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CHROMOSOME COUNTS OF TWELVE VASCULAR PLANT SPECIES FROM IRAN

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Twelve plant species belonging to seven families were collected from the wetlands of Kermanshah Province. Chromosome numbers for *Sinapis arvensis* L. (2*n* = 18), *Rumex cyprius* Murb. (2*n* = 18), *Calamagrostis pseudophragmites* var. *tartarica* (Hook. f.) R.R. Stewart (2*n* = 42) and *Scandix stellata* Banks & Sol. (2*n* = 20) are reported for the Flora of Iran for the first time. The karyomorphological parameters and Stebbins class were determined for all species.

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Keywords: Chromosome counts; Apiaceae; Poaceae; Asteraceae; Brassicaceae; Plantaginaceae; Amaranthaceae; Polygonaceae; Ideogram; Karyotype; Kermanshah Province; Iran

شمارش کروموزومی دوازده گونه از گیاهان آونددار ایران

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معصومه خان‌حسنی: مربی پژوهش مرکز تحقیقات کشاورزی و منابع طبیعی استان کرمانشاه، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرمانشاه، ایران

دوازده گونه گیاهی متعلق به هفت تیره از مناطق ماندابی استان کرمانشاه جمع‌آوری گردید. شمارش کروموزومی برای چهار گونه Calamagrostis pseudophragmites var. tartarica (Hook. f.) R.R. Stewart (2n = 42)، Rumex cyprius Murb. (2n = 18)، Sinapis arvensis L. (2n = 18) و Scandix stellata Banks & Sol. (2n = 20) برای اولین بار در فلور ایران گزارش شده است. پارامترهای کاریومورفولوژیکی و کلاس Stebbins نیز برای همه گونه‌ها تعیین شد.

INTRODUCTION

The formation of wetland habitats mainly takes place in mountainous areas due to the numerous small and large, permanent and non-permanent rivers and streams. These habitats are of great importance including biodiversity, groundwater treatment, preventing erosion around wetlands, managing water during heavy rains, providing shelter and nutrition for

creatures living there (Nafea, 2017). Few studies were conducted on the cytogenetics of wetland plants in Iran. A chromosomal count report from Mirzadeh Vaghefi and Jalili (2019) identified 14 species from 6 families of Iranian wetlands. The present study is part of a comprehensive project to determine the chromosomal number of plants in wetland habitats of Iran. A major goal of this study is to strengthen the information of Iran's plant cytogenetic database.

MATERIALS AND METHODS

Plants were collected from wetland areas of Kermanshah province in west of Iran. Plant samples were identified and herbarium specimen were prepared for examined species. For chromosome observation the seeds were rooted in Petri dishes. The roots were pretreated with α-Bromonaphthalene solution, fixed with Carnoy solution, hydrolyzed with 1 N hydrochloric acid solution, and stained with 4% hematoxylin, then the slides were prepared for microscopic examination. (Shariat & al., 2003; 2013; Shariat and Sefidkon, 2021). Optical microscopy (Nikon Coolpix P90 digital camera interfaced to a BH2- RFCA Olympus microscope) was used for mitotic studies. Karyotypic parameters and asymmetry indices were measured as follows: Total chromosome length of the haploid complement (HCL = $\sum CL$), Total form percentage TF% = ($\sum S/\sum CL$)* 100, Arano index of karyotype asymmetry (AsK% = ($\sum L/\sum CL$)×100) (Arano, 1963), Intrachromosomal asymmetry index (A1= $\sum_{i=1}^{n}(S_i/L_i)/n$), Interchromosomal asymmetry index (A2 =S_{CL}/X_{CL}) (Romero-Zarco (1986), Symmetry index, (S% = (CL_{min}/CL_{max})×100); Mean centromeric index (X_{CI}= $\sum CL/n$), Degree of karyotype asymmetry (A=$\sum_{i=1}^{n}[(L_i - S_i)/(L_i + S_i)]/n$) (Watanabe & al.,1999), Mean centromeric asymmetry (X_{CA} = A×100), Coefficient of variation of chromosome length (CV_{CL} = (S_{CL}/X_{CL})×100 = A_2×100), Coefficient of variation of centromeric index (CV_{CI} = (S_{CI}/X_{CI}) ×100), Asymmetry index (AI = ($CV_{CL} \times CV_{CI}$)/100) (Paszko, 2006).

