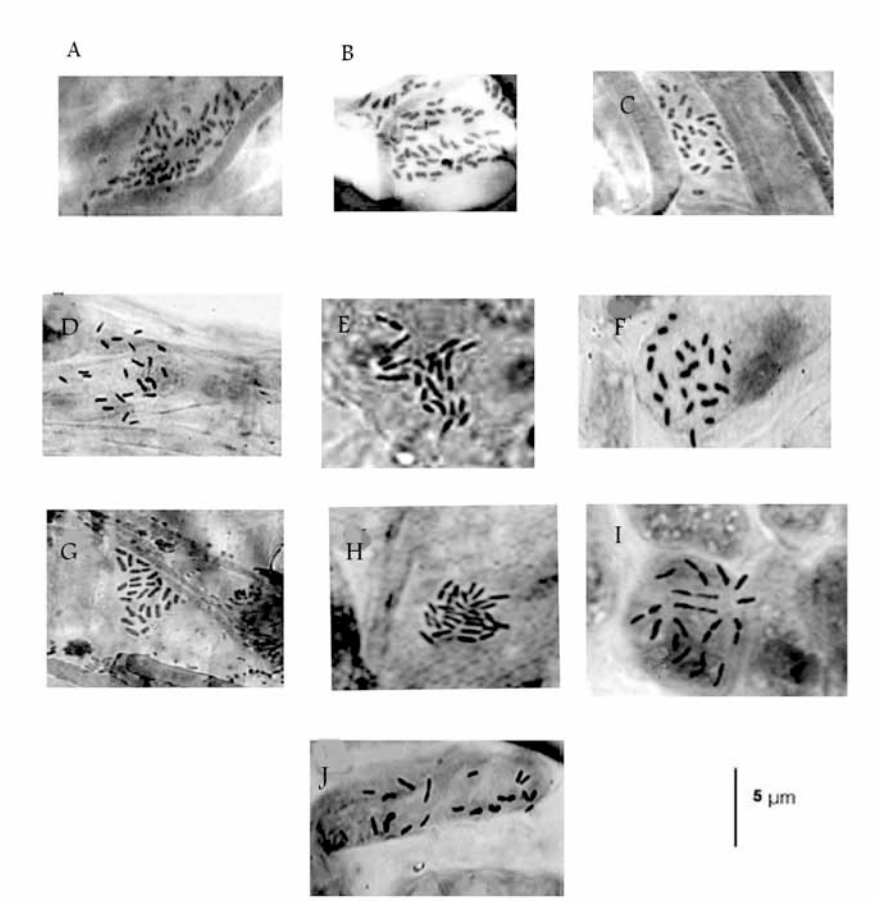

Figure1. Photomicrograph of mitotic division in somatic cells of five *Nepeta* species. A, B: *N. schiraziana* (2n=54), C, D: *N. pungens* (2n=22), E, F: *N. fissa* (2n=28), G, H: *N. juncea* subsp. *desertorum* (2n=26), I, J: *N. glomerulosa* subsp. *carmanica* (2n=18).

