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CYTOLOGICAL STUDIES ON FOUR ENDEMIC SPECIES OF NEPETA L. (LAMIACEAE)

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In the present study we report somatic chromosome numbers of four *Nepeta* species including: *N. laxiflora* (2n=18), *N. depauperata* (2n=34), *N. archibaldii* (2n=16) and *N. dschuparensis* (2n=18). These species are endemics of Iran and the chromosome number for these species are reported here for the first time.

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Key words: Lamiaceae; Chromosome number; new counting; *Nepeta*; Iran

مطالعه سیتولوژیکی چهار گونه انحصاری Nepeta L. (نعناییان) در ایران

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تحقیق حاضر عدد کروموزوم سلول‌های غیر جنسی چهار گونه از جنس Nepeta L. شامل N. laxiflora (2n=18)، N. depauperata (2n=34)، N. archibaldii (2n=16)، N. dschuparensis (2n=18) را گزارش می‌کند. این گونه‌ها انحصاری ایران هستند و عدد کروموزومی آنها برای اوّلین بار گزارش می‌گردد.

INTRODUCTION

The genus *Nepeta* L. belongs to Lamiaceae family. It includes about 300 species of herbaceous perennial, annual, and fruticose plants (Rechinger 1982; Jamzad & al. 2003a; Kaya & Dirmenci 2008). *Nepeta* (catmint) is economically an important genus in the Nepetoideae. *Nepeta* species are significantly distributed in Eurasia, North Africa and Canary Islands (Jamzad & al. 2003a). Endemic plants are determined as plants confined to small geographic ranges characterized by low population size and habitat specificity (Kruckeberg & Rabinowitz 1985). Of the 2300 endemic species in Iran, 85% are present in the Irano-Turanian area and Iran is one of the centers of variety for the genus *Nepeta* (Jamzad & al. 2003b). Endemic species are scientific and economically significant. Therefore, the recognition of these plants, their conservation and genetic studies are of interest to the scientific society (Ghaffari & al. 2005). Many karyological data concerning chromosome numbers of the genus have already been reported as x= 7, 8, 9, 13, 17, 18. (Goldblatt & Johnson 1979–2017) and there are a few reports from Iran (Aryavand 1977; Ghaffari & Kelich 2006;

Kharazian & al. 2013; Payandeh & al. 2015, Akbarpur Mamagani & al. 2016). The three chromosome number in *Nepta* are x=8, 9 and 17 as the most common primary and secondary base numbers (Gill 1979; Saggoo & al. 1983, 1984). The numerical variation in chromosome numbers within a genus is quite common. Previous studies indicate that the genus *Nepeta* has a heterogeneous set of chromosome numbers (Srivastava 2012). This study aimes to determine chromosome number, ploidy level and karyotype characteristics of four endemic species of *Nepeta* not studied previously.

MATERIALS AND METHODS

Four *Nepeta* species including *N. laxiflora* (two populations), *N. depauperata* (one population), *N. archibaldii* (one population) and *N. dschuparensis* (one population) have been studied (table 1). Vouchers are deposited in the herbarium of the Research Institute of Forests and Rangelands of Iran (TARI).

For cytological study, rootlets were collected from germinated seeds on wet filter paper in petri dishes at 25°C temperature, when they reached 0.5–1 cm in length, rootlets were separated. The root tips meristems treated with 0.5% saturated α-Bromo naphthalene at 4°C for 1 h and washed and fixed in Carnoy solution (3:1 absolute ethanol:glacial acetic acid) overnight. Then the root tips were rinsed for 1h in distilled water. Hydrolysis was carried out with NaOH (1 normal) at 60ºC for 20-25minutes and used hematoxylin-iron for chromosome staining for 1-2 h at room temperature. Root tips were squashed in a droplet of 45% acetic acid. The root tips were hydrolyzed for 5-8 min in 1 N HCl at room temperature, washed and stained in 2% Hematoxylin for 1 h. OLYMPUS BH-2 photomicroscope provided the clearest mitotic metaphase among 5 cells. Karyotypes were prepared and chromosome pairs were classified according to Levan & al. (1964). The chromosomes were arranged according to their lengths. The long arm (LA), short arm (SA), and total chromosome length (CL) were measured.