RESULTS AND DISCUSSION

Amaranthaceae

Amaranthus retroflexus L. (2*n* = 34), (figs. 1a, 2a)

Iran: Kermanshah Province, Sarab Sahneh, 1530 m, 23.09.2019, 34°29'40"N, 47°42'10.4"E. Khan Hasani and Khodakarami 4110 (RANK).

This species has a symmetrical karyotype and is placed in 1B of Stebbins classification. The karyotype formula was 1M + 27m + 6sm (table 1). This report confirms the previous number of chromosomes reported by Sheidai and Mohammadzadeh (2008), who reported that the number of gametic chromosomes in the *Amaranthus retroflexus* populations collected from Tehran and Tabriz was *n* = 17.

Apiaceae

Scandix stellata Banks & Sol. (2*n* = 20), (figs. 1b, 2b)

Iran: Kermanshah Province, Sarab Sahneh, 1530 m, 23.09.2019, 34°29'40"N, 47°42'10.4"E. Khan Hasani and Khodakarami 8255 (RANK).

The karyotype formula was 2M+18m. The total chromosome length of the haploid complement was 27.1 µm. Karyotype was symmetrical and placed in Stebbins 1B category of symmetry (table 1). This is the first report of the chromosome count of this species from Iran.

Torilis leptophylla (L.) Reichenb (2*n* = 12), (figs. 1c, 2c)

Iran: Kermanshah Province, Sarab Niloufar, 1331 m, 27.07.2019, 34°24'49"N, 46°51'32"E. Khan Hasani and Khodakarami 8150 (RANK).

The karyotype consisted of four metacentric, five submetacentric, and three subterminal chromosomes (4m + 5sm + 3st). The total chromosome length of the haploid complement was 65.5 µm. The karyotype was symmetrical and classified as Stebbins 1B symmetry (table 1). Our sample was diploid with 2*n* = 12. This count agrees with the previous report by Shner & al. (2004) from different parts of Iran.

Asteraceae

Anthemis odontostephana Boiss. (2*n* = 18,), (figs. 1d, 2d)

Iran: Kermanshah Province, Sarab Sahneh, 1530 m, 23.09.2019, 34°29'40"N, 47°42'10.4"E. Khan Hasani and Khodakarami 9461 (RANK).

The karyotype formula represented 1M + 14m + 3sm. The total chromosome length of the haploid complement was 51.5 µm (table 1). Karyotype tended to be symmetrical and is placed in Stebbins 1A category of symmetry. Ghaffari (1999) reported for the first time, the number of chromosomes in *Anthemis odontostephana* Boiss. var. *tubicina* (Boiss. & Hausskn.) Bornm., as $n = 9$. Our study is the second report which is in agreement with the results of the previous study.

Centaurea solstitialis L. ($2n = 16$), (figs. 1e, 2e)

Iran: Kermanshah Province, Sarab Niloufar, 1331 m, 27.07.2019, 34°24'49"N, 46°51'32"E. Khan Hasani and Khodakarami 8170 (RANK).

The karyotype formula is 16m + 2sm. The total chromosome length of the haploid complement is 23.5 µm. The karyotype is mostly symmetrical and classified as Stebbins 1A symmetry (table 1). Species of this genus are mainly diploid ($2x$) or tetraploid ($4x$) and the number of base chromosomes varies from $x = 7$ to $x = 16$. Mitotic and meiotic studies by other researchers indicated that the number of chromosomes in *Centaurea solstitialis* was $2n = 16$ (Ghaffari, 1989; Bancheva and Greilhuber, 2006; Gagnidze & al., 2006), while Jasiewicz and Mizianty (1975) and Tonian (1980) reported $2n = 18$ chromosomes.

Cichorium intybus L. ($2n = 18$), (figs. 1f, 2f)

Iran: Kermanshah Province, Sarab Bistoon, 1284 m, 23.10.2019, 34°23'21.5"N, 47°26'20.7"E. Khan Hasani and Khodakarami 8112 (RANK).

The karyotypic formula of this species is 1M + 14m + 3sm and in terms of symmetry, it is classified in group 1A of Stebbins. Other karyotypic symmetry indices studied, including A1, A2, AsK%, S%, X_{CA}, A_I, also indicated high symmetry of this species (table 1). Nowruzi & al. (2016) reported the number of chromosomes of populations of Mazandaran and Golestan provinces as $2n = 2x = 18$. Similar results were obtained by Ghaffari (1989) on the population of Mardabad in Karaj and Teston & al., (2017) on the Italian population

Brassicaceae

Sinapis arvensis L. ($2n = 18$), (figs. 1g, 2g)

Iran: Kermanshah Province, Sarab Niloufar, 1331 m, 21.02.2021, 34°24'49"N, 46°51'32"E. Khan Hasani and Khodakarami 8289 (RANK).

