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First karyotype analysis of *Nerium oleander* populations in Iran

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Abstract

Somatic chromosome numbers and karyotypes of five populations of *Nerium oleander* in Iran were determined. karyological studies showed that, all the studied populations were diploid (2n = 2x = 22). The present study is the first report of the karyotype characteristics of *N. oleander*. The highest values of mean chromosome length (1.45 µm) and haploid chromosome length (15.9 µm) were found in the Chabahar population. The general shape of the karyotypes, indicated symmetry for all populations, as all chromosomes were metacentric type. According to Stebbin's classification (1971), populations fall in class 1A, thus indicating occurrence of chromosome symmetry among the populations. In addition, a detailed account of karyomorphology and estimates of asymmetry indices showed that, all of the populations were symmetric, among which Khash and Kuhdasht populations were considered as the most asymmetrical and symmetrical karyotypes, respectively. Based on karyotypic parameters, the considered populations were categorized into two groups viz. Chabahar, Fanuj, and Khash populations which were previously reported as *N. indicum* in Flora Iranica, separated from Karaj and Kuhdasht populations.

Keywords: *Apocynaceae*, asymmetry index, chromosome number, karyotype, taxonomy

نخستین تجزیه کاریوتیپ جمعیت‌های خرزهره در ایران*

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خلاصه

تعداد کروموزوم‌های سوماتیک و کاریوتیپ‌های پنج جمعیت خرزهره (*Nerium oleander* L.) در رویشگاه‌های مهم این گیاه در ایران مورد بررسی قرار گرفت. مطالعات کاریولوژیک نشان داد که تمام جمعیت‌های مورد مطالعه دیپلوییید (2n = 2x = 22) بودند. مطالعه حاضر، نخستین گزارش از مشخصات کاریوتیپ *N. oleander* است. بیشترین مقادیر میانگین طول کروموزوم (۱/۴۵ میکرومتر) و طول کروموزوم هاپلوییید (۱۵/۹میکرومتر) در جمعیت چابهار یافت شد. همه کروموزوم‌ها از نوع متاسنتریک بودند و شکل کلی کاریوتیپ‌ها تقارن را برای همه جمعیت‌ها نشان داد. طبق طبقه‌بندی استبین (Stebbin 1971)، جمعیت‌ها در کلاس 1A قرار می‌گیرند که نشان‌دهنده تقارن کروموزوم در میان جمعیت‌ها است. به علاوه، مطالعات کاریومورفولوژی و برآورد شاخص‌های عدم تقارن نشان داد که همه جمعیت‌ها متقارن هستند. در این میان، جمعیت‌های خاش و کوهدشت به ترتیب نامتقارن‌ترین و متقارن‌ترین کاریوتایپ‌ها محسوب می‌شوند. براساس پارامترهای کاریوتیپی، جمعیت‌های در نظر گرفته شده چابهار، فنوج و خاش که در فلورا ایرانیکا به عنوان *N. indicum* Mill. گزارش شده‌اند، از جمعیت‌های کرج و کوهدشت متمایز بودند.

واژه‌های کلیدی: تعداد کروموزوم، تیره خرزهره، رده‌بندی، شاخص عدم تقارن، کاریوتیپ

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Introduction

The genus *Nerium* L., commonly known as oleander, belongs to the tribe *Nerieae* of the subfamily *Apocynoideae* (*Echitoideae*) in the family *Apocynaceae* (Rechinger 1974, Pagen 1988, Sennblad & Bremer 2002). It is originated in the Mediterranean region and Indo-Pakistan subcontinent (Sinha & Biswas 2016). The Indian oleander, as it grows wild in Iran, Afghanistan, Pakistan, Northern India, Nepal, and China, differs from the Mediterranean kind in some characteristics of flowers, leaves and plant growth habits. In 1737, the oleander of the Indian kind was considered by Linnaeus as a variety of the common Mediterranean oleander (Pagen 1988), while Miller (1768) distinguished it as a separate species (*Nerium indicum* Mill.). Rechinger (1974) and Van Steenis (1981) also defined *N. indicum* as distinct from *N. oleander* L. In addition, several other *Nerium* species have been distinguished (Pagen 1988). However, Leeuwenberg (1984) concludes that, they all should be considered as belonging to a single species: *N. oleander* L., a conclusion that makes the genus *Nerium* monotypic.