Table 1. The voucher specimens and collecting data of studied *Nepeta* species.

No	Species	Geographical Location
1	*Nepeta archibaldii* Rech. f.	Iran: Chaharmahal and Bakhtiari, right side of Nasir Abad Dam, north slope, 2674m, Ajani and Hasaninejad , 107083, TARI.
2	*N. depauperata* Bent.	Iran: Hormozgan, N. Bandar Abbas, N. slope of Mt. Bokhon, 1603 m, Ajani, 105673, TARI.
3	*N. dschuparensis* Bornm.	Iran: Kerman, Saruyeh to Jiroft-Bob Gorgi, 2935 m, Pourmirzae, 107088, TARI.
4	*N. laxiflora* Benth.	Iran: Chaharmahal va Bakhtiari, S. Chelgard Doabe Samsami to Bazoft, close to Telecommunication station, 2903m, 107085, TARI Iran: Chaharmahal va Bakhtiar, Shahrekord, to Morgh malek, 2623m, Ajani & Hasaninejad, 107084, TARI.

RESULTS

Karyotypic features

Details of karyotypic features of studied *Nepeta* species are provided in tables 2 &3 and figs. 1 & 2.

Nepeta archibaldii is a narrow endemic species. It grows in a limited geographical area in west Iran. This specie showed a diploid chromosome number 2n=2x=16 (fig. 1 A) and the basic chromosome number of x=8. The karyotype was formed of seven pairs of submetacentric and one pair of metacentric chromosomes (tables 2, 3; fig. 2 A). The chromosome length ranged from 1.3-2.8µm.

N. depauperata is an Irano-Turanian endemic growing in west and south of Iran. The results showed that this specie is also diploid with chromosome number of 2n=34 (fig.1 B). Karyotype consisted of 17 pairs of metacentric chromosomes (tables 2, 3; fig. 2 B). The mean length of chromosome varied from 1.02-1.56µm.

N. dschuparensis is an endemic perennial species found in few localities in south Iran. The studied specimens showed a diploid chromosome number of 2n=2x=18 in this taxon (fig. 1 C) and basic chromosome number of x=9. Karyotype in this taxon consisted of 9 pairs of submetacentric chromosomes (Tables 2, 3; fig. 2 C). Total karyotype length is 1.26-1.98µm.

N. laxiflora is an endemic perennial species, with a distribution range in central and west of Iran. Chromosome number of 2n=2x=18 and somatic chromosome count in this specie showed an x=9 (fig. 1 D). Karyotype included 8 pairs of submetacentric and one pair of metacentric chromosomes in this specie (tables 2, 3; fig. 2 D). The chromosome length varied from 1.19-1.73µm.

Table 2. The chromosome number (2n), basic chromosome number, ploidy levels and karyotype formulae in four *Nepeta* species.

No	Species	Chromosome number	Basic chromosome number	Ploidy levels	Karyotype formula	Chromosome length range (µm)
1	*Nepeta archibaldii*	16	X=8	diploid	7sm+ 1m	1.3-2.8
2	*N. depauperata*	34	X=17	diploid	17m	1.02-1.56
3	*N. dschuparensis*	18	X=9	diploid	9sm	1.26-1.98
4	*N. laxiflora*	18	X=9	diploid	8sm+1m	1.19-1.73

Table 3. Karyomorphological parameters (arm ratio and centromeric index, short arms, long arms, total chromosome length) of *Nepeta* species.

