Chromosomal Evolution in Some Iranian *Artemisia* L. using Numerical Analysis of Karyotypes

Hamid Reza Naseri^{1,*}, Hossein Azarnivand¹ and Mohammad Jafari¹

¹ Department of Arid and Mountainous Rangelands Reclamation, Faculty of Natural Resources, University of Tehran, P.O. Box 315854314 Karaj, Iran

Received February 4, 2009; accepted March 9, 2009

Summary A karyological study of seven taxa (four sections) of genus *Artemisia* L. from Azerbaijan region (Iran) is presented. We found the two usual basic numbers in the genus $x=9$, the most common one (in three diploid and two tetraploid populations) and $x=8$ (in two diploid populations). The intra- ($A1$) and interchromosomal ($A2$) asymmetry index, which does not depend on chromosome number or chromosome size showed asymmetry index based on $A1$ value has indicated that the chromosomes in some Iranian *Artemisia* evolved in order of section *Absinthium* and *Artemisia* meanwhile section *Seriphidium* and *Dracunculus* are close to each other but they can separate from Section *Absinthium* and *Artemisia*.

Key words *Artemisia*, Asteraceae, Chromosome, Karyotype.

The Anthemideae is an important tribe Asteraceae that contains about 8% of total genera and 13% of the species of the Asteraceae (Heywood *et al.* 1977). The genus *Artemisia* one of the largest and most widely distributed of the nearly 100 genera in the tribe Anthemideae. This genus contains a great number (more than 200 to more than 500, depending on the authors; (Ling 1991a, b, Bremer and Humphries 1993, Mabberley 1997, Vallès and Siljak-Yakovlev 1997, Torrell *et al.* 1999, Torrell and Vallès 2001) of species.

The 400 and more known species of *Artemisia* are mainly found in Asia, Europe and North America, but a few species occurs in most temperate countries. Asia seems to show the greatest concentration of species with 150 accessions for China (Hu 1965), 174 in the ex U.S.S.R (Poljakov 1961), 50 in Japan (Kitumara 1939, 1940) and about 37 reported to occur in Iran (Mozafarian 1998). These species of the genus in Iran are found from seacoast of Caspian Sea to high mountains, and most of them can be found in vast steppes of Iran.

According to Naseri *et al.* (2006), they include some introduced species, which have been cultivated for food use in Iran. *Artemisia* genus have a high economic value in several fields: food (absinth, *A. absinthium* L. and other taxa, such as *A. genipi*, used to make liquors; spices such as tarragon or estragon, *A. dracunculus* L.), medicine (many antihelminthic species such as *A. santonicum* L. and related taxa, antimalarian such as *A. annua* L.), forage (*A. herba-alba* Asso and related species in African and Asian steppes and semi deserts and *A. tridentata* Nutt. and related species in North American shrub lands), ornamentals (*A. absinthium* among many others). Some taxa, such as *A. verlotiorum* Lamotte, are invasive weeds which can adversely affect harvests.

Artemisia has been divided classically into four groups treated as sections or subgenera (*Artemisia*, *Absinthium*, *Dracunculus* and *Seriphidium*), but this number has increased with the proposal of the Tridentatae, which is endemic to North America (McArthur *et al.* 1981). Ling (1982, 1991a, b, 1995a, b) proposed the separation of one of the sections as an independent genus, *Seriphidium* (Besser ex Hook.) Fourr., which was accepted by Bremer and Humphries (1993) and Bremer (1994) in their respective cladistic revisions of the tribe Anthemideae and the family Asteraceae.

* Corresponding author, e-mail: hrnaseri@ut.ac.ir

Artemisia has two basic chromosome numbers, with ploidy levels ranging from diploid to dodecaploid for $x=9$ and from diploid to hexaploid for $x=8$ (Vallès and Siljak-Yakovlev 1997, McArthur and Sanderson 1999, Vallès and McArthur 2001). Palaeontological data suggest that the mountain region of *Artemisia* was most probably in the mountain regions of north-western Asia in mid-Cenozoic (Ling 1982). The most important center of diversity is temperate Asia, although there are dispersed foci of section in temperate and cold region of Eurasia and North America (Ling, 1982). The finding of different ploidy levels in these and other Artemisiinae species in Asia also supports the hypothesis of this origin of speciation.

Many species of *Artemisia* are not always well known and a world revision of the genus, from a systematic point of view is needed (Oliva and Vallès 1994). Karyological data may be very helpful in establishing systematic and evolutionary ship within the genus. In spite of the very large number of counts in the tribe, chromosomal data on Iranian populations are scarce.

