KARYOTYPE ANALYSIS AND NEW CHROMOSOME NUMBERS OF SOME SPECIES OF EUPHORBIA L. (EUPHORBIACEAE) IN IRAN

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The somatic chromosome numbers and karyotype features of 15 species of *Euphorbia* (Euphorbiaceae) from Iran were analyzed. The chromosome counts of four species including *E. inderiensis* (2n=18), *E. polycaulis* (2n=18), *E. phymatosperma* (2n=16) and *E. gypsicola* (2n=54) are reported for the first time, while the chromosome number of 11 more studied species are confirmed. We also confirm the occurrence of two different cytotypes in *E. microsciadia*: one diploid (2n=18) and the other tetraploid (2n=36). The karyotypes are often symmetrical composing mainly of metacentric and submetacentric chromosomes. The results also confirm the presence of different basic chromosome numbers including x= 7, 8, 9 and 10 within the genus, indicating the potential evolutionary importance of such data in the genus.

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Key words: Cytotypes; *Euphorbia*; flora of Iran; karyology; taxonomy; evolution

مطالعه کاریوتیپی و گزارش عدد کروموزومی جدید در برخی گونه‌های سرده فرفیون، تیره Euphorbiaceae در ایران

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عدد کروموزومی و کاریوتایپ ۱۵ گونه از سرده فرفیون از بخشه‌های مختلف موجود در ایران مورد بررسی قرار گرفت. عدد کروموزومی چهار گونه شامل، E. polycaulis (2n=18)، E. inderiensis (2n=18)، E. phymatosperma (2n=16) و E. gypsicola (2n=54) برای اولین بار گزارش می‌شود. همچنین نتایج مطالعه حاضر برای ۱۱ گونه دیگر مورد مطالعه، تایید کننده پژوهش‌های پیشین بود. وجود دو تیپ هسته‌ای دیپلویید وتتراپلویید برای گونه E. microsciadia تایید می‌شود. کاریوتایپ‌ها متقارن بوده و اغلب شامل کروموزوم‌های متاسنتریک و ساب‌متا سنتریک هستند. همچنین نتایج ما تایید کننده وجود اعداد کروموزومی پایه متفاوت شامل x = 7, 8, 9, 10 برای این سرده است که بر اهمیت تکاملی چنین داده‌هایی دلالت دارد.

INTRODUCTION

Euphorbia L. (Euphorbiaceae), with approximately 2000 recognized species and a nearly global distribution, is among the largest genera of flowering plants (Govaerts & al. 2000; Radcliffe-Smith 2001; Riina & al. 2013). The genus comprises remarkable life form variability from annual to perennial herbs, shrubs, trees, succulent and xerophyte forms (Riina &

al. 2013). Despite this diversification in habit and the number of species, the genus can be easily recognised by a distinctive morphological synapomorphy, the cyathium – an aggregation of reduced flowers which acts as a pseudanthium (Steinmann & Porter 2002; Horn & al. 2012).

In Iran, *Euphorbia* is represented by about 90 species (Pahlevani & al. 2011) with majority of species corresponding to subgen. *Esula* Pers. (ca. 480 species worldwide: Riina & al. 2013; 73 species in Iran), and subgen. *Chamaesyce* (Rafin.) Gray (ca. 600 species worldwide: Yang & al. 2012; and 7 species in Iran), respectively. The most important characters in discrimination of species and higher ranks in the genus are: plant habit, cyathium structure, capsule shape and surface, seed shape, size and ornamentation as well as its caruncle (Salmaki & al. 2011; Pahlevani & al. 2015).

Members of the family Euphorbiaceae particularly genus *Euphorbia*, exhibit a great diversity of chromosome number and size (Perry 1943). Many *Euphorbia* species have basic chromosome number of x=8, whereas some other species include chromosome number of x=6, 7, 9 and 10, which is related to both aneuploidy and polyploidy (Perry 1943; Hans 1973). Previous cytological studies indicated various ploidy levels ranging from diploid, tetraploid, hexaploid to octaploid (2n=12–120), indicating a significant role of polyploidy in the speciation and evolution of the genus (Hans 1973; Hassal 1976; Strid & Frazen 1981; Franzen & Gustavsson 1983; Dalgaard 1985; Vicens & al. 1991; Benedi & Blanche 1992; Vogt & Oberprieler 1994; Yan-Hong & al. 1999).

