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شمارش کروموزومی نو در برخی از گونه‌های گیاهی شرق و جنوب شرقی ایران

فیروزه بردبار* و منصور میرتاج الدینی

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چکیده. در این مطالعه عدد کروموزومی و کاریوتیپ برخی از گونه‌های گیاهی انحصاری که در شرق و جنوب شرقی ایران پراکنش دارند، مورد بررسی قرار گرفت. شمارش کروموزومی میتوزی برای شش گونه متعلق به سه تیره از نهان‌دانگان شامل: *Chaenorhinum grossecostatum* (2n=24)، *Nanorrhinum campyloceras* (2n=18)، *Linaria iranica* (2n=12) (بارهنگیان)، *Gaillonia bruguieri* (2n=22) (روناسیان)، *Nepeta rivularis* و *N. assurgens* (2n=18) (نعنائیان) برای اولین بار ارائه شده است.

واژه‌های کلیدی. ایران، عدد کروموزومی، کاریوتیپ، گونه انحصاری، میتوز

New chromosome counts in some plant species from east and south-east Iran

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Abstract. In this study, we investigated the chromosome numbers of some endemic species distributed in East and South-East Iran. The mitotic chromosome counts were presented for the first time for six species in three angiosperm families including: *Linaria iranica* (2n=12), *Nanorrhinum campyloceras* (2n=18), *Chaenorhinum grossecostatum* (2n=24) (Plantaginaceae), *Gaillonia bruguieri* (Rubiaceae) (2n=22), *Nepeta rivulari*s and *N. assurgens* (Lamiaceae) (2n=18).

Keywords. chromosome number, endemic species, Iran, karyotype, mitosis

INTRODUCTION

Chromosomal investigations were beneficial to systematics and evolutionary biology and are powerful sources for the clarification of the affinity among the taxa in molecular systematics (Soltis, 2014). In this study six taxa mostly endemic, growing in different localities of east and southeast of Iran were examined. The aim of this study was to determine the somatic chromosome number and karyotypes for *Linaria iranica* Hamdi & Assadi, *Chaenorhinum grossecostatum* Speta, *Nanorrhinum campyloceras* (Aellen & Esfand.) Naanaie, Assadi & Tavassoli, *Gaillonia bruguieri* A.Rich. ex DC., *Nepeta assurgens* Hausskn. & Bornm. ex Bornm. and *Nepeta rivularis* Bornm. for the first time.

MATERIALS AND METHODS

For the study of karyotypes, online data bases, 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 (Table 1). The seeds of *Linaria iranica* were obtained from the isotype specimen. The vouchers were deposited in MIR herbarium. We used squash method according to Payandeh *et al.* 2015.

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For this purpose, seeds were germinated on wet filter paper in Petri dishes at 25 °C. Roots with 1–2 cm length were pretreated for 5 h in α-monobromonaphthalene at 4 °C and washed and fixed in Carnoy I solution (3:1 absolute ethanol:glacial acetic acid) for 24 hours. At the next fixation step, hydrolysis was carried out in 1N HCl at 60 °C for 2 min. Then, the roots were washed and stained in aceto-iron hematoxilin for 24 h at 30 ºC. The roots were gently squashed in 45% acetic acid on a glass slide. The images of the clearest mitotic metaphases of 5-10 cells were taken using an Olympus BH-2 light microscope equipped with camera photomicrograph system.

Table. 1. Name of the plant species, locality information and chromosome numbers.

Taxon	Locality information	Chromosome number
Plantaginaceae		
Linaria iranica Hamdi & Assadi	Iran, SE, Kerman, Jiroft, Rud farq, 03.IV.1996, Mirtadzadini 2000 (MIR)	2n=12
Chaenorhinum grossecostatum Speta	Iran, SE, Kerman, Rayen, Hossein Abad, N29° 20' 39.79", E57° 28' 35.22", 2267 m, 31.V.2015, Bordbar 2907 (MIR)	2n=24
Nanorrhinum campyloceras (Aellen & Esfand.) Podlech & Iranshahr	Iran, SE, Kerman, Jiroft to Deh-Bakri, N28° 49' 09.02", 57° 48' 09.02", 1269 m, 01.VI.2015, Bordbar 2908 (MIR)	2n=18
Rubiaceae		
Gaillonia bruguieri A.Rich. ex DC.	Iran, E: Khorasan, N Nehbandan, E Kalata Seied Ali village, N31° 37' 15.5", E6 0° 01' 13.8", 1435 m, 02.V.2017, Mirtadzadini and Jafari 2373 (MIR).	2n=22
Lamiaceae		
Nepeta assurgens Hausskn. & Bornm. ex Bornm.	Iran, SE, Kerman, North of mt. Lalehzar, near the point N29° 28' 20.14", E56° 48' 28.06", ca. 3000 m, 08.VIIII.2017, Bordbar 2909 (MIR).	2n=18
Nepeta rivularis Bornm.	Iran, SE, Kerman, North of mt. Lalehzar, ca. 3000 m, Mirtadzadini 2384 (MIR).	2n=18

RESULTS AND DISCUSSION

The karyotypes of the taxa examined are presented in Fig. 1.