The karyotype formula is 2M+16m. The total chromosome length of the haploid complement is 28.4 µm (table 1). The karyotype tended to be symmetrical and classified as Stebbins 1A symmetry. The number of chromosomes in this study is consistent with the results of other researchers from Poland (Pogan, 1983), Canada (Mulligan, 1984), Czech (Krahulcová, 1992), Sweden (Lövkvist and Hultgård, 1999) who also worked on the number of gametic (Mulligan, 1984) and somatic chromosomes. This is the first count of Chromosome of this species for the flora of Iran.

Plantaginaceae

Plantago major L. *(*$2n = 12$), (figs. 1h, 2h)

Iran: Kermanshah Province, Sarab Niloufar, 1331 m, 10.07.2020, 34°24'49"N, 46°51'32"E. Khan Hasani and Khodakarami 8228 (RANK).

The karyotypic formula represented two median and 10 metacentric chromosomes (2M + 10m). The total chromosome length of the haploid complement is 20.1 µm. The karyotype tended to be symmetrical and classified as Stebbins 1A symmetry (table 1). According to this study, the number of chromosomes in the Kermanshah population was similar to the number recorded in East Azerbaijan (Aryavand, 1977), Hamedan (Lessani& Sanei Shariat Panahi, 1979), Mazandaran (Aryavand, 1980), and Fars (Mohsenzadeh & al., 2008).

Poaceae

Bromus danthoniae subsp. *Pseudodanthoniae* (Drobow) H. Scholz ($2n = 28$), (figs. 1i, 2i)

Iran: Kermanshah Province, Talab Hashilan, 1310m, 15.07.2019, 34°35'5"N, 46°53'30.5"E. Khan Hasani and Khodakarami 7074 (RANK).

The total chromosome length of the haploid complement is 268.3 µm. The karyotype formula is 1M + 22m + 5sm. The karyotype is symmetrical and classified as Stebbins 1B symmetry (table 1). The number of gametic chromosomes reported for *Bromus danthoniae* Trin is 7 and the number of somatic chromosomes in several reports is $2n = 2x = 14$ (Moinuddin *&* *al*., 1994; Ghukasyan. 2004; Goukasian and Nazarova. 1998), while *B. danthoniae* subsp. *Pseudodanthoniae* (Drobov) H. Scholz was tetraploid and the number of chromosomes was $2n = 4x = 28$. This report is the second report on this variety, which is the

same as the previous report.

Bromus tomentellus Boiss. (2*n* = 28), (figs. 1j, 2j)

Iran: Kermanshah Province, Sarab Sahneh, 1530 m, 23.09.2019, 34°29'40"N, 47°42'10.4"E. Khan Hasani and Khodakarami 7509 (RANK).

The karyotypic formula represented one median, 25 metacentrics, two submetacentric chromosomes (1M + 25m + 2sm). The total chromosome length of the haploid complement is 181 µm (table 1). The karyotype is symmetrical and classified as Stebbins 1B symmetry. The number of chromosomes in the present study is consistent with the results of other researchers who worked on the populations of Ardabil, Zanjan, Tabriz, Ghazvin, and Tehran (Mirzaie-Nodoushan & al., 2000, 2006; Mirzaie-Nodoushan and Shariat, 2002).

Calamagrostis pseudophragmites var. *tartarica* (Hook. f.) R.R. Stewart (2*n* = 42), (figs. 1k, 2k)

Iran: Kermanshah Province, Talab Hashilan, 1310 m, 9.06.2020, 34°35'5"N, 46°53'30.5"E. Khan Hasani and Khodakarami 8162 (RANK).

The karyotype formula is 1M + 35m + 6sm. It is categorized in type 1B of Stebbins classification. The total chromosome length of the haploid complement is 185.1 µm (table 1). This is the first report on the chromosome number of *Calamagrostis pseudophragmites* var. *tartarica* (Hook. f.) R.R. Stewart in Iran. Our result is consistent with the results of the previous count from India (Koul and Gohil, 1991).

Polygonaceae

Rumex cyprius *Murb.* (2*n* = 18), (figs. 1l, 2l)

Iran: Kermanshah Province, Alvand, Ghasre Shirin, 1310 m, 15.07.2019, 34°35'5" N, 46°53'30.5" E. Khan Hasani and Khodakarami 8254 (RANK).