Nerium oleander is a small evergreen shrub with 2–5 m in height and all parts of the plant being toxic. It is cultivated worldwide as an ornamental plant due to its profuse flowering which are long lasting along with their moderate hardiness. Oleander is one of the most poisonous commonly grown garden plants (Kuete 2014, Aslani 2018, Sinha & Biswas 2016). In addition, the plant is widely used in traditional medicine to treat microbial and fungal diseases and cancers (Kuete 2014, Ebrahimi *et al.* 2018).

Karyotype analysis and chromosome counting provide valuable information in identifying species and inferring some closely related taxa (Fallahi *et al*. 2020, Rajabi Mazaher *et al*. 2021). Karyological information on *Nerium* is restricted mostly to chromosome counting (Noori-Daloii *et al*. 1996), which is from a few decades ago. So far, karyotypic parameters of *N. oleander* have not been reported, which may be due to the small size of its chromosomes. The main aim of this study was to determine chromosome number and general information on karyotype characteristics of five populations of *N. oleander* (for the first time), which were collected from different areas of Iran.

Materials and Methods

The plant materials were collected from natural habitats in different localities of Iran (Table 1). The voucher specimens have been deposited in the Herbarium of Institute of Medicinal Plants (IMPH), Academic Center for Education, Culture and Research (ACECR), Karaj, Iran.

The seeds were germinated between moist Whatman papers in Petri dishes. The root tips were cut off and pretreated in 2 mM 8-hydroxyquinoline for 3 h at room temperature. Then, they were fixed in Carnoy's fixative (3:1 ethanol: glacial acetic acid) at room temperature overnight. Hydrolysis was carried out with 1 M HCl for 12 min at 60 °C. Subsequently, root tips were stained with aceto-orcein for 3–4 h at room temperature. The stained root tips were afterwards squashed in a droplet of 45% (v/v) acetic acid. At least five metaphase cells were used to determine chromosome numbers and karyological characteristics. The metaphase chromosomes were photographed by a DP25 digital camera attached to the BX51 Olympus microscope.

The morphology of chromosome is explained using nomenclatures proposed by Levan *et al.* (1964). Eight chromosomal parameters were either measured or calculated, including long (LA) and short (SA) arms, chromosome length (CL), arm ratio (AR), r-value, relative length of chromosome (RL%), chromosome form percentage (F%) and centromeric index (CI%). Moreover, 13 different methods of karyotype asymmetry were used comprising: total chromosome length of the haploid complement (HCL), total form percentage (TF%; Huziwara 1962), coefficient of variation of chromosome length and centromeric index (CV_{CL} and CV_{CI}; Paszko 2006), mean centromeric asymmetry (X_{CA}; Peruzzi & Eroglu 2013), mean centromeric index (X_{CI}), asymmetry index (AI; Paszko 2006), degree of karyotype asymmetry (A; Watanabe *et al.* 1999), percentage of karyotype symmetry (S%), intra- and inter-chromosomal

asymmetry indices (A_1 and A_2; Romero Zarco 1986), percentage karyotype asymmetry index (AsK%; Arano 1963), and Stebbin's classification (1971) method.

To visualize the genetic relationships among populations, PAST software was used to construct the Neighbor Joining (NJ) hierarchical classification.

Results

All five populations were identified as diploid (2n = 2x = 22). Mitotic chromosomes of *Nerium* were very small sized. Comparison of length of large and small chromosome arms and mean chromosome length (0.81, 0.64, and 1.45 µm, respectively) indicated that Chabahar had remarkable the longest chromosomes. On the other hand, the shortest mean chromosome length was observed in Kuhdasht (0.99 µm). The highest values of CI%, r-value and F% as well as the lowest value of AR were determined in Kuhdasht (45.4, 0.83, 2.06 and 1.20, respectively), while the lowest values of CI%, r-value and F% as well as the highest value of AR were observed in Khash (43.1, 0.76, 1.96, and 1.32, respectively) (Table 2). The RL% value was the same in all populations. Using Levan *et al.* (1964) chromosome nomenclature, all populations showed karyotypes formed by a set of metacentric chromosomes (Table 2). No satellite was detectable on the arms of the chromosomes. Representative somatic metaphase plates and idiograms of studied *Nerium* populations are demonstrated in figure 1.