Despite the importance of karyological studies in explaining speciation processes and recognizing evolutionary relationships in *Euphorbia* as well as existence of many available chromosome data for the genus worldwide, cytological studies on *Euphorbia* in Iran, are restricted to a few reports (Zehzad 1978; Ghaffari 1986, 2006, 2008; Sheidai & al. 2010; Naseh, 2013).

In the present study, chromosome number and karyotype features of 15 species of *Euphorbia* growing in Iran are reported.

MATERIALS AND METHODS

Karyological studies on 15 species of Euphorbia were performed. The list of studied species with associated collection information are presented in table 1.

For mitotic chromosome preparation, fresh grown root tips were pre-treated with 0.002 mol 8-hydroxyquinolin for 2.5 h at room temperature and then fixed in ethanol: acetic acid (3:1) for 24 h. Hydrolysis was made at 50–60 °C in 1 N HCL for 10–15 minutes.

Table 1. Collection data from *Euphorbia* specimens examined in this study.

Species	Subgenus/ Section	Collection Data
Euphorbia aucheri Boiss.	*Esula/ Herpetorrhizae*	Khorassan, 103 km to Dargaz from Ghouchan; Zarre, Salmaki & Ebrahimi, 38188 (TUH)
E. buhsei Boiss.	*Esula/ Esula*	Semnan: 35km to Firuzkuh from Sorkheh; Zarre, Salmaki & Ebrahimi, 38018 (TUH)
E. chamaesyce L.	*Chamaesyce/ Anisophyllum*	W. Azerbaijan, about 8km to Jolfa from Siahrud; Salmaki & al., 39837 (TUH)
E. densa Schrenk	*Esula/ Herpetorrhizae*	Khorassan, Sabzevar, 3 km after Parvand toward Parvarz mts.; Zarre, Salmaki & Ebrahimi, 38200 (TUH)
E. gypsicola Rech.f. & Aellen	*Esula/ Pithyusa*	Semnan: Sorkheh; Zarre & Salmaki, 43792 (TUH)
E. helioscopia L.	*Esula/ Helioscopia*	Tehran: Pardisan Park, s.n. (TUH)
E. inderiensis Less. ex Kar. & Kir.	*Esula/ Oppositifoliae*	Khorassan: Dizbad-e Sofla, road of Imam-Ali to Neyshabour; Zarre, Salmaki & Ebrahimi, 38215 (TUH)
E. macroclada Boiss.	*Esula/ Pithyusa*	Kermanshah, SW. Kerend, on the deviation of Radar station; Zarre & al., 39523 (TUH)
E. microsciadia Boiss.	*Esula/ Pithyusa*	Semnan: 45 km to Meyamey from Shahrud; Zarre, Salmaki & Ebrahimi, 38043 (TUH)
E. myrsinites L.	*Esula/ Myrsiniteae*	Qazvin: 20 km to Rajaei-Dasht from Rashteghon; Salmaki & al., 39898 (TUH)
E. peplus L.	*Esula/ Tithymalus*	Bandar Abbas, 47km to Manoujan, Kahnouj to Bandar abbas; Salmaki & Zarre, 39949 (TUH)
E. phymatosperma Boiss.	*Esula/ Lagascae*	Lorestan, Khorram Abaad, 4km to Sarab-e- Doureh, Kouhsefid; Zarre & al., 41004 (TUH)
E. polycaulis Boiss. & Hohen.	*Esula/ Pithyusa*	Markazi, 2km after Dojoft village toward Boroujerd; Zarre & al., 39467 (TUH)
E. stricta L.	*Esula/ Helioscopia*	Qazvin, 3 km after Kouhin Pass toward Loshan, 3 km Bekandi; Salmaki & al., 39748 (TUH)
E. szovitsii Fisch. & C.A.Mey.	*Esula/ Szovitsiae*	Khorassan, NE Mashhad, 3 km after Taghiabad to Amirabad; Zarre & al., 38184 (TUH)