In this study we provide karyomorphological data with somatic chromosome numbers 7 populations belonging to the 7 taxa, *Artemisia fragrans* Willd., *A. spicigera* Willd., *A. vulgaris* Rat., *A. campestris* L., *A. scoparia* Waldst. and Kit, *A. absinthium* L. and *A. chamaemelifolia* Vill. in Iran.

Materials and methods

Plants and ripe achenes were collected from natural population in different ecological regions of Eastern Azerbaijan province (Iran). Herbarium vouchers of the populations studied are deposited in the Herbarium of RIFR (Research Institute of Forests and Rangelands, Tehran). The species studied are listed in Table 1.

For the study we used root tip meristems from seedlings obtained from the germination of achenes on wet filter paper in Petri dishes or from plants cultivated in pots. Ripe achenes were placed on wet filter in Petri dishes and left in the dark at germinator temperature (20°–22°C). Adults' plants also collected and were grown in pots in greenhouse conditions. Root tip meristems were obtained from the seedling or from the plants living in pots. They were pretreated with saturated solution of α - bromonaphthalene at room temperature for 4 h at 15°C. These specimens fixed in absolute ethanol and glacial acetic acid (3 : 1) for at least 12 h at 4°C. Root tips were hydrolysed in 1 M HCL for 5 min at 60°C, squashed in a droplet of 45% acetic acid, dyed by Aceto- Iron-Hematoxylin. The preparation was observed with an optical microscope at magnification of 1000X. The best metaphasical plates were drawn with a camera and photographed. The counts were carried out bearing in mind the comments of Favarger (1978) to avoid mistakes in chromosome counting (basically the following: not being influenced by previous reports; obtaining good metaphases, especially in polyploid taxa; having the slides examined by different experienced people in case of doubt; and making a correct plant material determination). At least ten metaphases were examined per population, with the five best selected for making idiograms.

The following parameters were estimated in each metaphase plate to characterize the karyotypes numerically; (1) total karyotype length (TKL); (2) mean chromosome length (MCL); (3) chromosome length range (CLR); (4) lengths ratio of long and short arms of chromosome according to Levan *et al.* (1964) (L/S); (5) intrachromosomal asymmetry index ($A1$) = $1 - [\Sigma(b/B)/n]$; and (5) interchromosomal asymmetry index ($A2$) = s/x , where b and B are the mean length of short and long arms in every homologous chromosome groups, respectively, n is the number of homologues, s is the standard deviation, and x the mean chromosome length. Karyotype asymmetry was estimated using the mean centromeric index, the ratio of the shortest/longest pair and according to the $A1$ and $A2$ indices (Romero 1986). Symmetry classes (SC) according to Stebbins (1971) of the populations studied were done and Chromosomal formula according to Levan *et al.* (1964) obtained for all species studied.

Results

Our Results showed five population belong to taxa with basic number $x=9$, the most common one in the genus. Among this population we find three diploid and two tetraploid mean while two diploid population belong to taxa basic number $x=8$. Chromosome number in seven Iranian *Artemisia* species counted were listed follows; $2n=36$ for *A. spicigera*, *A. campestris* and $2n=18$ for *A. fragrans*, *A. absinthium*, *A. chamaemelifolia* and finally $2n=16$ for *A. vulgaris* and *A. scoparia*. Table 2 presented the data on poloidy level, chromosome formula, TKL, MCL, CLR L/S, interchromosomal asymmetry index (A_2), interchromosomal asymmetry index (A_2) and SC.

Their mitotic metaphase chromosomes were shown in Fig. 1 and Haploid ideograms of seven Iranian *Artemisia* species were indicated in Fig. 2.

Artemisia scoparia showed the shortest ($48.56 \mu\text{m}$) and *A. spicigera* the longest ($122.99 \mu\text{m}$) TKL of all species. *A. scoparia* and *A. fragrans* had smallest MCL ($3.04 \mu\text{m}$) mean while the largest MCL belonged to *A. absinthium* ($3.85 \mu\text{m}$). Other species were significantly different from each other ($3.09\text{--}3.67 \mu\text{m}$). *A. scoparia*, *A. campestris*, *A. chamaemelifolia* and *A. fragrans*, had lowest L/S that weren't significantly different ($1.47\text{--}1.65$). *A. vulgaris* and *Artemisia absinthium* showed highest L/S ($2.88\text{--}3.14$) meanwhile *A. spicigera* had medium L/S (2.20).

