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KARYOTYPE ANALYSIS OF THREE SPECIES FROM THE WESTERN REGIONS OF IRAN

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Three plant species belonging to three genera of Apiaceae, Boraginaceae & Poaceae were collected from wetlands in western regions of Iran. The chromosomal count of *Leiotulus porphyrodiscus* (Stapf & Wettst.) Pimenov & Ostr. (2*n* = 22) is reported for the first time, and the chromosome count of *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl (2*n* = 28) is reported for the first time for the flora of Iran. The number of chromosomes in *Echium italicum* L. (2*n* = 16) is reported from Kermanshah Province. Karyomorphological parameters such as karyotypic formula, Romero-Zarco indices, Stebbins class, and other karyotypic symmetry indices were determined for all species.

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Keywords: Chromosome counts; ideogram; symmetry indices; karyotype; flora of Iran

بررسی کاریوتیپی سه گونه از مناطق غربی ایران

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سه گونه گیاهی متعلق به سه تیره Apiaceae, Boraginaceae, Poaceae از مناطق ماندابی غرب کشور جمع‌آوری گردید. شمارش کروموزومی برای گونه Leiotulus porphyrodiscus (Stapf & Wettst.) Pimenov & Ostr. (2n = 22) برای اولین بار و شمارش کروموزومی گونه Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl (2n = 28) برای اولین بار در فلور ایران و در گونه Echium italicum L. نیز 2n = 16 از استان کرمانشاه گزارش می‌گردند. پارامترهای کاریومورفولوژیکی از جمله فرمول کاریوتیپی، شاخص‌های رومروزرکو، کلاس Stebbins و سایر شاخص‌های تقارن کاریوتیپی برای همه گونه‌ها تعیین شد.

INTRODUCTION

Historically, the study of wetland habitats in Iran has primarily focused on Anzali, Gavkhooni, and Jazmourian wetlands while the scattered mountain wetland conditions have been less studied (Khanhasani & al. 2021). Plants that grow in such ecosystems are

called hydrophytic plants. These plants have adapted to special ecological conditions, such as inorganic soils or soft soils that are flooded with poor drainage. Aquatic plants can complete their reproductive cycle in water. However, in addition to the mentioned aquatic plants, other aquatic plants that need a dry environment to complete their life cycle are also considered real aquatic plants. (Den Hartog & van der Velde 1988). The Kermanshah Province occupies about 1.5 percent of Iran with an area of 25000 square kilometers. In the Zagros Mountain range with mountains such as Shahu, Dalakhani, Sefidkooh, differences in altitudes, and climates, have led to a significant diversity of plant species. There are more than one hundred open springs (mirage) as the source of rivers or their tributaries and five permanent rivers and several wetlands and reservoirs in Kermanshah province. Khanhasani & al. (2021) with the aim of preparing a distribution map of wetland plants in Kermanshah province, studied 31 wetland habitats and reported a total of 288 plant species. Few studies have been done on the cytogenetic characteristics of wetland plants in Iran. Shariat & al. (2021a) reported the karyotypic characteristics of 12 wetland species in Kermanshah province. In other research, Mirzadeh Vaghefi and Jalili (2019) represented a chromosome count of 14 species from six families of Iranian wetlands. The present study is part of a comprehensive project to determine the chromosome number of plants in wetland habitats of Iran. It is worth noting that some studied species were not wetlands, but due to favorable environmental conditions around wetlands, they have grown there. A major goal of this study is to strengthen the information on Iran's plant cytogenetic database.

MATERIALS AND METHODS

Plants were collected from two wetland areas of Kermanshah province named Hashilan, and Gamasiab. Plant samples were identified and herbarium specimens were prepared for examined species. For karyotypic studies, the seeds were first germinated in sterilized Petri dishes. The roots were pretreated with α-Bromonaphthalene solution (2 h), then fixed in Carnoy solution for 24 hours (3 ethanol: 1 glacial acetic acid v/v). The roots were kept in 70% alcohol until the time of testing. The roots were then hydrolyzed using 1 N hydrochloric acids at 60 °C for 10 minutes and stained with 4% hematoxylin for 6 hours at 60 °C and finally squashed in 45% (v/v) acetic acid (Shariat & al. 2013; Shariat and Sefidkon 2021b). An optical microscope (Nikon Coolpix P90 digital camera interfaced to a BH2- RFCA Olympus microscope) was used for mitotic studies. Karyotypic parameters and asymmetry indices were measured by Ideokar software (Mirzaghaderi & Marzangi 2015) as described in table 1.

Table 1. Measures and formulae of karyotypic parameters and asymmetry indices. Abbreviations: L, length of long arm; S, length of short arm; I, homologous group number; s, standard deviation; X, mean; n, haploid chromosome number of an individual or taxon.