The material was then stained in 2% aqueous aceto-orcein. Chromosome number and karyotype details were studied in at least 5 well-prepared metaphase plates.
The cells were photographed by digital camera (Canon PowerShot G5) and the chromosomes were measured by Micro Measure 3.3 software (Reeves & al. 2000).
Chromosome pairs were identified and arranged on the basis of their length and some more karyomorphological features, including karyotype composition and symmetry. The nomenclature used for describing karyotype composition followed Levan & al. (1964) and karyotype symmetry was determined according to Stebbins (1971). Other karyotype parameters like size of the longest chromosome (LC), size of the shortest chromosome (SC), haploid total chromosome length (T) [L+S], ratio of the longest to shortest chromosome (LC/SC), mean chromosome length (X) and intra-chromosomic asymmetry index (A1), inter-chromosomic asymmetry index (A2) (Romero Zarco 1986) were evaluated.

RESULTS

The somatic chromosome numbers and details of karyotypic features of 15 studied species of *Euphorbia* are presented in table 2 and figures 1-2. Our cytological study revealed different basic chromosome numbers including x=7, 8, 9, and 10 with different ploidy levels among studied species. *Euphorbia aucheri*, *E. buhsei*, *E. densa*, *E. inderiensis*, *E. myrsinites* and *E. szovitsii* with basic chromosome number x=10, *E. chamaesyce*, *E. macroclada* and *E. polycaulis* with x=9, *E. peplus* and *E. phymatosperma* with x = 8 and *E. stricta* with x=7 were diploids, while *E. microsciadia* was found to be tetraploid (2n=36) with x=9. Moreover, *Euphorbia gypsicola* (2n=54) and *E. helioscopia* (2n=42) were also hexaploid (figs. 1-2).
The obtained karyotypes were more or less symmetrical due to high proportion of metacentric and submetacentric chromosomes (fig. 2), and all of them belong to 1A and 2A classes of Stebbins karyotype symmetry which consider as the most primitive karyotypes.
Based on our results the intrachromosomal (A1) and interchromosomal (A2) asymmetry index varied from 0.31 to 0.49 and 0.10 to 0.24, respectively (table 2). Among the species in class 1A, the highest value of A1 (0.48) was shown in *E. buhsei*. This species has also had the lowest value of A2 (0.122) showing the most asymmetric karyotype. In contrast, *E. peplus* with the lowest A1 (0.31) and the highest A2 (0.24) values was considered as the most symmetric karyotype. Within class 2A, the most asymmetric karyotype with the highest A1 (0.49) value was observed in *E. macroclada*, while the lowest A1 (0.41) value with the most symmetric karyotype occured in *E. gypsicola* (table 2).
Total haploid chromosome length (TL) of class 1A varied from 8.75 µm in *E. peplus* to 27.65 µm in *E. helioscopia* and it also differed from 12.84 µm in *E. szovitsii* to 48 µm in *E. gypsicola* within members of class 2A. Moreover, the highest mean haploid chromosome length (X) was observed in *E. myrsinites* (3.44 µm) and *E. aucheri* (2.48 µm) within classes 1A and 2A respectively, while the lowest values occurred in *E. inderiensis* (0.94 µm) and *E. szovitsii* (1.28 µm). The highest ratio of longest to shortest chromosome (LC/SC) was observed in *E. aucheri* and *E. gypsicola*, where as the lowest values of LC/SC was observed in *E. chamaesyce*, *E. inderiensis* and *E. szovitsii* (Table 2).