This species has a symmetrical karyotype and fell in 1B of Stebbins classification. The karyotype formula is 11m + 5 sm + 2st. The asymmetry index (AI) was 246.8 (table 1). Higher values of the AI index are considered to indicate higher levels of karyotypic heterogeneity. As the index gets lower, it indicates greater karyotype symmetry. The number of chromosomes in the genus *Rumex* varies from 18 to 60 in different species. One of the reasons for the variable number of chromosomes is the ability of this genus to hybridize in several species (Pye, 2008). In this study *Rumex cyprius* Murb. was counted for the first time in Iran. The number of chromosomes in this study is similar to other counts (Díaz Lifante & al., 1992; Slavīk & al., 1993; Vogt and Aparicio, 1999).

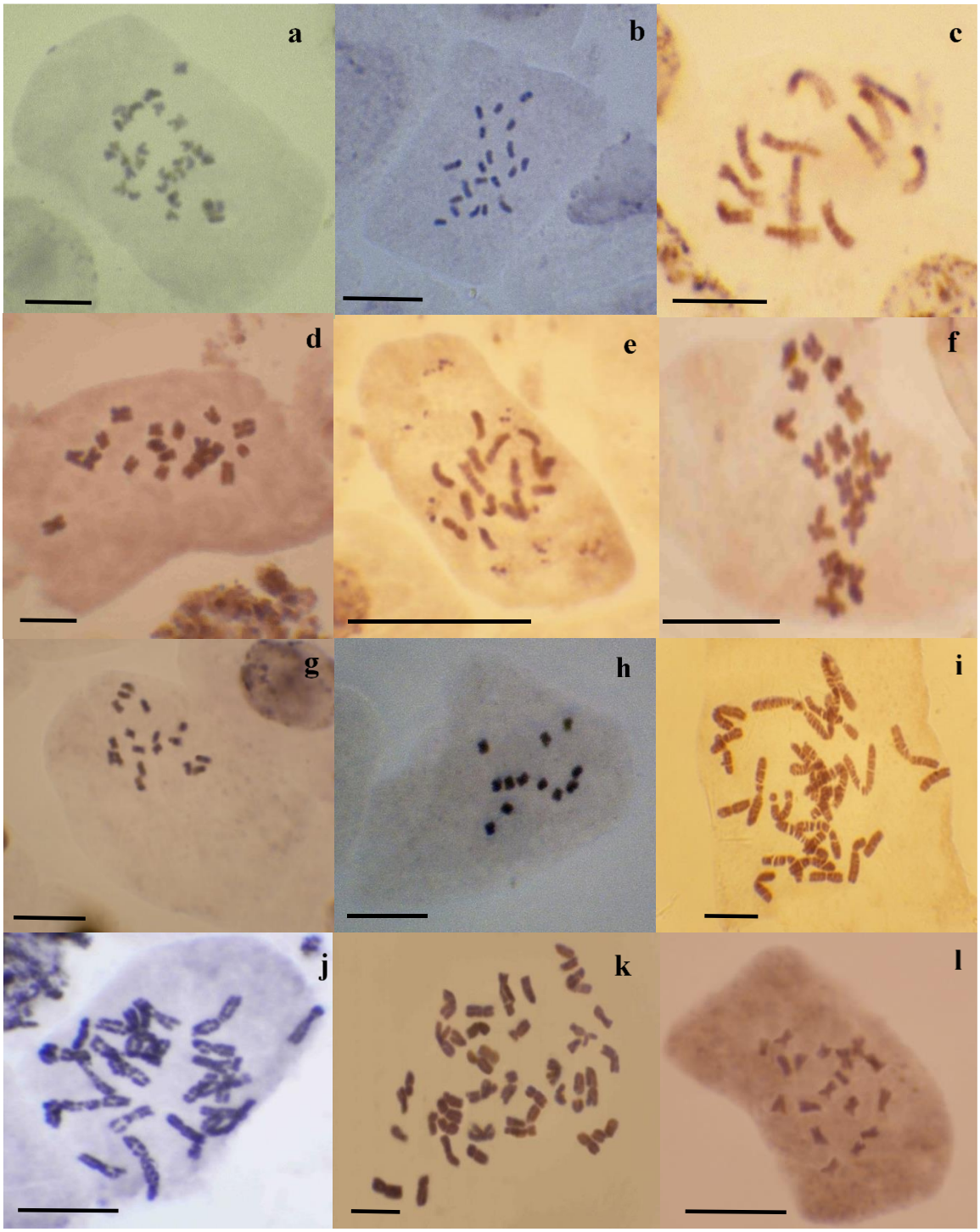

Fig. 1. Karyotypes of 12 wetland species. a, *Amaranthus retroflexus* L. (2*n* = 34); b, *Scandix stellata* Banks & Sol. (2*n* = 20); c, *Torilis leptophylla* (L.) Reichenb (2*n* = 12); d, *Anthemis odontostephana* Boiss. (2*n* = 18); e, *Centaurea solstitialis* L. (2*n* = 16); f, *Cichorium intybus* L. (2*n* = 18); g, *Sinapis arvensis* L. (2*n* = 18); h, *Plantago major* L. *(2n* = 12); i, *Bromus danthoniae* subsp. *Pseudodanthoniae* (Drobow) H. Scholz (2*n* = 28); j, *Bromus tomentellus* Boiss. (2*n* = 28); k, *Calamagrostis pseudophragmites* var. *tartarica* (Hook. f.) R. R. Stewart (2*n* = 42); l, *Rumex cyprius Murb.* (2*n* = 18). Bar = 10 µm.