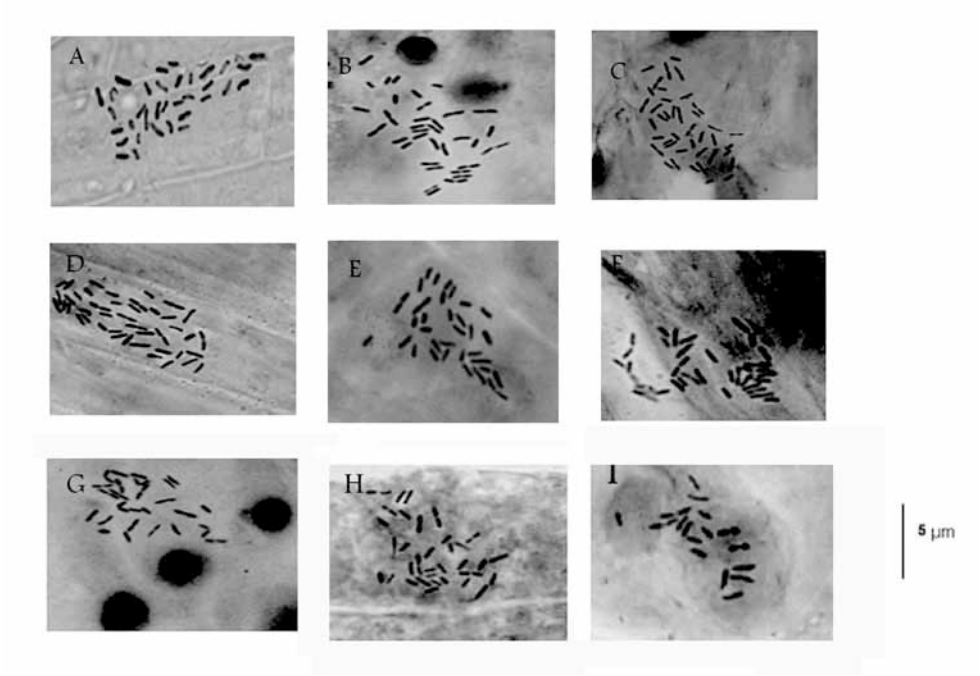

Figure 2. Photomicrograph of mitotic division in somatic cells of six *Nepeta* species. A, B: *N. persica* (2n=36), C, D: *N. oxyodonta* (2n=42), E, F: *N. daenensis* (2n=32), G: *N. sessilifolia* (2n= 26), H: *N. kotschyi* (2n= 34), I: *N. bakhtiarica* (2n= 18).

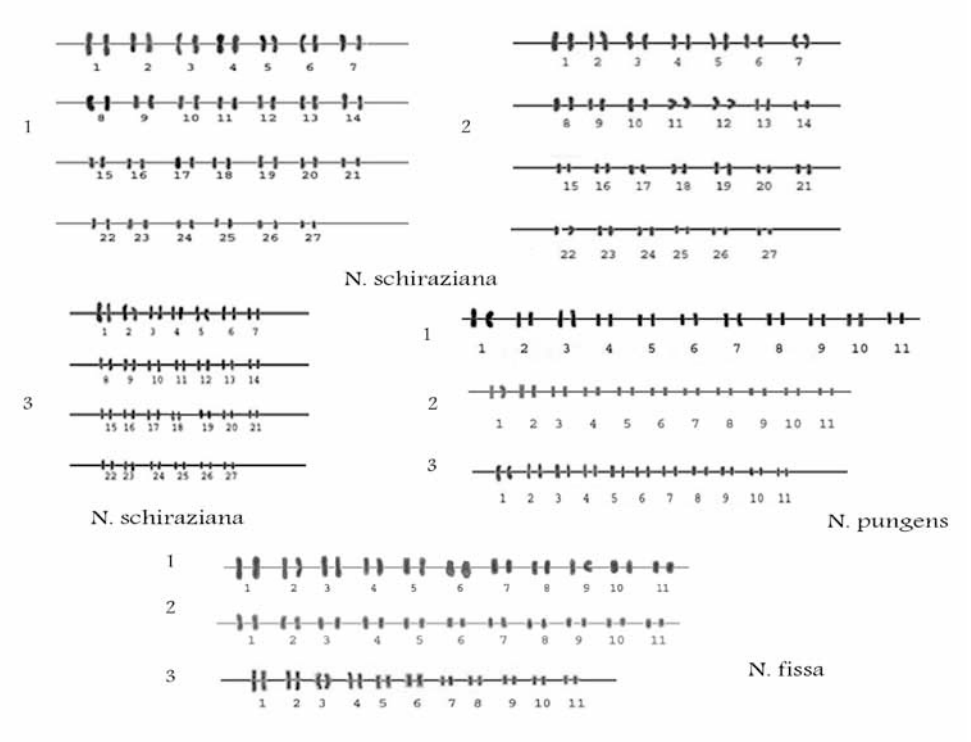

Figure3. Representative of ideogram in four *Nepeta* species.