DISCUSSION

The chromosome numbers of *E. inderiensis* (2n=18), *E. phymatosperma* (2n=16), *E. polycaulis* (2n=18) and *E. gypsicola* (2n=54) are reported for the first time in the present study. Moreover, the results obtained from other species are in agreement with previous studies (Lessani & al. 1979; Chariat-Panahi & al. 1982; Zehzad 1978; Sheidai & al. 2010; Nasseh 2013). Our results as well as previous investigations (Sheidai & al. 2010; Nasseh 2013) reveal the symmetric karyotype comprising of a variable number of metacentric and submetacentric chromosomes as a common karyological feature of *Euphorbia*.
Karyological studies have also revealed extensive chromosomal variation in the genus *Euphorbia* which is probably resulted through polyploidy and aneuploidy (Hans 1973; Urbatsch & al. 1975). Of the ten different reported (x=5, 6, 7, 8, 9, 10, 11, 12, 13, 17) basic chromosome numbers of the genus four are observed in the present study (x=7, 8, 9, 10)
Among the studied species, *E. polycaulis*, *E. macroclada*, *E. microsciadia* and *E. gypsicola*, belong to sect. *Pithyusa* (Riina & al. 2013) with similar basic chromosome number (x=9) but different ploidy levels including diploid (*E. macroclada* and *E. polycaula*), tetraploid (*E. microsciadia*), and hexaploid (*E. gypsicola*). Basic chromosome number x=9 is also reported in some more Iranian species of sect. *Pithyusa* such as *E. cheiradenia*, *E. teheranica* and *E. seguieriana* (Zehzad, 1978). *Euphorbia microsciadia* is known as a taxonomically and phylogenetically complex species. Cytological studies also confirm this complexity (Ghaffari, 2006; Zehzad, 1978). Ghaffari (2006) reported diploid level for *E. microsciadia* (2n=18), while Zehzad (1978) reported the same

number as our result (2n=36) which could be indicative of the presence of different cytotypes for this species.

Euphorbia stricta and *E. helioscopia* belong to sect. *Helioscopia*. There are several different kinds of reports on their chromosome number. These species show the basic chromosome number x=7 as diploid (2n=14), 2n=10 (Hayirlioğlu-Ayaz & al. 2002) and hexaploid (2n=42), respectively (Vicens & al. 1991; Strid & Frazen 1981). Our results also confirm the former reports for the annual *E. peplus* and *E. szovitsi* by Pavone & al. (1981), Sheidai & al. (2010) and Nasseh (2013) as well as Zehzad (1978), while the chromosome number for *E. phymatosperma* is recorded for the first time in the present study. *Euphorbia peplus* (sect. *Tithymalus*) and *E. phymatosperma* (sect. *Lagascae*) have the same chromosome number (2n=16) based on x=8, while the chromosome number for *E. szovitsii* is 2n=20 based on x=10 (Zehzad 1978). A different ploidy level for *E. szovitsii* (2n=40) is reported by Nasseh (2013) which has a discrepancy with our results and Zehzad (1978). This incongruity might be related to the existence of different cytotypes in this species. *Euphorbia chamaesyce*, the only representative of subgenus *Chamascyse* has the chromosome number 2n=20 and 2n=18 which corroborate the previous results (Vignal & Reynaud 1992).

One interesting result of our study is the highest ploidy level (hexaploid) observed in *E. helioscopia* and *E. gypsicola*. Both species show extraordinary ecological adaptations: the former species is an invasive weed and the latter one is adapted to severe dried conditions on gypsum hills in north of Dasht-e Kavir (South of Alborz mountain range). It has been previously shown that higher ploidy level facilitates the invasiveness of a species or population of a certain species (Schlaepfer 2008) mainly through increasing survival rates and fitness in the earliest establishment phase (Beest & al. 2011). Furthermore, recent studies have also demonstrated that polyploid genomes can be highly dynamic and undergo rapid structural and functional alterations (Parisod & al. 2009) favoring the frequency of polyploid species under harsh conditions.