Artemisia fragrans had the lowest intrachromosomal asymmetry index A_1 (0.17), and *A. campestris*, *A. spicigera*, *A. vulgaris*, *A. scoparia*, and *A. chamaemelifolia*, showed relatively small A_1 ($0.18\text{--}0.34$). The largest A_1 belonged to *A. absinthium* (0.60). *A. scoparia* showed the lowest (0.04) and *A. absinthium* the highest (0.26) A_2 values of all species. None of other species were significantly different from each other ($0.07\text{--}0.13$). These results of A_1 and A_2 were shown graphically in Fig. 3 and Fig. 4 for both species and sections. Symmetry types of Stebbins in these seven *Artemisia* species showed all species were located in same class (1A).

Discussions

The results obtained confirm the existence of two basic chromosome numbers in the genus. Five of the studied taxa have $x=9$, the most common basic number in the genus *Artemisia*, the tribe Anthemideae and the family Asteraceae as well (Solbrig 1977, Schweizer and Ehrendorfer 1983, Oliva and Valle's 1994, Valle's and Silja-Yakovlev, 1997). Among the considered taxa, *Artemisia vulgaris* and *A. scoparia* have the less common basic number $x=8$, this is evidence for the descending dysploidy that occurs in the genus (Vallès and Siljak-Yakovlev 1997, Torrel *et al.* 2001). This phenomenon is common in many genera of the family Asteraceae (Fernandez Casas and Susanna 1986, Siljak-Yakovlev 1996, Vallès *et al.* 2001, 2005).

In this study, we have provided additional karyomorphological parameter using intra- (A_1) and interchromosomal asymmetry index (A_2), which does not depend on chromosome number or chromosome size (Romero 1986). Fig. 3 indicates scatter diagram that showing the relation between intra- and interchromosomal asymmetry. The section *Absinthium* shows high value for (A_2) 1 and it is distinguished separately meanwhile for the section *Artemisia*, *Dracunculus* and *Seriphidium* are difficult to distinguish in their interchromosomal asymmetry index (A_2). On the other hand, the intrachromosomal asymmetry index (A_1) indicates a difference between four groups of Iranian *Artemisia* Fig. 4. The section *Dracunculus* and *Seriphidium* show relatively smaller A_1 values ($0.17\text{--}0.22$), section *Artemisia* intermediate A_1 value ($0.29\text{--}0.34$) and section *Absinthium* higher A_1 value (0.60) (Table 2). Each section seems to be more affected with A_1 values than with A_2 value. Stebbins (1971) has suggested that asymmetrical karyotypes are more advanced than symmetrical ones in relation to phylogeny and evolutionary processes. Our asymmetry index based on A_1 value has showed that the A_1 parameter is useful for estimation of evolutionary relationships among some subgenera or sections of genus *Artemisia*. These results have been obtained by other authors (Mato-

Table 1. Origin of populations studied and herbarium vouchers

Taxon	Location	Altitude	Collector and Date	Herbarium voucher
Sect. <i>Seriphidium</i>				
<i>A. fragrans</i> Willd.	Tabriz-Ahar Road, Research Station of Khajeh village 38°9'N, 46°39'E	1548	H. R. Naseri and Y. Asri 25 VIII 2004	84423
<i>A. spicigera</i> C. Koch.	Tabriz-Ahar Road, Research Station of Khajeh village 38°9'N, 46°39'E	1550	H. R. Naseri and Y. Asri 25 VIII 2004	84424
Sect. <i>Artemisia</i>				
<i>A. vulgaris</i> L.	Ahar-Kalibar Road, Peyam village 38°48'N, 47°9'E	1271	H. R. Naseri and Y. Asri 14 IX 2004	84422
<i>A. chamaemelifolia</i> Vill.	Arasbaran Forest, Akdash Region 38°49'N, 46°49'E	2472	H. R. Naseri and Y. Asri 25 IX 2004	84418
Sect. <i>Dracunculus</i>				
<i>A. campestris</i> L.	Ahar-Kalibar Road, Peyam village 38°48'N, 47°9'E	1279	H. R. Naseri and Y. Asri 14 IX 2004	84421
<i>A. scoparia</i> Waldst. & Kit	Arasbaran Forest, Vayghan Cross 38°55'N, 46°47'E	1340	H. R. Naseri and Y. Asri 22 IX 2004	84420
Sect. <i>Absinthium</i>				
<i>A. absinthium</i> L.	Arasbaran Forest, Akdash Region 38°49'N, 46°49'E	2500	H. R. Naseri and Y. Asri 25 IX 2004	84419