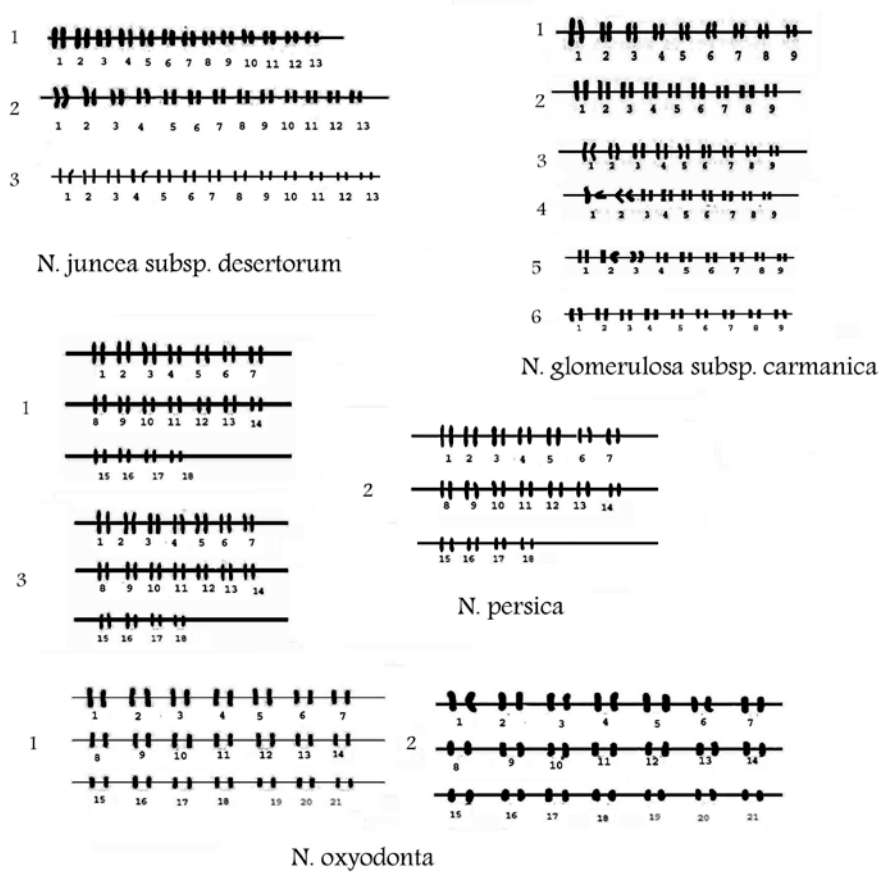

Figure 4. Representative of ideogram in four *Nepeta* species

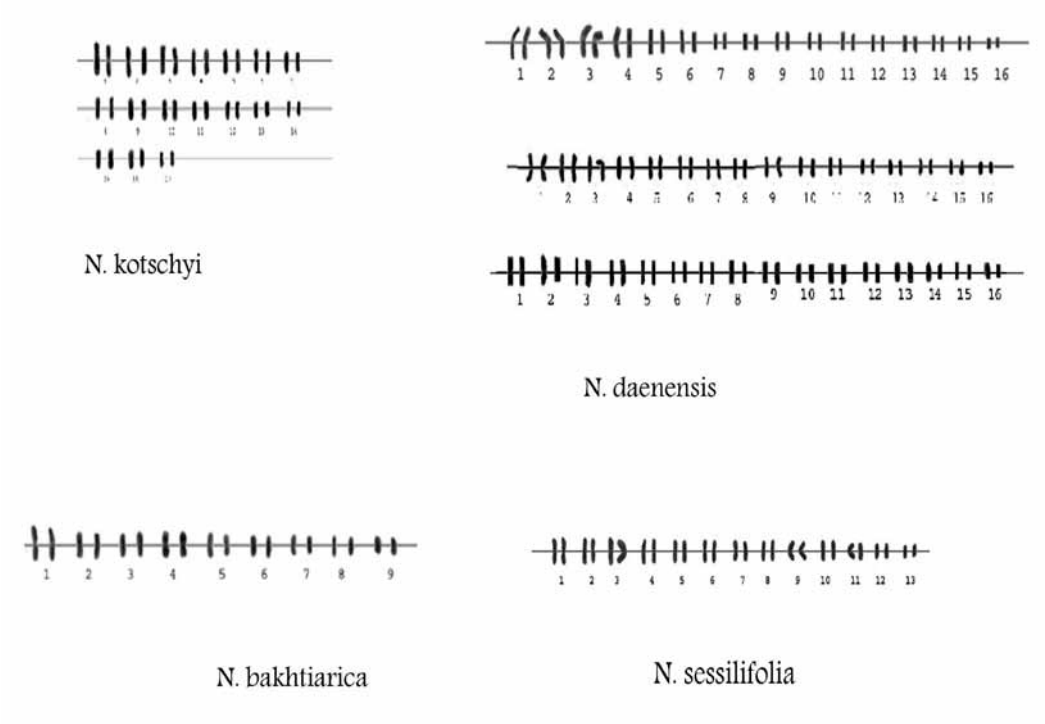

Figure 5. Representative of ideogram in four *Nepeta* species

Table 2. the chromosome number, basic chromosome number, ploidy levels and karyotype formulae in eleven *Nepeta* species

Species	Chromosome number	Basic chromosome number	Ploidy levels	Karyotype formulae
N. bakhtiarica	2n=2x=18	X=9	diploid	5M+4m
N. glomerulosa subsp. *carmanica*	2n=2x=18	X=9	diploid	3M+3m+3sm,5M+4m, 4M+5m,5M+2m+2sm, 7M+1m+1sm,8M+1sm
N. fissa	2n=2x=22	X=11	diploid	9M+1m+1sm, 10M+1m, 8M+2m+1sm
N. pungens	2n=2x=22	X=11	diploid	3M+8m, 11M, 7M+4m
N. persica	2n=4x=36	X=9	tetraploid	7M+10m+1sm, 11M+6m+1sm,8M+4m+6sm
N.sessilifolia	2n=2x=26	X=13	diploid	11M+2m
N. juncea subsp. decertorum	2n=2x=26	X=13	diploid	11M+1m+1sm,3M+10m, 7M+4m+2sm
N. kotschyi	2n=2x=34	X=17	diploid	4M+6m+6sm
N. daenensis	2n=4x=32	X=8	tetraploid	13M+3m,11M+4m+1sm,12M+2m+2sm
N. oxyodonta	2n=6x=42	X=7	hexaploid	19M+2m,13M+8m
N. schiraziana	2n=6x=54	X=9	hexaploid	15M+4m+8sm, 11M+3m+13sm, 11M+ 11m+5sm