Table 1. Geographical information of studied *N. oleander* populations in Iran

Location	Latitude	Longitude	Altitude (m)	Collector	Herbarium No.
Sistan & Baluchestan prov.: Chabahar	25°35′06″N	60°62′59″E	37	M. Ghorbani Nohooji	IMPH-7200
Sistan & Baluchestan prov.: Fanuj	26°58′43″N	59°62′48″E	185	M. Ghorbani Nohooji	IMPH-7204
Sistan & Baluchestan prov.: Khash	28°25′96″N	61°23′85″E	1350	M. Ghorbani Nohooji	IMPH-7201
Alborz prov.: Karaj	35°91′33″N	50°99′31″E	1425	M. Ghorbani Nohooji	IMPH-7203
Lorestan prov.: Kuhdasht	33°32′06″N	47°36′22″E	1220	F. Ebrahimi	IMPH-7002

Table 2. Karyotype characteristics of *Nerium oleander* populations

Population	2*n*	LA (µm)	SA (µm)	CL (µm)	CI%	AR	r-value	RL%	F%	CT
Chabahar	22	0.81 a	0.64 a	1.45 a	44.0 bc	1.27	0.79	4.54	2.00	m
Fanuj	22	0.60 b	0.49 b	1.09 b	44.6 ab	1.24	0.80	4.54	2.03	m
Khash	22	0.62 b	0.47 b	1.10 b	43.1 c	1.32	0.76	4.54	1.96	m
Karaj	22	0.56 bc	0.44 b	1.01 b	44.0 bc	1.27	0.79	4.54	2.00	m
Kuhdasht	22	0.54 c	0.45 b	0.99 b	45.4 a	1.20	0.83	4.54	2.06	m

2n: Somatic chromosome number, LA: mean long arm, SA: mean short arm, CL: mean chromosome length, CI%: centromeric index, AR: arm ratio, RL%: relative length of chromosome, F%: chromosome form percentage, CT: chromosome type.

Karyotypes of all populations were classified in the 1A class of Stebbins classification (Stebbins 1971). Based on 13 different methods, the karyotype asymmetry was assessed (Table 3). The highest values of TF% (45.3) and X_{CI} (0.45) as well as the lowest values of AsK% (54.7), A (0.09), X_{CA} (9.4) and A_1 (0.17) were detected in Kuhdasht (the most symmetric). On the other hand, the lowest values of TF% (42.9) and X_{CI} (0.43) as well as the highest values of AsK% (57.1), A (0.14), X_{CA} (14.1), and A_1 (0.23) were observed in Khash (the most asymmetric). In addition, the lowest values of HCL (10.9 µm), CV_{CI} (5.9) and AI (0.75) were identified in Kuhdasht, while the highest values of these parameters were found in Chabahar (15.9 µm) and Fanuj (9.4 and 1.22), respectively. The highest value of S% (71.0) and the lowest values of CV_{CL} (10.9) and A_2 (0.10) were distinguished in Karaj, while the highest values of CV_{CL} (15.3) and A_2 (0.15) were demonstrated in Chabahar. Furthermore, the lowest value of S% (62.2) was identified in Fanuj.