No	Karyotypic parameters	Formula	Reference
1	Total chromosome length of the haploid complement	HCL = $\sum CL$	Levan & al. (1964)
2	Total form percentage	TF% = $(\sum S/\sum CL)$* 100	Huziwara (1962)
3	Arano index of karyotype asymmetry	AsK% = $(\sum L/\sum CL)$×100	Arano (1963)
4	Intrachromosomal asymmetry index	A1= $\sum_{i=1}^{n}(S_i/L_i)/n$	Romero-Zarco (1986)
5	Interchromosomal asymmetry index	A2 =S_{CL}/X_{CL}	Romero-Zarco (1986),
6	Symmetry index	S% = (CL_{min}/CL_{max})×100	Watanabe & al. (1999)
7	Mean centromeric index	X_{CI}= $\sum CL/n$	Paszko (2006)
8	Degree of karyotype asymmetry	A=$\sum_{i=1}^{n}[(L_i - S_i)/(L_i + S_i)]/n$	Watanabe & al. (1999)
9	Mean centromeric asymmetry	X_{CA} = A×100	Paszko (2006)
10	Coefficient of variation of chromosome length	CV_{CL} = (S_{CL}/X_{CL})×100 = A_2×100	Paszko (2006)
11	Coefficient of variation of centromeric index	CV_{CI} = (S_{CI}/X_{CI}) ×100	Paszko (2006)
12	Asymmetry index	AI = (CV_{CL} × CV_{CI})/100	Paszko, (2006

RESULTS AND DISCUSSION

Apiaceae

Leiotulus porphyrodiscus (Stapf & Wettst.) Pimenov & Ostr. (2*n* = 22), (Fig. 1, Table 2)

Iran: Kermanshah Province, Hashilan, 1316 m, 03.08.2021, 34°35'4.9"N, 46°53'46"E. Khanhasani and Rahimi 8899 (RANK).

The karyotypic formula represented 13 median, seven metacentric, and two subterminal chromosomes (13 m + 7 sm + 2 st). The karyotype is relatively symmetrical and classified as Stebbins 1B symmetry. The total chromosome length of the haploid complement is 69.8 µm (Table 2). This is the first chromosome number report of this species.

The chromosome counts from the other species of the genus, including *L. pastinacifolius* (Boiss. & Balansa) M.G. Pimenov & T.A. Ostroumova, *L. secacul* (Mill.) M.G. Pimenov & T.A. Ostroumova, and *L. dasyanthus* (K. Koch) M.G. Pimenov & T.A. Ostroumova from the Mediterranean region have previously been reported as 2*n* = 20, 2*n* = 44, and 2*n* = 22 respectively (Shner 2004 & 2005).

Boraginaceae

Echium italicum L. (2*n* = 16), (Fig. 1, Table 2)

Iran: Kermanshah Province, Hashilan, 1316 m, 03.08.2021, 34°35'4.9"N, 46°53'46"E. Khanhasani and Rahimi 8234 (RANK).

This species has a symmetrical karyotype and fell in 1B of Stebbins classification. The karyotype formula is 2M + 10m + 2sm + 2st. The total chromosome length of the haploid complement is 37.5 µm. A value of TF = 44.1% indicates intrachromosomal differences. A higher value of TF indicates greater symmetry of chromosomes, while S = 32.7% indicates the difference between the minimum and maximum length of chromosomes. In addition to the two mentioned indices, Romero-Zarco indices are in agreement with the values of S% and TF%, or in other words, with the increase of TF, the value of A1 decreases and with the decrease of S, the value of A2 increases. It can be concluded that in *Echium italicum*, the difference between the short and long arms is small, while there is a significant difference between the length of the longest and shortest chromosomes, or in other words, the intrachromosomal differences are minimal, while the interchromosomal differences are significant. The results of this study are in agreement with the results of Azoush & al. 2014 and Ghaffari 1996, who worked on the populations of Tehran (Fasham) and Karaj, respectively.

Poaceae

Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl (2*n* = 28), (Fig. 1, Table 2)

Iran: Kermanshah Province, Gamasiab, 1290 m, 14.07.2021, 34°27'8.3"N, 47°26'19.8"E. Khanhasani and Rahimi 8122 (RANK).

The karyotype formula is 3M +17m + 6sm + 2st. This species has a symmetrical karyotype and fell in 1B of Stebbins classification. The total chromosome length of the haploid complement is 140.4 µm (Table 1). This is the first report on the chromosome number of *Arrhenathrum elatius* for the flora of Iran. Our result is consistent with the results of previous counts from Norway (Engelskjon 1979) and Armenia (Ghukasyan 2004).

Table 2. Chromosomal parameters and karyotype asymmetry indices of 3 species from Kermanshah Province.

Species	HCL	TF	AsK%	S%	Xci	A	XcA	CVcl
Leiotulus porphyrodiscus	69.8	39.4	60.6	41.5	0.38	0.23	23.3	17.5
Echium italicum	37.5	44.1	55.9	32.7	0.44	0.12	11.6	36.2
Arrhenathrum elatius	140.4	40.5	59.5	30.8	0.41	0.18	18.2	26.8

Table 2. Continued.