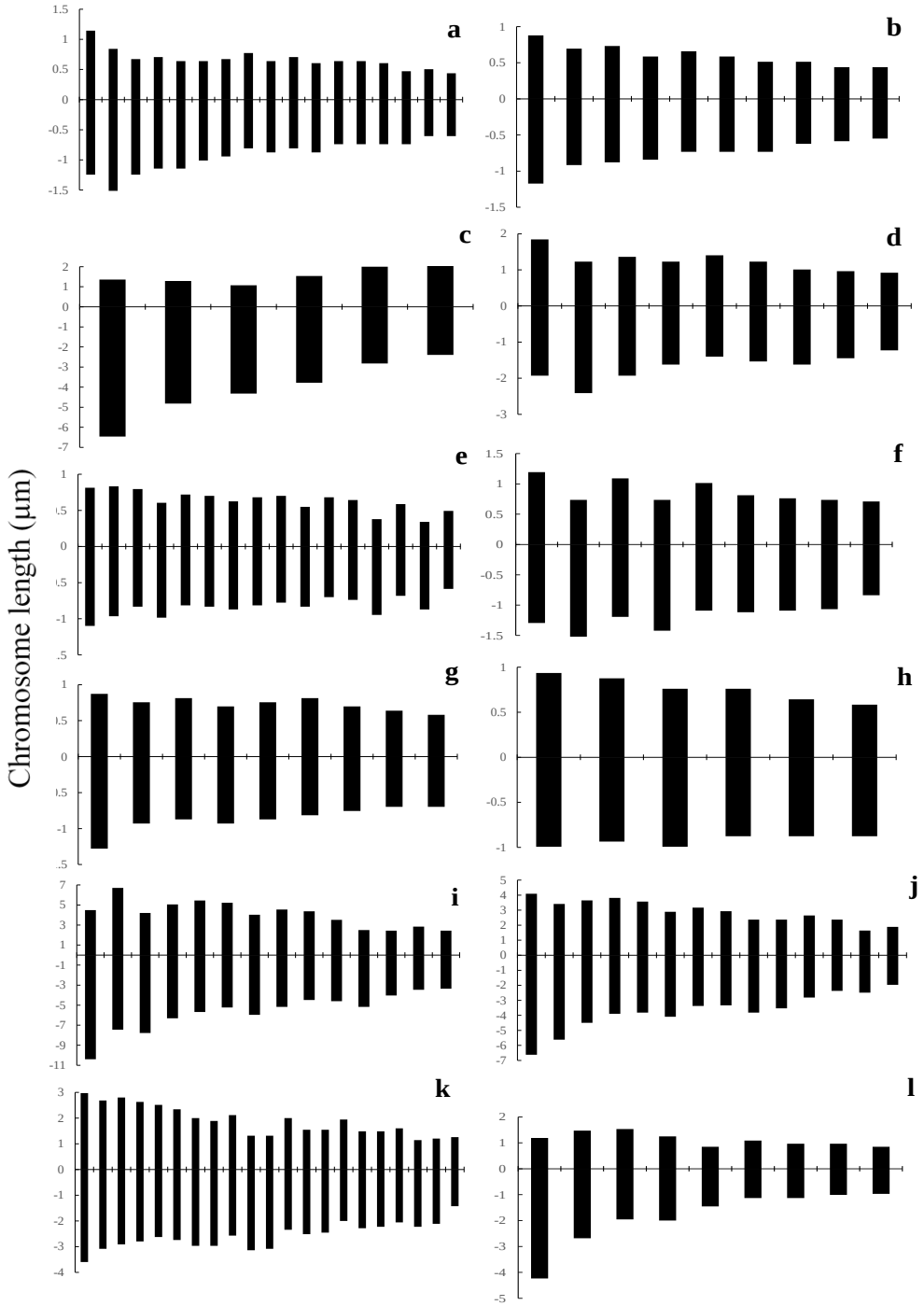

Fig. 2. Ideograms of 12 wetland species. a, *Amaranthus retroflexus* L. (2*n* = 34); b, *Scandix stellata* Banks & Sol. (2*n* = 20); c, *Torilis leptophylla* (L.) Reichenb (2*n* = 12); d, *Anthemis odontostephana* Boiss. (2*n* = 18); e, *Centaurea solstitialis* L. (2*n* = 16); f, *Cichorium intybus* L. (2*n* = 18); g, *Sinapis arvensis* L. (2*n* = 18); h, *Plantago major* L. *(*2*n* = 12); i, *Bromus danthoniae* subsp. *Pseudodanthoniae* (Drobow) H. Scholz (2*n* = 28); j, *Bromus tomentellus* Boiss. (2*n* = 28); k, *Calamagrostis pseudophragmites* var. *tartarica* (Hook. f.) R. R. Stewart (2*n* = 42); l, *Rumex cyprius Murb.* (2*n* = 18).

Table 1- Chromosomal parameters and karyotype asymmetry indices of the 12 examined species.

Species	HCL	TF	AsK%	S%	Xci	A	XcA	CVcl	CVci	AI	Stebbine C	Asymmetry indices (Romero-Zarco, 1986)		KF
												A1	A2	
Amaranthus retroflexus L.	53.6	42.7	57.3	42.3	0.43	0.14	14.19	23.12	11.22	205.9	1B	0.24	0.23	1M + 27m + 6sm sm
Scandix stellata Banks & Sol.	27.1	44.9	55.1	42.9	0.45	0.10	10.03	22.70	5.51	412.1	1B	0.18	0.23	2M + 18 m