No	Species	AR (µm)	CI (µm)	SA (µm)	LA (µm)	CL (µm)
1	*Nepeta. archibaldii*	0.5	0.33	0.65	1.32	1.97
2	*N. depauperata*	0.75	0.43	0.54	0.73	1.27
3	*N. dschuparensis*	0.5	0.33	0.51	1.03	1.55
4	*N. laxiflora*	0.57	0.36	0.52	0.92	1.44

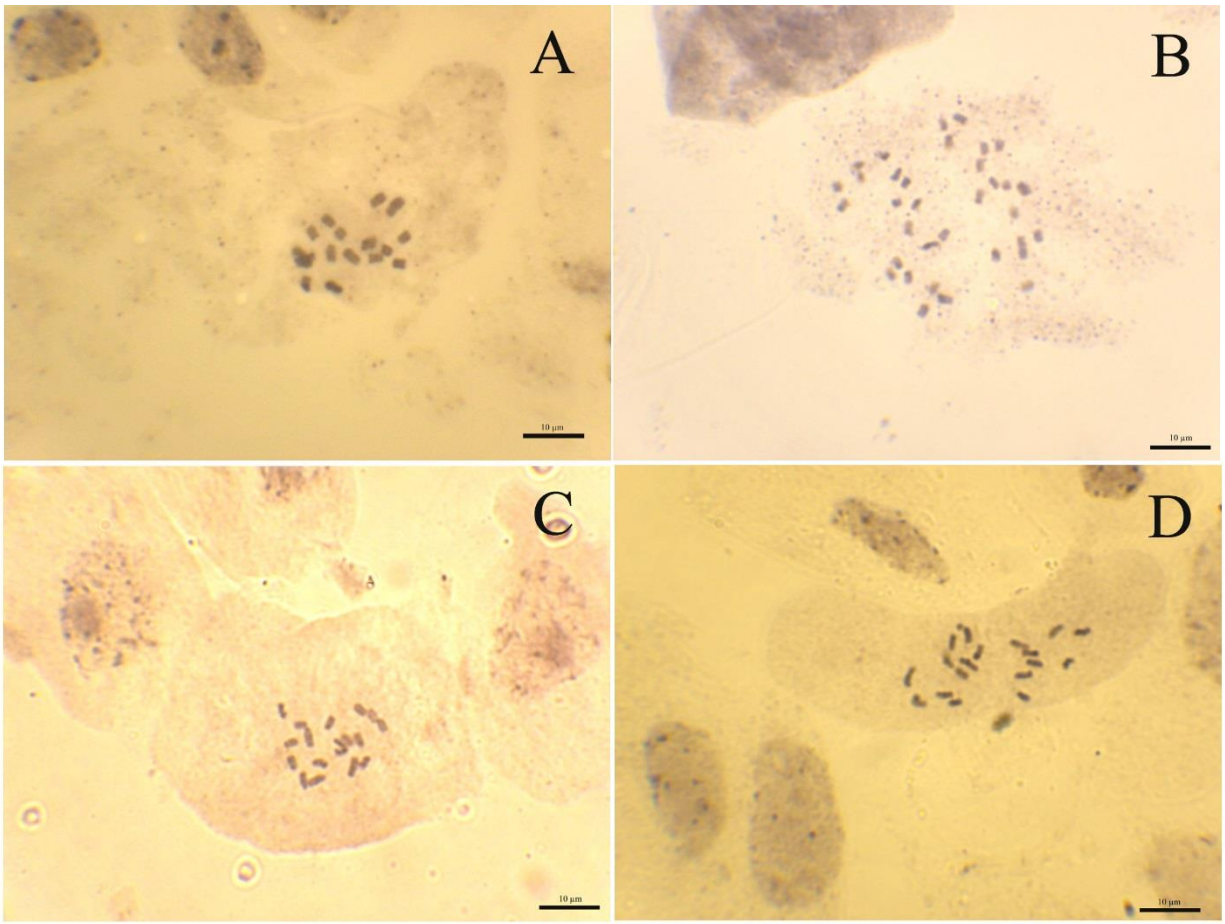

Fig. 1. Somatic chromosomes of *Nepeta*. A, *N. archibaldii* (2n=16); B, *N. depauperata* (2n=34); C, *N. dschuparensis* (2n=18); D, *N. laxiflora* (2n=18). Scale bars: A, B, C, D 10 µm.