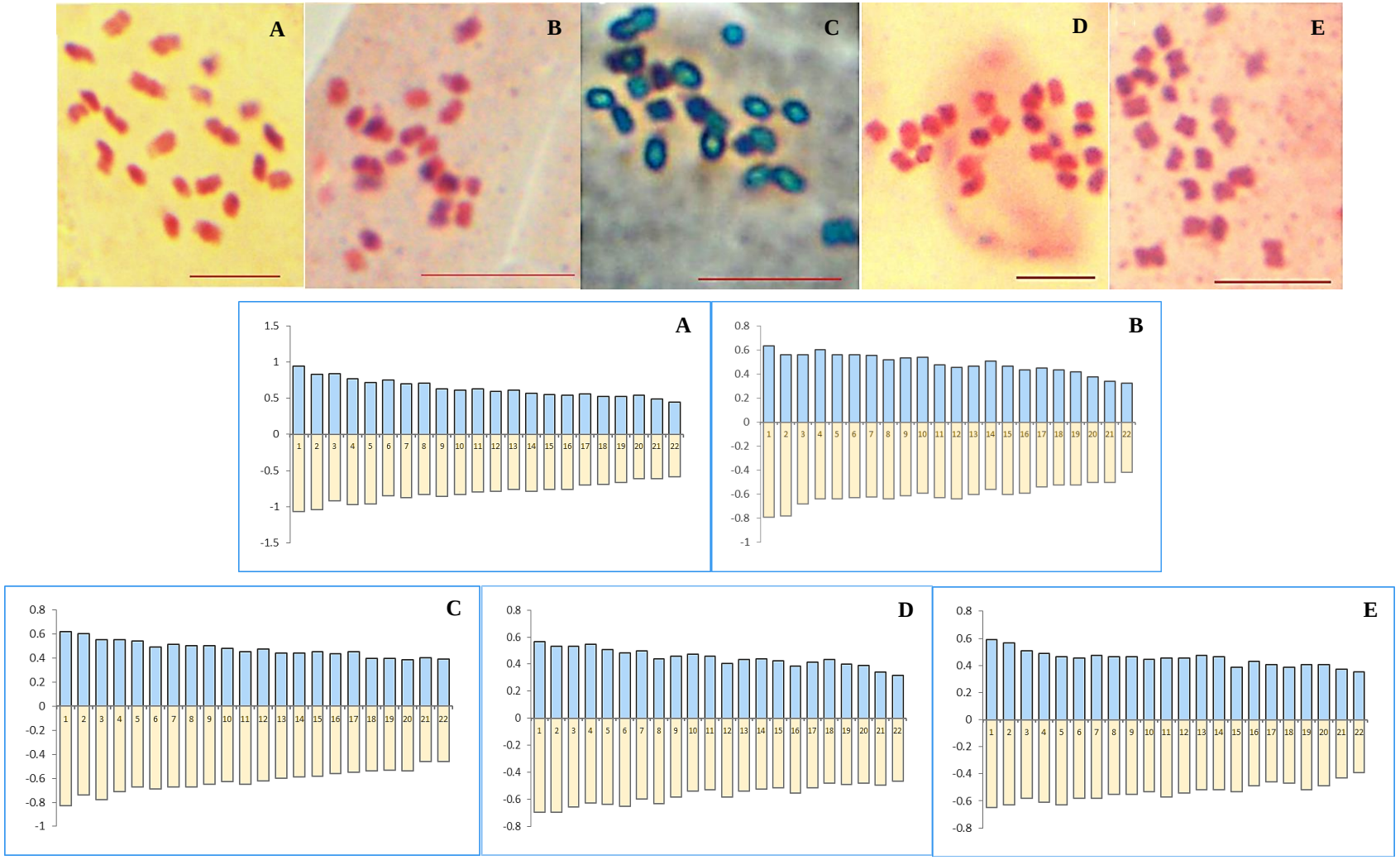

Fig. 1. Somatic metaphase chromosomes along with their ideograms of *Nerium oleander* populations: A. Chabahar, B. Fanuj, C. Khash, D. Karaj, E. Kuhdasht (Bar = 5 µm).