Linaria iranica (Plantagiaceae)

The results of this study revealed 2n=12 and x=6 for *Linaria iranica* (Fig. 1. A). *Linaria* Miller with about 200 taxa is the largest and worldwide distributed genus of the tribe Antirrhineae Chav. (Sutton, 1988). *Linaria* species include 35 species of annual or perennial herbs in Iran, which are distributed in all parts of the country (Hamdi & Assadi, 2011). According to Hamdi & Assadi (2011) *Linaria iranica* belongs to sect. *Versicolores* (Benth.) Wettst. This species is an annual herb and narrow endemic species restricted to a very small area in South-East Iran (Hamdi & Assadi, 2011; Podlech & Iranshahr, 2015). Since the first report (Hamdi *et al*., 2008), no additional observations of the species have been made in the field. This can be an indication of its endangered conservation status. The basic chromosome numbers reported for *Linaria* up till now are x= 6, 7, 9, 12 and 13, however, the highest frequent chromosome number reported is 2n=12 (Ranjbar & Nouri, 2015). Therefore, the chromosome number 2n=12 for *L. iranica* is in accordance with previous counts for *Linaria*.

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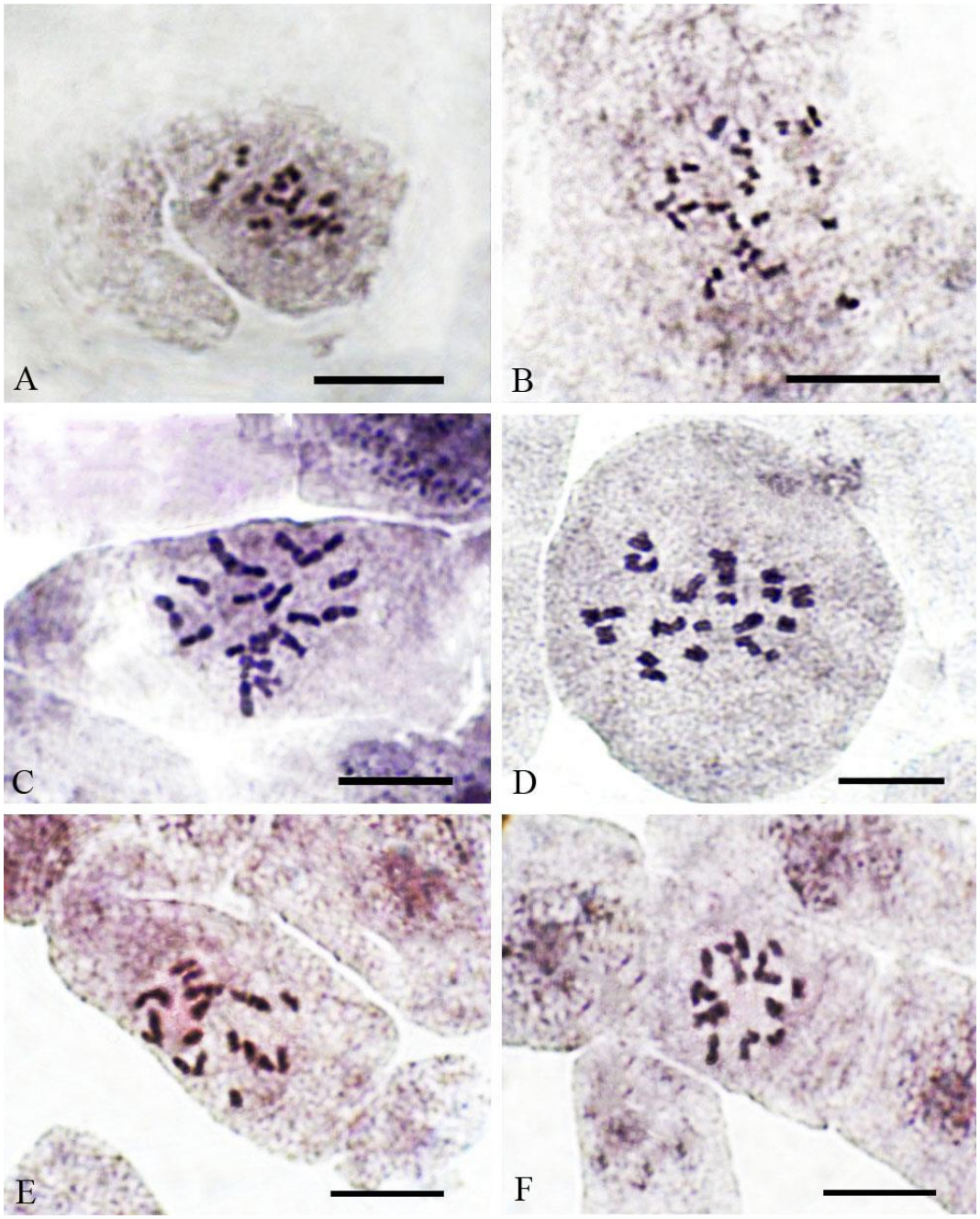