Euphorbia myrsinites as the type of sect. *Myrsiniteae* has the chromosome number 2n=20 based on x=10. Several species of this section were examined in the previous studies: all indicating the same chromosome number (2n=20) based on x=10 in this section (Strid & Frazen 1981; Nasseh 2013).

Our results suggest that *E. peplus* and *E. chamaesyce* represent the most symmetrical, and probably the most primitive karyotypes (according to Sharma 1990) among the studied species. Moreover, Hans (1973) considered that most members of the genus *Euphorbia* belong to a primary system of x=8 and a secondary system of x=6, 7, 9 and 10 which implies that the fifteen studied *Euphorbia* species distributed in Iran belong to different series of primary and secondary basic chromosome numbers that might possibly have resulted from aneuploidy and polyploidy.

Table 2. Karyotype features of *Euphorbia* species studied. Abbreviations: T= haploid total chromosome length (µm), L= size of the longest chromosome (µm), S= size of the shortest chromosome (µm), LC/SC= ratio of the longest to shortest chromosome, X= mean chromosome length, A_1 and A_2 =Romero- Zarco indices, ST= Stebbins class, KF= karyotype formula**.** The species indicated with asterisk have very small or overlapped chromosomes and could not be measured appropriately. Therefore the size and indices of karyotypes are not mentioned for them.

Species	**2n**	**T**	**L**	**S**	**L/S**	**X**	**A_1**	**A_2**	**ST**	**KF**
E. aucheri	20	24.78	3.21	1.76	1.82	2.48	0.372	0.179	1A	7m+3sm
E. busei	20	21.95	2.67	1.73	1.54	2.19	0.484	0.122	1A	2m+8sm
E. chamaesyce	18	9.89	1.33	0.92	1.45	1.09	0.346	0.124	1A	9m
E. densa	20	14.31	1.78	1.18	1.51	1.43	0.386	0.129	1A	7m+3m
E. gypsicola	54	48	2.38	1.23	1.93	1.78	0.419	0.144	2A	11m+16sm
E. helioscopia	42	27.65	1.70	1.03	1.65	1.32	0.395	0.131	1A	12m+9sm
E. inderiensis	20	9.37	1.12	0.77	1.45	0.94	0.371	0.125	1A	9m+1sm
E. macroclada	18	21.82	3.16	1.85	1.71	2.42	0.492	0.165	2A	9sm
E. microsciadia	36	38.9	2.87	1.62	1.77	2.16	0.420	0.171	2A	7m+11sm
E. myrsinites	20	34.41	4.16	2.64	1.58	3.44	0.464	0.131	2A	2m+8sm
E. peplus	16	8.75	1.28	0.84	1.52	1.09	0.311	0.247	1A	8m
E. polycaula	18	16.75	2.31	1.41	1.64	1.86	0.430	0.158	2A	3m+6sm
E. szovitsii	20	12.84	1.48	1.08	1.37	1.28	0.435	0.101	2A	3m+7sm
*E. phymatosperma**	16	-	-	-	-	-	-	-	-	-
E. stricta *	14	-	-	-	-	-	-	-	-	-