Table 3. Karyotypic parameters and asymmetry indices of *N. oleander* populations

Population	HCL (µm)	TF%	AsK%	S%	X_{CI}	A	X_{CA}	CV_{CL}	CV_{CI}	AI	SC	A_1	A_2
Chabahar	15.9 a	44.1 ab	55.8 ab	62.4	0.44 ab	0.12 ab	11.9 ab	15.3	6.7 bc	1.02	1A	0.21 ab	0.15 a
Fanuj	12.0 b	44.3 a	55.6 b	62.2	0.44 ab	0.11 bc	11.4 b	13.0	9.4 a	1.22	1A	0.19 bc	0.13 ab
Khash	12.1 b	42.9 b	57.1 a	66.9	0.43 b	0.14 a	14.1 a	13.0	9.0 ab	1.17	1A	0.23 a	0.13 ab
Karaj	11.2 b	44.1 ab	55.9 ab	71.0	0.44 ab	0.11 bc	11.8 ab	10.9	7.8 abc	0.85	1A	0.20 bc	0.10 b
Kuhdasht	10.9 b	45.3 a	54.7 b	67.6	0.45 a	0.09 c	9.4 b	12.7	5.9 c	0.75	1A	0.17 c	0.13 ab

HCL: total chromosome length of the haploid complement, TF%: total form percentage, AsK%: percentage karyotype asymmetry index, S%: percentage of karyotype symmetry, X_{CI}: mean centromeric index, A: degree of karyotype asymmetry, X_{CA}: mean centromeric asymmetry, CV_{CL}: coefficient of variation of chromosome length, CV_{CI}: coefficient of variation of centromeric index, AI: asymmetry index, SC: Stebbins' classification, A_1: intrachromosomal index, A_2: interchromosomal index.

As shown in figure 2, NJ cluster analysis divided the studied populations into two main distinct groups based on karyological features. The first cluster consisted of Khash, Fanuj and Chabahar populations, while Karaj and Kuhdasht were placed in the second cluster.

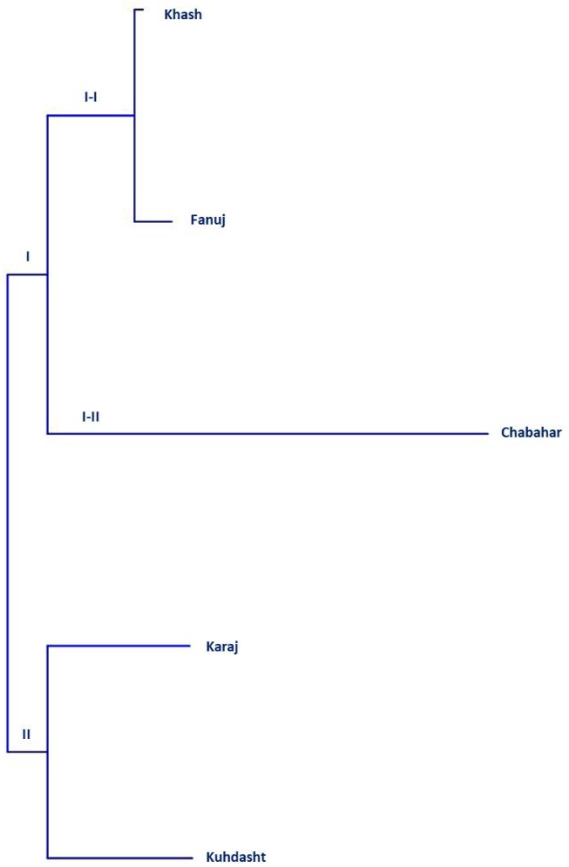

Fig. 2. The dendrogram of cluster analysis using NJ method on karyotype parameters in *N. oleander* populations.

Discussion

The present investigation, is the first attempt to karyomorphological study of *Nerium*. The all studied *Nerium* populations were diploid (2n=2x=22), which was in agreement with previous studies (Chauhan & Raghuvanshi 1977, Löve 1977, Noori-Daloii *et al*. 1996, Roy Tapadar 1964, Tjio 1948). Only Schürhoff & Müller (1937) mentioned the basic chromosome number of *N. oleander* as x = 8, which was not confirmed by other researchers. The chromosomes of these populations are of the type metacentric. The mean length of chromosomes was from 0.99 µm to 1.45 µm. In vascular plants, chromosomes about 2 µm long or less, are considered as small, while those over 10 µm are called as large (Stace 2000). The small size of *Nerium* chromosomes makes its karyotype problematic (Noori-Daloii *et al*. 1996). As far as we know, no report has been published on the karyotypic parameters of *Nerium* so far.