Torilis leptophylla (L.) Rchb.f.	65.5	31.2	68.8	51.1	0.35	0.29	29.20	21.60	29.10	74.3	1B	0.50	0.19	4m + 5sm + 3st
Anthemis odontostephana Boiss.	51.5	41.6	58.4	57.0	0.42	0.17	16.91	17.65	10.86	162.5	1A	0.28	0.18	1M + 14m + 3sm
Centaurea solstitialis L.	23.5	43.2	56.8	56.4	0.43	0.14	14.08	14.25	15.08	94.5	1A	0.23	0.14	16m + 2sm
Cichorium intybus L.	39.0	43.1	56.9	52.0	0.43	0.14	13.57	18.64	12.29	151.7	1A	0.22	0.19	1M +14m + 3sm
Sinapis arvensis L.	28.4	46.3	53.7	54.1	0.46	0.07	7.01	14.36	5.74	250.1	1A	0.13	0.14	2M + 16 m
Plantago major L.	20.1	46.1	53.9	72.7	0.46	0.08	7.89	10.38	6.92	150.2	1A	0.14	0.10	2M + 10m
Bromus danthoniae subsp. *Pseudodanthoniae* (Drobow) H. Scholz	268.3	43.4	56.6	32.4	0.44	0.13	12.97	28.71	13.30	215.9	1B	0.21	0.29	1M + 22m + 5sm
Bromus tomentellus Boiss.	181.0	44.0	56.0	32.6	0.44	0.13	12.54	27.65	12.36	223.8	1B	0.21	0.28	1M + 25m + 2sm
Calamagrostis pseudophragmites var. *tartarica* (Hook. f.) R.R.	185.1	42.7	57.3	30.4	0.43	0.15	14.87	21.23	14.27	148.8	1B	0.24	0.21	1M + 35m + 6sm
Rumex cyprius Murb.	54.1	37.2	62.8	26.8	0.40	0.21	20.81	41.21	16.70	246.8	1B	0.32	0.41	11m + 5 sm + 2st

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