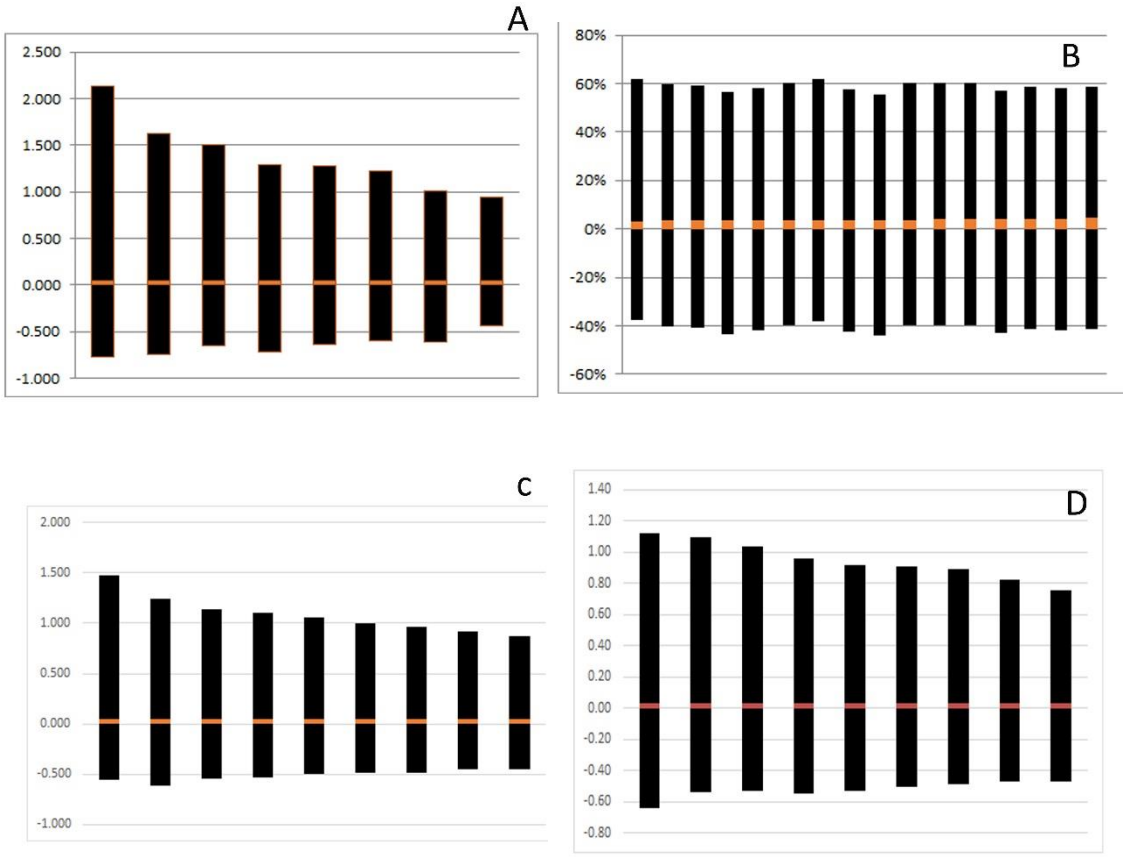

Fig. 2. Idiograms of the karyotypes of *Nepeta*. A, *N. archibaldii* (2n=16); B, *N. depauperata* (2n=34); C, *N. dschuparensis* (2n=18); D, *N. laxiflora* (2n=18). Scale bars: A, B, C, D 10 µm.

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