Table 3. the chromosome features in eleven and 33 accessions of *Nepeta* species

Species/accession	S	L	L/S	Tl	A1	A2	%TF	DRL
N. schiraziana	0.31	0.32	0.96	0.64	0.02	0.35	48	2.79
N. schiraziana	0.37	0.39	1.05	0.78	0.04	0.37	47	2.31
N. schiraziana	0.44	0.45	1.02	0.88	0.03	0.37	49	2.50
C.V.	16.2	15.7	3.96	15.7	33.3	3.05	2.08	9.48
N. pugens	0.46	0.61	1.32	1.07	0.24	0.28	43	4.71
N. pugens	0.47	0.58	1.23	1.05	0.17	0.19	44	2.76
N. pugens	0.33	0.45	1.37	0.78	0.31	0.23	42	2.85
N. pugens	0.33	0.36	1.09	0.69	0.07	0.27	47	3.22
N. pugens	0.47	0.61	1.3	1.1	0.35	0.2	42	2.32
C.V.	17.1	21.1	7.93	19.3	50	17.39	4.74	28.7
N. fissa	0.44	0.58	1.32	1.02	0.23	0.33	42	4.69
N. fissa	0.63	0.84	1.33	1.47	0.23	0.33	42	4.69
N. fissa	0.37	0.45	1.22	0.82	0.22	0.25	43	3.5
C.V.	27.1	30.6	4.65	30	2.27	13.7	1.34	15.8
N. juncea subsp. *decertorum*	0.54	0.64	1.18	1.19	0.12	0.26	46	2.89
N. juncea subsp. *decertorum*	0.5	0.7	1.4	1.21	0.32	0.2	41	2.77
N. juncea subsp. *decertorum*	0.64	0.74	1.15	1.38	0.15	0.23	46	3.51
C.V.	20	7.24	10.48	7.9	52.6	13.04	6.57	12.7
N. glomerulosa subsp. *carmanica*	0.74	0.96	1.29	1.7	0.35	0.18	42	3.65
N. glomerulosa subsp. *carmanica*	0.73	0.97	1.32	1.7	0.27	0.18	42	2.96
N. glomerulosa subsp. *carmanica*	0.67	1.04	1.55	1.71	0.41	0.29	38	4.99
N. glomerulosa subsp. *carmanica*	0.69	0.88	1.27	1.58	0.22	0.19	43	3.2
N. glomerulosa subsp. *carmanica*	0.86	1.13	1.31	2	0.31	0.17	42	5.65
N. glomerulosa subsp. *carmanica*	0.57	0.79	1.38	1.36	0.28	0.22	41	2.86
C.V.	12.6	15.4	7.4	11.9	20	20	4.23	29.8
N. persica	0.5	0.56	1.12	1.07	0.08	0.22	48	2.42
N. persica	0.49	0.52	1.06	1.02	0.04	0.22	48	2.63
N. persica	0.58	0.63	1.08	1.22	0.06	0.22	48	2.64
C.V.	7.69	9.64	2.77	9.1	33.3	5	0.02	4.68
N. oxyodonta	0.47	0.59	1.25	1.25	0.51	0.19	46	1.55
N. oxyodonta	0.4	0.43	1.07	0.83	0.06	0.26	48	2.17
C.V.	9.3	21.5	10.34	27.8	75	18.8	3	23.1

Table 3. (continued)

N.sessilifolia	0.79	0.95	1.20	0.93	0.16	0.21	45	2.59
N. kotschyi	0.73	0.93	1.27	1.66	0.22	0.23	43	2.50
N. daenensis	0.5	0.53	1.06	1.04	0.06	0.35	48	3.83
N. daenensis	0.61	0.66	1.08	1.27	0.06	0.17	48	1.79
N. daenensis	0.43	0.49	1.13	0.92	0.09	0.26	46	2.51
C.V.	17.6	14.2	3.3	15.8	80.7	34.6	2.32	38
N. bakhtiarica	0.64	0.77	1.20	1.43	0.16	0.21	45	4.15