Table 2. Means of the parameters chromosome formula, TKL, MCL, CLR, L/S, intra- (*A1*), interchromosomal asymmetry index (*A2*) and Symmetry Classes

Taxon	2n	x	Chromosome formula	TKL (μm)	MCL (μm)	CLR (μm)	L/S	A1	A2	SC
Sect. <i>Seriphidium</i>										
<i>A. fragrans</i>	18	9	18m	54.72	3.04	2.40–3.96	1.65	17.0	0.08	1A
<i>A. spicigera</i>	36	9	32m+4sm	122.92	3.41	2.08–4.58	2.20	0.22	0.07	1A
Sect. <i>Artemisia</i>										
<i>A. vulgaris</i>	16	8	14m+2sm	58.78	3.67	1.88–5.42	2.88	0.29	0.13	1A
<i>A. chamaemelifolia</i>	18	9	16m+2sm	55.68	3.09	2.4–3.65	1.52	0.34	0.04	1A
Sect. <i>Dracunculus</i>										
<i>A. campestris</i>	36	9	32m+4sm	120.64	3.35	2.71–4.06	1.49	0.18	0.10	1A
<i>A. scoparia</i>	16	8	16m	48.56	3.04	2.40–3.54	1.47	0.19	0.04	1A
Sect. <i>Absinthium</i>										
<i>A. absinthium</i> L.	18	9	12m+6sm	69.38	3.85	1.65–5.18	3.14	0.60	0.26	1A

Fig. 1. Somatic chromosomes at metaphase of seven species of Iranian *Artemisia*. A: *A. fragrans* ($2n=18$). B: *A. spicigera* ($2n=36$). C: *A. campestris* ($2n=36$). D: *A. vulgaris* ($2n=16$). E: *A. scoparia* ($2n=16$). F: *A. absinthium* ($2n=18$). G: *A. chamaemelifolia* ($2n=18$). Scale bar represents 5 μm for A to G.

Fig. 2. Haploid ideogram of seven species of Iranian *Artemisia*. A: *A. fragrans* ($2n=18$). B: *A. spicigera* ($2n=36$). C: *A. campestris* ($2n=36$). D: *A. Vulgaris* ($2n=16$). E: *A. scoparia* ($2n=16$). F: *A. absinthium* ($2n=18$). G: *A. chamaemelifolia* ($2n=18$).

ba *et al.* 2007).

Comparing between our results and other results from different region showed in most species we can confirm other results.

Section *Seriphidium*: Three reports, also of $2n=18$, have been published to evaluate for *A. fragrans* by Kawatani and Ohno (1964), Nazarova and Pogolian (1970) and Torrel *et al.* (2001). Our results now confirm this chromosome number from plants collected directly from a natural population. Result for *A. spicigera* is $2n=36$ and this is the second count on this taxon in Iran. Our count agrees from the only previous report Rabiee (2001). This group being the only one in the genus (together with the North American endemic section *Tridentatae*, not treated here) which

Fig. 3. Graphic representation of the asymmetry indices of Romero (1986) of the species of *Artemisia* studied.

Fig. 4. Graphic representation of the asymmetry indices of Romero (1986) of the sections of *Artemisia* studied.

lacks dysploidy, with $x=9$ as the unique basic chromosome number.

Section *Artemisia*: Many counts with different results ($2n=16, 18, 24, 36$ and 45) have been published for very common species *A. vulgaris* all over the world (Fedorov 1969, Ornduff 1969, Moore 1973, Goldblatt 1981, Agapova *et al.* 1990), with no correlation between chromosome number and geographical distribution. Most of the counts report $2n=16$ chromosomes, as in the present study. Our count confirms other results from Iran (Rabiee 2001, saedi 2003). *A. chamaemelifolia* is largely distributed in Eurasian mountains: Spanish Sierra Ne- presvada, Cantabrian chain, Pyrenees, Alps, Carpathians, Caucasus and the mountains of Iran (Peccenini 1994). previous studies dealing with the chromosome number of this taxon; from Stockholm, probably from Botanic Garden material, as the species is not wild there (Kawatani and Ohno 1964), Cantabrian chain and Pyrenees (Torrell *et al.* 1999), and Iran (Tavassoli and Derakhshandeh-Peikar 1993, Torrell *et al.* 2001). In all cases the reported chromosome number is $2n=18$. Our results confirm pervious studies for this taxon.