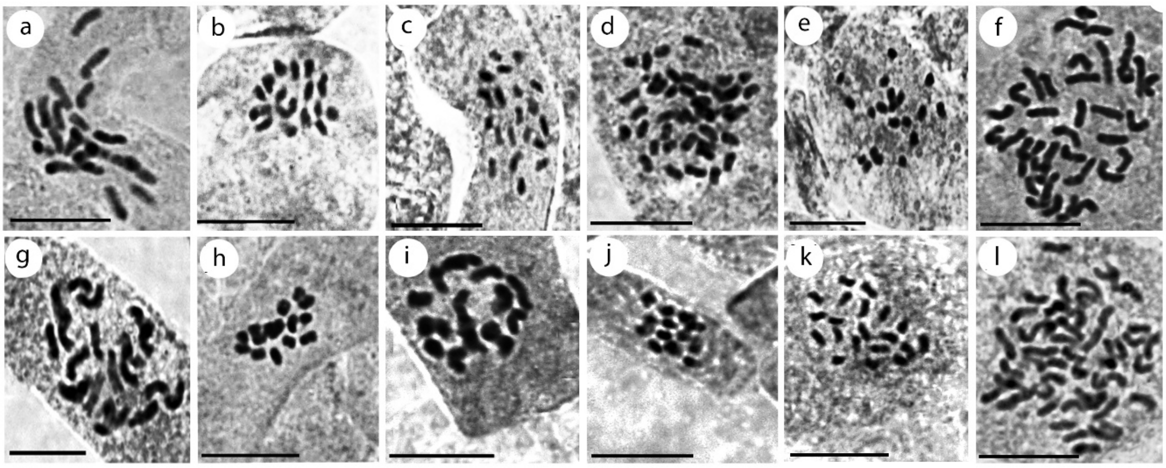

Fig.1. Representatives of somatic cells of *Euphorbia* species studied. a, *Euphorbia aucheri* with 2n=20; b, *E. chamaesyce* with 2n=18; c, *E. densa* with 2n=20; d *E. helioscopia* with 2n=42; e, *E. inderiensis* with 2n=20; f, *E. microsciadia* with 2n=36; g, *E. myrsinites* with 2n=20; h, *E. peplus* with 2n=16; I, *E. polycaulis* with 2n=18; j, *E. stricta* with 2n=14; k,:*E. szovitsii* with 2n=20; l *E. gypsicola* with 2n=54. Scale bar= 10µm.