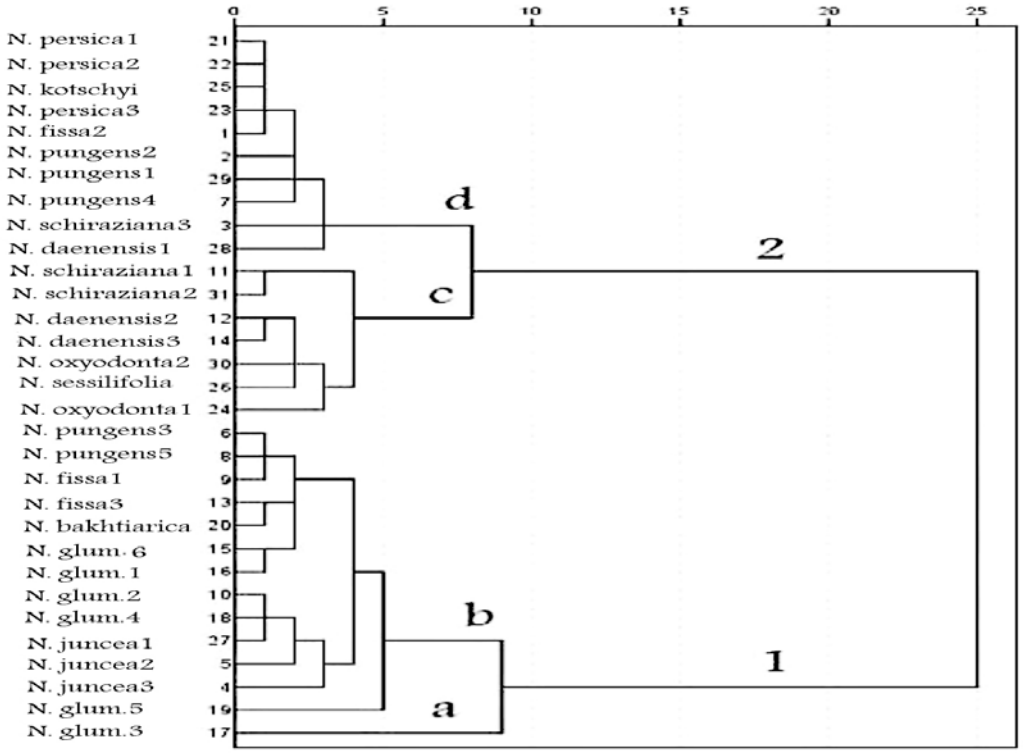

Figure 6. dendrogram of cytological characters and 31 accessions of *Nepeta* species in Iran

4. Conclusions and discussion

The current study presents the somatic chromosome numbers, basic chromosome numbers, and levels of ploidy and karyotype details of 11 species of *Nepeta* in Iran.

The ploidy levels of *Nepeta* species are diploid, tetraploid and hexaploid, and chromosome numbers are 2n= 18, 22, 26, 32, 34, 36, 42, 54. From the basic chromosome number reported above, it appears that *Nepeta* has more than one basic chromosome number. Consequently, these *Nepeta* species are thought to be diploid, tetraploid and hexaploid which is consistent with the literature. To validate this viewpoint, studies in the microsporogenesis of these species will be necessary to substantiate the process. The import of the foregoing is to provide reasonable evidential support for the differences and similarities of the *Nepeta* species studied in the context of the report.

According to the literature, some of the *Nepeta* species are characterized by several basic chromosome number such as x= 7 (Snogerup, 1985; Saggoo et al., 2011), 8 (Aryavand, 1977; Gill, 1981; Baden, 1983; Saggoo et al., 2011), 9 (Aryavand, 1977; Baden, 1983; Ubera, 1983; Gill, 1984; Budantsev et al., 1992; Khatoon and Ali, 1993; Ghaffari and Kelich, 2006; Saggoo et al., 2011), 13 (Saggoo et al., 2011), 17 (Casas, 1976; Ubera, 1983; Budantsev et al., 1992; Blatisberger and Huber, 1993) and 18 (Aryavand, 1977; Gill, 1979, 1984; Ubera, 1983; Seidenbinder and Verlaque, 1985; Khatoon and Ali, 1993). Based on the chromosome number Casas (1976), Aryavand (1977), Gill (1979, 1981,