Section *Dracunculus*: The chromosome number $2n=36$ obtained in population of *A. campestris* agrees with other reports of tetraploid level for this taxon (Kawatani and Ohno 1964, Kuzmanov 1993, Oliva and Valle's 1994) for which diploid population have also been reported (Moore 1982, Stahevich and Wojats 1988, Valle's and Torrell 1995).

Many chromosome counts have been published for *A. scoparia*, this widespread Eurasian species, our results for this Iranian populations agree with most of them: Khoshoo and Sobti (1958), Chuksanova *et al.* (1968) from Russian far east, Bhat *et al.* (1974) and Kaul and Bakshi (1984) from Kashmir and other Himalayan regions, Podlech and Bader (1974) from Afghanistan, Maájovsky and Murín (1987) from Slovakia, Kuzmanov (1993) from Bulgaria, Volkova and Boyko (1986) from Russian far east, Krasnikov and Lomonosova (1990) from Siberia, and Tavassoli and Derakhshandeh-Peikar (1993) from Iran. At present study $2n=16$ for *A. scoparia* is confirmed. Torrell *et al.* (2001) stated that a typographic mistake ($2n=18$ is written instead of $2n=16$ in the text, even though the karyograms contain 16 chromosomes) is reproduced by Goldblatt and Johnson (1990), who erroneously report $2n=18$. Nevertheless, there are some other counts indicating a diploid ($2n=18$) or tetraploid ($2n=36$) level based on $x=9$ (Kawatani and Ohno 1964, Kovanda 1972, Yan *et al.* 1989, Qiao *et al.* 1990).

Section *Absinthium*: We found $2n=18$ chromosomes in population of *A. absinthium*. This number agrees with previous reports from Iran like Derakhshandeh-Peikar (1993), Rabiee (2001) and Saedi (2003). Our results confirmed reports from other authors (Kawatani and Ohno 1964, Vallès and Torrell 1995). Our count agrees with many others from different territories (Watanabe 2002 and references therein). This number is shown to be very stable, because it is also the same in many other reports based on plants from different regions.

It is also being noted that Life cycle also correlates with genome size (Bennett 1995). It has been proposed that, within a genus, an increase in nuclear DNA content implies a longer cell cycle, thus annual plants usually have lower DNA amounts than perennial ones (Rees and Narayan 1981). Nagl and Ehrendorfer (1974) reported this tendency in some members of the tribe Anthemideae, including two (one annual and one perennial) *Artemisia* species. This is now confirmed, in this genus, by the annual species *A. scoparia* which shows the lowest TKL amounts of all the present populations, its TKL value is lower than that of the perennial species and TKL strongly correlated with genome size and DNA content.

Two of the seven populations are tetraploid, which occurs frequently in *Artemisia* and is consistent with assessments of many authors (Ehrendorfer 1980, McArthur *et al.* 1981, Oliva and Vallès 1994, Matoba *et al.* 2007) who considered polyploidy as common and one of the main evolutionary mechanisms in plants in general and in the Anthemideae and *Artemisia* in particular. Most of *Artemisia* that colonizes extreme and arid habitats is polyploids. This fact supports the hypothesis that polyploids have more tolerance of extreme environmental conditions (Otto and Whitton 2000).

The high frequency of polyploidy in plants studied here and other research leads us to consider that *Artemisia* is continuously developing genetic and ecological evolutionary mechanisms leading to diversification and speciation, particularly in Asia which is regarded as the main center of diversification of the genus.

Acknowledgements

We are indebted to Y. Asri, K. Saedi, J. Pour Rezaee, and Z. Mehrabanfar N. for their help in the collection of plant material and references. A. Jalili, (RIFR) is thanked for supporting us in the filed and lab work, and Mrs. Rabiee her suggestions, which improved our study.