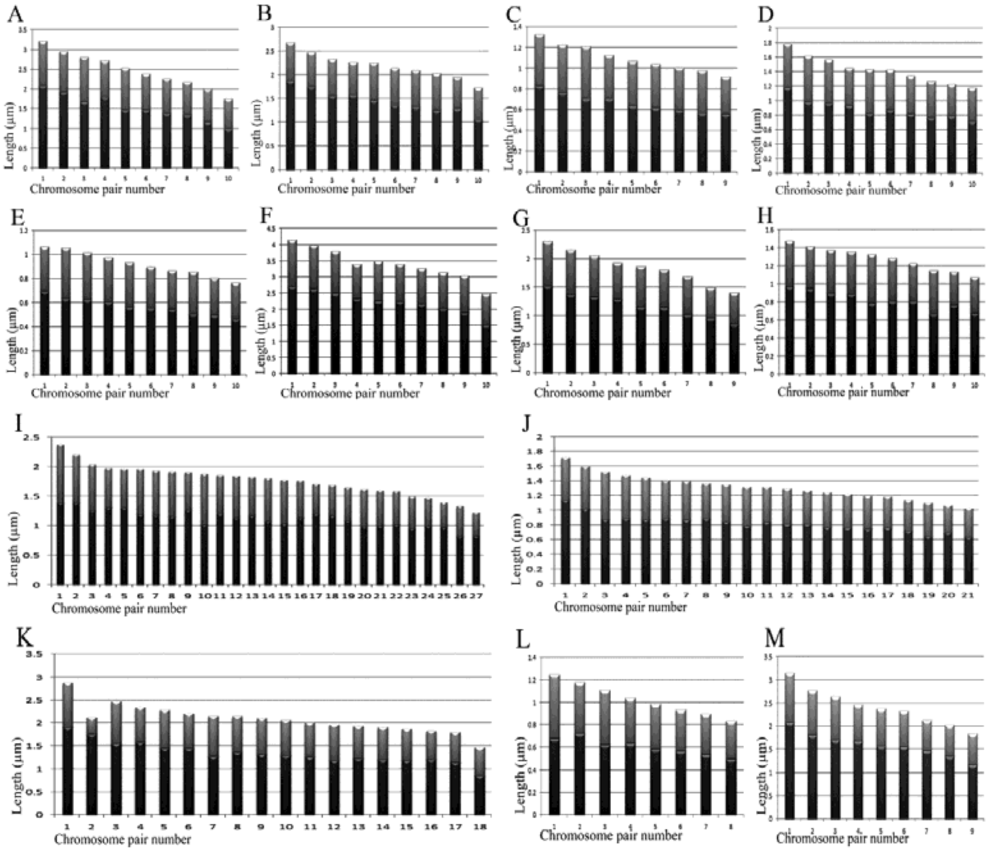

Fig. 2. Idiograms of A, *Euphorbia aucheri*; B, *E. buhsei*; C, *E. chamaesyce*; D, *E. densa*; E, *E. inderiensis*; F, *E. myrsinites*; G, *E. polycaula*; H, *E. szovitsii*; I, *E. gypsicola*; J, *E. helioscopia*; K, *E. microsciadia*; L, *E. peplus*; M, *E. macroclada.*

REFERENCES:

Beest, Te M., J.J., Richardson, D.M., Anne K. Brysting, A.K., Suda, J., Kubešová M., & Pyšek, P. 2012: The more the better? The role of polyploidy in facilitating plant invasions. – Ann. Bot. 109: 10-45.

Benedi, C., & Blanche, C. 1992: IOPB chromosome data 4. – International Organization of Plant Biosystematics Newsletter, 18/19: 6.

Chariat-Panahi, M.S., Lessani, H. & Cartier, D. 1982: Étude caryologique de quelques especes de la flore de Iran. – Rev. Cytol. Veg. Biot. 5: 189-196.

Dalgaard, V. 1985: Chromosome studies in flowering plants from Madeira. – Willdenowia 15: 137–156.

Franzen, R. & Gustavsson, L.A. 1983: In chromosome numbers in flowering plants from the high mountains of Sterea Ellas, Greece. – Willdenowia 13: 101-106.

Ghaffari, S.M. 1986: IOPB Chromosome number reports XCI. –Taxon 35: 407.

Ghaffari, S.M. 2006: New or rare chromosome counts of some angiosperm species from Iran. – Iran. J. Bot. 11(2): 185-192.

Ghaffari, S.M. 2008: Chromosome reports for some plant species from Iran. – Iran. J. Bot. 14: 39-46.

Govaerts, R., Frodin, D.G. & Radcliffe-Smith, A. 2000: World checklist and bibliography of Euphorbiaceae (and Pandaceae) – The Royal Botanical Garden, Kew.

Hans, A.S. 1973: Chromosomal conspectus of the Euphorbiaceae. – Taxon 22: 591–636.

Hayirlioğlu-Ayaz, S., Inceer, H., Beyazoğlu, O. 2002: Cytotaxonomic studies on some *Euphorbia* L. (Euphorbiaceae) species in Turkey. – Pak. J. Bot. 34(1): 494-500.

Horn, J.W., van Ee, B.W., Morawetz, J.J., Riina, R., Steinmann, V.W., Berry, P.E. & Wurdack, K.J. 2012: Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). – Mol. Phylogenet. Evol. 63: 305-326.

Khatoon, S. & Ali, S.I. 1993: Chromosome Atlas of the Angiosperms of Pakistan. – Department of Botany, University of Karachi, Karachi.

Lessani, H. & Chariat-Panahi, A. 1979: In IOPB chromosome number reports LXV. – Taxon 28: 635-636.

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

Urbatsch, L.E., Bacon, J.D., Hartman, R.L., Johnston, M.C., Watson, T.J., Webster, J.R., & Webster, G.L. 1975: Chromosome numbers for North American Euphorbiaceae. – Amer. J. Bot. 62(5): 494-500.

Nasseh, Y. 2013: Chromosome numbers of some Iranian species of the genus *Euphorbia* (Euphorbiaceae). – Takhtajania 2: 127-128

Mehra, P.N. & Choda, S.P. 1978: Cyto-taxonomical studies in the genus *Euphorbia* L. – Cytologia 43: 217-235.

Pahlevani, A.H., Maroofi, H. & Joharchi, M.R. 2011: Notes on six endemic or rare species of *Euphorbia* subg. *Esula* (Euphorbiaceae) in Iran. – Willdenowia 41: 267-276.