Species	CVci	AI	Stebbins Class	Asymmetry indices (Romero- Zarco)		
				A1	A2	KF
Leiotulus porphyrodiscus	21.7	80.8	1B	0.35	0.18	13 m + 7 sm + 2 st
Echium italicum	16.4	221.0	1B	0.18	0.36	2M + 10m + 2sm + 2st
Arrhenathrum elatius	18.8	142.1	1B	0.28	0.27	3M +17m + 6sm + 2st

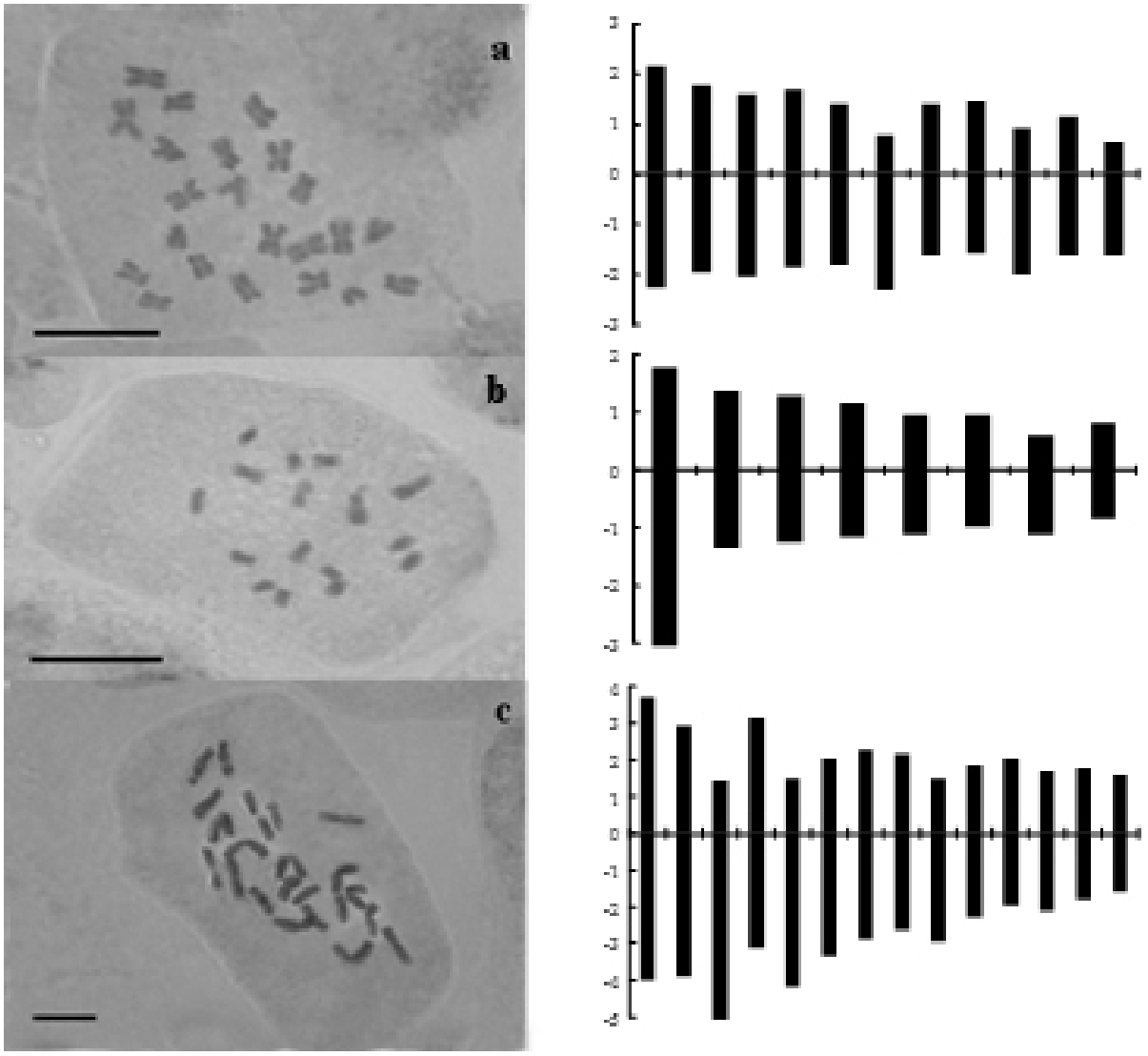

Fig. 1. Karyotypes and ideograms of the examined species. a, *Leiotulus porphyrodiscus* (2*n* = 22); b, *Echium italicum* (2*n* = 16); c, *Arrhenathrum elatius* (2*n* = 28); Bar = 10 µm.

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