Fig. 1. Somatic chromosomes of **A:** *Linaria iranica*, **B:** *Chaenorhinum grossecostatum*, **C:** *Nanorrhinum campyloceras*, **D:** *Gaillonia bruguieri*, **E:** *Nepeta assurgens* and **F:** *Nepeta rivularis* .Scale bar = 10 µm.

Chaenorhinum grossecostatum (Plantagiaceae)

The results of this study revealed 2n=24 for *Chaenorhinum grossecostatum* (Fig. 1. B). *Chaenorhinum* (DC.) Rchb., including annual herbs, is a genus belonging to the sect. *Microrrhinum* (Endl.) D.A.Sutton, tribe Antirrhineae that has four species in Iran (Podlech & Iranshahr, 2015). This is a narrow endemic species which is distributed in Kerman province, located in South-East Iran (Hamdi & Assadi, 2011). Among *Chaenorhinum* species, the chromosome count was reported for *Chaenorhinum rodriguezii* (Porta) L. Sáez & Vicens (2n=14) (Castro & Rosselló, 2007) and according to Index to Plant Chromosome Numbers (IPCN) (Goldblatt & Johnson, 1979–) for *Ch. minus* (L.) Lange (2n=14 and 28), *Ch. origanifolium* (L.) Fourr. (2n=14), *Ch. tenellum* Lange (2n=14) and *Ch. villosum* Lange (2n=14). Therefore, 2n=24 (in *C. grossecostatum*) has not been reported before among *Chaenorhinum* species.

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***Nanorrhinum campyloceras* (Plantagiaceae)**

The results of this study revealed 2n=18 and x=9 for *Nanorrhinum campyloceras* (Fig. 1. C). *Nanorrhinum* Betsche, including perennial subshrubs or herbs, belongs to the tribe Antirrhineae, which has four species in Iran (Podlech & Iranshahr, 2015) of which *N. campyloceras*, is an endemic species distributed in South and South-East Iran (Hamdi & Assadi, 2011). The chromosome number of 2n=18 have been previously reported for *Nanorrhinum* species *(N. asparagoides* (Schweinf.) Ghebr., *N. heterophyllum* (Schousb.) Ghebr., *N. ramosissimum* (Wall.) Betsche, and *N. woodii* (D.A.Sutton) Ghebr.) (Ghebrehiwet, 2001). Therefore, the results of the present work are in accordance with the results of previous reports.

***Gaillonia bruguieri* (Rubiaceae)**

The results of this study indicated 2n=22 and x=11 for *Gaillonia bruguieri* (Fig. 1. D). *Gillonia* A. Rich. ex DC. consists of small subshrub or perennial herbs mainly distributed in semi-deserts of Irano–Turanian region of the world (Ehrendorfer & Schönbeck–Temesy, 2005). *G. bruguieri* is the main and most abundant species of *Gaillonia* in Iran also existing in Afghanistan and Iraq. According to the literature, only the chromosome number of *G. macrantha* Blatt. & Hallb. has been recorded so far (Khatoon & Ali, 1993). The chromosome number of this species was 2n=22, so the number x=11 for *G. bruguieri* is in accordance with the previous report for this genus.

***Nepeta assurgens* and *N. rivularis* (Lamiaceae)**

The results of this study revealed 2n=18 and x=9 for both *Nepeta assurgens* and *N. rivularis* (Fig. 1. E and F, respectively). This is the first chromosome count for these species.

Nepeta L. belongs to the family Lamiaceae and subfamily Nepetoideae, tribe Mentheae (Cantino *et al*., 1992). *Nepeta* species are perennial, rarely annual, herbaceous and fruticose plants and consist of 300 species in the world (Jamzad *et al*., 2003). The majority of the recorded data in various studies revealed that x=8 and x=9 were the most common primary basic chromosome numbers of the genus and the original basic numbers for Lamiaceae family. The other basic chromosome numbers are considered to be secondarily evolved (Srivastava, 2012). Previous studies (Saggoo *et al*., 2011; Kharazian *et al*., 2013; Payandeh *et al*., 2015) have frequently reported 2n=18 in *Nepeta*, which support the classical view of x=9 as a primary basic number. *Nepeta rivularis* and *N. assurgens* are perennial herbs and narrow endemic species restricted to a very small area in South East Iran (Rechinger, 1982; Jamzad, 2012). The chromosome number of these species (2n=18) is in accordance with the previous report for this genus.

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