1984), Ubera (1983), Baden (1983), Snogerup (1985), Seidenbinder and Verlaque (1985), Budantsev et al. (1992), Khatoon and Ali (1993), Blatisberger and Huber (1993), and Ghaffari and Kelich (2006) reported chromosome variation of 2n= 14, 18, 32, 34, 36 and 54 in different *Nepeta* species which refer the tetraploid and hexaploid levels and agree with our results. The basic chromosome number of x= 7, 8, 9, 13, 17 were accorded with previous results, however the chromosome number of 2n= 22 and 42 and basic chromosome number of x= 11 were not based on the previous reports. From the literature, it appears that *Nepeta* has different levels of ploidy. In this study, *N. pungens, N. fissa, N. juncea* subsp. *desertorum, N. glomerulosa* subsp. *carmanica, N. sessilifolia, N. kotschyi* and *N. bakhtiarica* are dioloid whereas *N. persica* and *N. daenensis* are tetraploid, and *N. schiraziana* and *N. oxyodonta* are hexaploid. Based on previous data, the diploid, tetraploid and hexaploid *Nepeta* species were common (Casas, 1976; Aryavand, 1977; Gill, 1979, 1981; Baden, 1983; Ubera, 1983; Gill, 1984; Snogerup, 1985; Seidenbinder and Verlaque, 1985; Budantsev et al., 1992; Khatoon and Ali, 1993; Blatisberger and Huber, 1993; Ghaffari and Kelich, 2006). Moreover, Saggoo et al. (2011) were reported 2n= 18, 36 and x=9, and diploid and tetraploid levels for 14 *Nepeta* species from India. In addition, the tetraploid cytotypes in 15 *Nepeta* species have been reported. It seems that the diverse chromosome numbers and basic numbers of *Nepeta* species indicate that the group of species in different regions has differentiated independently after diffusion (Yang et al., 2004). Moreover, the high base number of 17 appears to be secondary in origin and might have arisen by amphiploidy (Gill, 1979). The different chromosome numbers indicate that aneuploidy occurs in these species (Esra et al., 2011). However, remarkable variation could reflect the nuclear DNA variation (Javadi et al., 2011).

The karyotype details of *Nepeta* species in this context have not exactly been reported so far. Baden (1983) first reported the metacentric and sub-metacentric for *N. sibthorpii* which supports our results. Baden (1983) argues that details karyotype studies are difficult due to the small size of chromosomes. In this study, the chromosome type is median point (M), median region (m) and sub-median region (sm) and the range of chromosome length in this study varies between 0.64-2 µm which is supported by Baden (1983) and mostly without constrictions which not corresponds with Baden (1983). Among the studied taxa, the highest length value was observed in *N. glomerulosa* subsp. *carmanica* (0.97-1.13µm) and the lowest was in *N. schiraziana* (0.31 µm). The karyotype and variation patterns in basic chromosome number were related to the specific speciation mechanisms (Sheidaei and Jalilian, 2008), which suggests an important role in speciation and evolution of *Nepeta* species. The differences in chromosome length might come from population growth in different regions (Esra et al., 2011).

B chromosomes, which are also recognized as accessory chromosomes, have been often detected in some of *Nepeta* species. Baden (1983) first reported 2n= 16+1-2B and 1-2 satellites for *N. sibthorpii*. In our results, there are not any B chromosomes and satellite in taxa studied.