Pahlevani, A.H., Liede-Schumann, S. & Akhani, H. 2015: Seed and capsule morphology of Iranian perennial species of *Euphorbia* (Euphorbiaceae) and its phylogenetic application. – Bot. J. Linn. Soc. 177: 335-377.

Parisod, C., Holderegger, R. & Brochmann, C. 2010: Evolutionary consequences of autopolyploidy. – New Phytol. 186: 5-17.

Pavone, P., Terrasi, C.M. & Zizza, A. 1981. In chromosome number reports LXXII. – Taxon 30: 695–696.

Perry, B.A. 1943: Chromosome number and phylogenetic relationships in the Euphorbiaceae. – Am. J. Bot. 30: 527-543.

Radcliffe-Smith, A. 2001. Genera Euphorbiacearum. – Royal Botanical Gardens, Kew. Rafinesque, C.S., 1837. Flora Telluriana 2. Rafinesque (self-published), Royal Botanical Gardens, Kew, UK.

Reeves, A. & Tear, J. 2000: Micro-measure software, Colorado State University.

Riina, R., Peirson, J.A., Geltman, D.V., Molero, J., Frajman, B., Pahlevani, A., Barres, L., Morawetz, J.J., Salmaki, Y., Zarre, S., Kryukov, A., Bruyns, P.V. & Berry, P.E. 2013: A worldwide molecular phylogeny and classification of the leafy spurges, *Euphorbia* subgenus *Esula* (Euphorbiaceae). – Taxon 62: 316– 342.

Romero Zarco, C. 1986: A new method for estimating karyotype asymmetry. – Taxon. 35(3): 526-530.

Salmaki, Y., Zarre, S., Esser, H.J. & Heubl, G. 2011: Seed and gland morphology in *Euphorbia* (Euphorbiaceae) with focus on their systematic and phylogenetic importance, a case study in Iranian highlands. – Flora 206: 957-973.

Schlaepfer, D.R. 2008: Ecological significance of ploidy level of native and invasive populations of *Solidago gigantean.* – Doctoral Thesis, ETH Zurich.

Sharma, A. 1990: Taxonomy as related to genetic diversity in plants. – J. Indian Bot. Soc. 69: 1-3.

Sheidai, M., Ghazi, M. & Pakravan, M. 2010: Contribution to cytology of the genus *Euphorbia* in Iran. – Cytologia 75 (4): 477-482.

Simon, J. & Vicens, J. 1999: E studis biosystemàtics en *Euphorbia* L., a la Mediterrània Occidental. – Institut d' Estudis Catalans. Arx. Secc. Ci. 122:704 pp. Barcelona.

Stebbins, G.L. 1971: Chromosomal evolution in higher plants. – Edward Arnold Press.

Steinmann, V.W. & Porter, J.M. 2002: Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on its and *ndhF* sequence data. – Ann. Missouri Bot. Gard. 89: 453-490

Strid, A. & Franzen, R. 1981: In chromosome number reports LXXIII. – Taxon 30: 829–842.

Vicens, J., Molero, J. & Blanché C. 1991: Mediterranean chromosome number reports 1 (38–44). – Flora Medit. 1: 241–243.

Vignal, C. & Reynaud, C. 1992: Mediterranean chromosome number reports 2 (118-119). – Flora Medit. 2: 272-273.

Vogt, R. & Oberprieler, C. 1994: Chromosome numbers of North African phanerogams. IV. – Candollea 49: 549–570.

Webster, G.L. 1994: Synopsis of the genera and subgeneric taxa of Euphorbiaceae. – Ann. Missouri Bot. Gard. 81: 33-144.

Yang, Y., Riina, R., Morawetz, J.J., Haevermans, T., Aubriot, X. & Berry, P.E. 2012: Molecular phylogenetics and classification of *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). – Taxon 61: 764–789.

Zehzad, B. 1978: Chromosome numbers for *Euphorbia* species from Iran. – Rev. Biol. Ecol. Mediterraneenne 1: 35-40.