One of the most important parameters in karyomorphology is the karyotype asymmetry (Astuti *et al*. 2017, Shamsolshoara *et al*. 2020). Changes to an asymmetric karyotype can arise by having chromosomes more or less of the same length (inter-chromosomal asymmetry) and/ or by the position of centromere along single chromosomes (intra-chromosomal asymmetry) (Astuti *et al*. 2017). In the present study, the karyotype asymmetry was assessed based on either qualitative or quantitative indices. According to Stebbin (1971), karyotypes of different populations of *Nerium* were in class 1A which are classified as symmetric. Since Stebbins' (1971) classification is a qualitative and very broad method to separate the different types of karyotype asymmetry (Oroji Salmasi *et al*. 2019), we considered more quantitative indices to achieve greater measurement accuracy. The AR, CV_{CI} and A_1 are indicators of intra-chromosomal asymmetry. In Khash, the highest AR and A_1 and high CV_{CI} value indicate the most intra-chromosomal asymmetry karyotype in this population. In contrast, Kuhdasht has the most intra-chromosomal symmetry karyotype with the lowest values of these

parameters. In case of karyotype asymmetry indices, all of the populations were symmetric, but by having the highest rate karyotype asymmetry indices of AsK%, A, X_{CA} and the lowest rates for CI%, r-value, F%, TF% and X_{CI}, Khash population was considered as the most asymmetrical karyotype. On the other hand, with having the largest rates of CI%, r-value, F%, TF%, X_{CI} and the lowest rates of HCL, AsK%, A, X_{CA}, CV_{CI}, and AI, Kuhdasht population was considered the most symmetrical karyotype. It should be noted that, a symmetric karyotype does not necessarily implies primitivity (Peruzzi & Eroğlu 2013).

Variations in the karyological indices of the studied populations was observed, which was significant enough to differentiate the populations and was confirmed by cluster analysis. Based on all karyotypic parameters and asymmetry indices, Chabahar, Fanuj and Khash populations, which were identified as *N. indicum* according to the Flora Iranica, were separated from Karaj and Kuhdasht by the NJ clustering method. Future investigation of chromosomal karyotype with enhancement of population number from different regions may be useful in clarifying their taxonomic relationships.

The results of this study showed some clear karyotypic differences between the populations of southeastern, central, and western regions of Iran, so that, three populations of *Nerium*, which were designated as *N. indicum* in the Flora Iranica, were grouped in distinct cluster. These differences are probably due to climatic condition of the plant, which can also indicate some taxonomic differences in the species or sub-species levels between studied populations. It is hoped that, these findings may contribute to the taxonomical studies of *Nerium*, however, it is necessary to examine these differences from other perspectives in plant taxonomy.

In conclusion, results derived from the present study showed that, the ploidy level of *Nerium* is 2n = 2x = 22. In this study, the karyotype analysis of *Nerium* has been reported for the first time. In addition, all chromosomes in all of the populations were metacentric, and the karyotype in this genus is symmetric. Asymmetry indices pointed out Khash and Kuhdasht populations were the most asymmetrical and symmetrical karyotypes, respectively.

References

Aslani, M.R. 2018. Cardiotoxicity of plants in Iran: a review. Iranian Journal of Veterinary Science and Technology 10(1): 1–12.

Astuti, G., Roma-Marzio, F. & Peruzzi, L. 2017. Traditional karyomorphological studies: can they still provide a solid basis in plant systematics? Flora Mediterranea 27: 91–98.

Chauhan, A.K.S. & Raghuvanshi, S.S. 1977. Cytogenetical studies of some members of Apocynaceae. Cytologia 42: 723–729.

Ebrahimi, F., Ghorbani Nohooji, M. & Miri, S.M. 2018. Agronomic and pharmacological aspects of *Nerium oleander*: an important medicinal plant. Proceedings of the 1st. National Congress and International Fair of Medicinal Plants and Strategies for Persian Medicine that Affect Diabetes. 9–11 Oct., Mashhad, Iran.