The chromosome number of *N. fissa* was previously reported 2n=18 from Iran, Teheran province by Aryavand (1977). In our results the chromosome number of this species was observed 2n= 22 which is contrary to the previous report. In this case of variability, Gill (1979) reported the intra-specific races for some of *Nepeta* species. Moreover, *N. schiraziana* was reported 2n=16 by Aryavand (1975), whereas in this research we found 2n=54 for this species. Aryavand (1977) also reported n= 8 for *N. persica* from Iran, Isfahan province. Nevertheless in this research we found 2n=4x=36 and n=9 for this species. Based on the chromosome number variation, Budantsev et al. (1992) were reported 2n=34, n=17 in *N. cataria* L. which differs from that of Sugiura (1940) and Gill (1979)' report, who obtained 2n=36. Saggoo (1983, PhD thesis) reported n= 9 for *N. distans* Royle ex Benth. and n=18 for *N. hindostana* (Roth.) Haines, but Gill (1984) reported n=18 for *N. diastans* and n= 9 for *N. hindostana*. Nakata et al. (2001) reported n=9 (2n=18) for *N. subsessilis* Maxim. with intra-specific polyploidy. Budanstsev et al. (1992) recorded 2n= 34 for *N. grandiflora* M. Bieb. and Krahulcova (1991) obtained 36 for this species. *N. racemosa* Lam. was first reported by Aryavand (1975) with n=18 and tetraploid level but Ghaffari and Kelich (2006) reported 2n=18, n=9 and diploid level for this species. Also, Baden (1983) reported 2n= 16 for *N. camphorata* although Gill (1981) obtained 2n=32 for this species. Budanstsev et al. (1992) reported 2n= 16 and 18 for *N. transcaucasica* Grossh. It can be inferred that the chromosome number of *Nepeta* species displayed high variations. Moreover, Saggoo et al. (2011) reported that aneupliody is operative both at diploid and polyploidy levels. They conclude that five species namely as *N. cataria* (2n=34, 36), *N. distans* (2n= 18, 26), *N. grandiflora* (2n= 34, 36), *N. nepetella* L. (2n= 34, 36) and *N. transcaucasica* (2n=16, 18) display intra-specific aneuploidy without effecting ploidy level. It might be concluded that out breeding systems may be responsible for the chromosome variations (Murray and Young, 2001).

Khatoon and Ali (1993) reported n=9 for *N. juncea*. In our results we found 2n=26 for *N. juncea* subsp. *desertorum*. Based on the variations of chromosome number in *Nepeta* subspecies, Seidenbinder and Verlaque (1985) obtained 2n=36 for *N. nepetella* but Ubera (1983) recorded n=17 for *N. nepetella* subsp. *aragonensis*.

Based on the results of cluster analysis, the diploid species as *N. fissa* (2n=2x=22), *N. pungens* (2n=2x=22), *N. juncea* (2n=2x=26), *N. bakhtiarica* (2n=2x=18) and *N. glomerulosa* (2n=2x=18) were clustered in one group. In addition, *N. daenesis* (2n=4x=32) with tetraploid level was closely clustered with *N. schiraziana* (2n=6x=54) and *N. oxyodonta* (2n=6x=42) as hexaploid species. It seems that haxaploid and tetraploid species display different groups with diploid species. Moreover, *N. persica* (2n=4x=36) as tetraploid species and *N. oxyodonta* as hexaploid species were also grouped with some of diploid species. The highest cytological diversity observed in *N. fissa, N. pugens, N. glomerulosa, N. schiraziana* and *N. daenensis*. Obviously, the diploid accessions have more diversity than the tetraploid and

hexaploid species as *N. daenensis, N. schiraziana* and *N. pugens*. Most of the diploid species were closely clustered, and the tetraploid and hexaploid species were grouped in one cluster. These differences between ploidy levels might be due to the high gene flow at infra-specific levels and chromosome variation of these species. Furthermore, the diploid species have high potential to initiate speciation (Sheidaei and Jalilian, 2008). Chromosomal variations at the diploid levels seem to play a leading role and sympatric speciation via hybridization and polyploidization (Zhiyum et al., 2004). The change in the chromosomal traits is one of the mechanisms of inter and intra species diversification (Kalvandi et al., 2012).

Finally, *Nepeta* is a genus with diverse chromosome numbers and in some of the species the variability in chromosome complements is common (Goldblatt and Johnson, 2003). However, changes in the chromosome number and variation of karyotype structure can be considered as the main device of species diversification and the predominant feature of chromosomal evolution of this genus (Zhiyum et al., 2004; Sheidaei and Jalilian, 2008). Identifying the chromosome number of eleven *Nepeta* species in this study provides a base for biosystematic studies. Consistent with the study objectives, it is concluded that Zagros region is one of the diversity and speciation centers for this genus in Iran and provide the evolutionary trends in this genus. Further research work could be advanced to uncover necessary differences and similarities where necessary in other to provide additional insights and perspectives regarding *Nepeta* genus and related species.

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