Fallahi, M., Mohammadi, A. & Miri, S.M. 2020. The natural variation in six populations of *Calendula officinalis* L.: A karyotype study. Journal of Genetic Resources 6(1): 34–40.

Huziwara, Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. American Journal of Botany 49: 116–119.

Kuete, V. 2014. Physical, hematological, and histopathological signs of toxicity induced by African medicinal plants. Pp. 635–657. *In*: Toxicological Survey of African Medicinal Plants (Kuete, V., ed.). Elsevier.

Leeuwenberg, A.J.M. 1984. Series of revisions of Apocynaceae XIII. Notes on *Nerium* L.

and *Tabernaemontana* L. Mededelingen Landbouwhogeschool Wageningen 83(7): 57–60.

Levan, A., Fredga, K. & Sandberg, A.A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

Löve, Á. 1977. IOPB chromosome number reports LVI. Taxon 26(2/3): 257–274.

Noori-Daloii, M.R., Aliyari, R. & Ebrahimzadeh, H. 1996. Study of chromosome and soluble proteins of *Rhazya stricta* Decasine. and *Nerium oleander* L. Journal of Sciences, Islamic Republic of Iran 7(4): 209–216.

Oroji Salmasi, K., Javadi, H. & Miri, S.M. 2019. Karyotype analysis of some *Allium* species in Iran. Journal of Plant Physiology and Breeding 9(2): 115–127.

Pagen, F.J.J. 1988. Oleanders: *Nerium* L. and the oleander cultivars. Agricultural University Wageningen, Netherlands. 113 pp.

Paszko, B. 2006. A critical review and a new proposal of karyotype asymmetry indices. Plant Systematics and Evolution 258: 39–48.

Peruzzi, L. & Eroğlu, H.E. 2013. Karyotype asymmetry: again, how to measure and what to measure? Comparative Cytogenetics 7(1): 1–9.

Rajabi Mazaher, A., Miri, S.M. & Mohammadi, A. 2021. A new chromosome number report in *Stachys* L. species by use of karyological analysis. Journal of Genetic Resources 7(1): 29–35.

Rechinger, K.H. 1974. Flora Iranica, vol. 103. Apocynaceae. Akademische Druck U Verlagsanstalt, Graz, Austria.

Romero Zarco, C. 1986. A new method for estimating karyotype asymmetry. Taxon 35: 526–530.

Roy Tapadar, N.N. 1964. Cytotaxonomic studies in Apocynaceae and delineation of the different evolutionary tendencies operating within the family. Caryologia 17(1): 103–138.

Sennblad, B. & Bremer, B. 2002. Classification of Apocynaceae s.l. according to a new approach combining Linnaean and phylogenetic taxonomy. Systematic Biology 51(3): 389–409.

Schürhoff, P.N. & Müller, H. 1937. Zytologische Untersuchungen über die Haploidgenerationem der Apocynaceen. Cytologia Fujii Jubilaei(1): 407–415.

Shamsolshoara, Y., Javadi, H. & Miri, S.M. 2020. Karyomorphological study of seven species of the genus *Astragalus* from Iran. The Iranian Journal of Botany 26(2): 172–178.

Sinha, S.N. & Biswas, K. 2016. A concise review on *Nerium oleander* L. An important medicinal plant. Tropical Plant Research 3(2): 408–412.

Stace, C.A. 2000. Cytology and cytogenetics as fundamental taxonomic resources for the 20th. and 21st. centuries. Taxon 49: 451–477.

Stebbin, G.L. 1971. Chromosomal Evolution in Higher Plants. Edward Arnold Press, UK.

Tjio, J.H. 1948. The somatic chromosomes of some tropical plants. Hereditas 34(1–2): 135–146.

Van Steenis, C.G.G.J. 1981. Rheophytes of the world. Springer, Netherlands. 424 pp.

Watanabe, K., Yahara, T., Denda, T. & Kosuge, K. 1999. Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. Journal of Plant Research 112: 145–161.