References

- Agapova, N. D., Arkharova, K. B., Vakhtina, L. I., Zemskova, E. A. and Tarvis, L. V. 1990. Chisla Khoromosom tsvetkoykh rastenii flory SSSR. Aceraceae-Menyanthaceae-Numeri chromosomatum. Nauka, Leningrad, Russia.
- Bennett, M. D. and Leitch, I. J. 1995. Nuclear DNA amounts in angiosperms. *Ann. Bot. (London)*, **76**: 113–176.
- Bhat, B. K., Bakshi, S. K. and Kaul, M. K. 1974. Reports. In: Löve Å, ed. IOPB chromosome number reports, XLVI. *Taxon* **23**: 809.
- Bremer, K. and Humphries, C. J. 1993. Generic monograph of the Asteraceae-Anthemideae. *Bull. Br. Mus. (Nat. Hist.) Bot.* **23**: 71–171.
- 1994. Asteraceae. Cladistics and classification. Portland: Timber Press.
- Chuksanova, N. A., Sveshnikova, L. I. and Alexandrova, T. V. 1968. Materialy k kariologii semeistva Slozhnotsvetnykh/Data on karyology of the family Compositae Giseke. *Citologija* **10**: 198–206.
- Ehrendorfer, F. 1980. Polyploidy and distribution. In *Polyploidy: Biological relevance*. Edited by W.H. Lewis. Plenum Press, New York. pp. 45–60.
- Favarger, C. 1978. Philosophie des comptages de chro- Scimosomes. *Taxon* **27**: 441–448.
- Fedrov, A. A. (editor). 1969. Khromosomnye chisla tsvetkovykh rastenii. Chromosome numbers of flowering plant. Nauka, Leningrad, Russia.
- Fernandez Casas, J. and Susanna, A. 1986. Monografia la section Chamaecyanus Willkomm del genero Centaurea L. Treballs de l'Institut Botanic de Barcelona **10**: 5–174.
- Goldblatt, P. 1981. Index to plant chromosome numbers 1975–1978. *Monogr. Syst. Bot. Miss. Bot. Gard.* **5**.
- and Johnson. 1990. Index to plant chromosome numbers 1986–1987. *Monographs in Systematic botany from the Missouri Botanical Garden*, vol. 30. Missouri Botanical Garden, St. Louis, MO
- Heywood, V. H. Harborne, J. B. and Turner, B. L. 1977. The biology and Chemistry of the Compositae, Academic Press, London, vol. 1, chapter1, pp. 852–888.
- Hu, S. Y. 1965. The compositae of China I. Q.J. Taiwan Mus. **18**: 1–136.
- Kaul, M. K. and Bakshi, S. K. 1984. Studies on the genus *Artemisia* L. in north-West Himalaya with particular reference to Kashmir. *Folia Geobotanica et Phytotaxonomica* **19**: 299–306.

- Kawatani and Ohno. 1964. Chromosome numbers in *Artemisia*. Bull. Natl. inst Hyg. Sci. (Tokyo) **82**: 183–193. (in Japanese)
- Khoshoo, T. N and Sobti, S. N. 1958. Cytology of Indian Species of *Artemisia* [sic]. Nature **181**: 853–854.
- Kitamura, S. 1939. A classification of *Artemisia*. Acta Phytotax. Geobot. **8**: 62–66.
- 1940. Compositae Japonicae. Pars Secunda. Mem. Coll. Sci. Kyoto Univ. 25(3) art. **9**: 286–446.
- Kovanda, M. 1972. Somatic chromosome numbers for some Asteraceae. Rhodora **74**: 102–116.
- Krasnikov, A. and Lomonosova, M. 1990. Chisla khromosom predstavitelei nekotorykh semestv sosudistyykh rastenii flory Novosibirskoi oblasti, I. Chromosome numbers in representatives of some families of vascular plants in the flora of the Novosibirsk region, I. Botanicheskii Zhurnal **75**: 116–11.
- Kuzmanov, B. 1993. Chromosome numbers of Bulgarian angiosperms: An introduction to a chromosome atlas of the Bulgarian flora. Flora Mediterranea **3**: 19–163.
- Levan, A., Fredga, K. and Sandberg, A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.
- Ling, Y. R. 1995a. The New World *Artemisia* L. In: Hind DJN, Jeffrey C, Pope GV, eds. Advances in Compositae Systematics. Kew: Royal Botanic Gardens, 255–281.
- 1995b. The New World *Seriphidium* (Besser) Fourr. In: Hind DJN, Jeffrey C, Pope GV, eds. Advances in Compositae Systematics. Kew: Royal Botanic Gardens, 283–291.
- 1991a. The Old World *Seriphidium* (Compositae). Bull. Bot. Lab. N.-East. For. Inst. **11**: 1–40.
- 1991b. The Old World *Artemisia* (Compositae). Bull. Bot. Lab. N.-East. For. Inst. **12**: 1–108.
- 1982. On the system of the genus *Artemisia* L. and the relationship with its allies. Bulletin of the Laboratory of the North-East Forest Institute **2**: 1–60.
- Májovsky, J., Murín, A. eds. 1987. Karyotaxonomicky pre-h'ad flóry Slovenska. Bratislava: Veda vydavateľ'stvo Slovenskej Akadémie Vied.
- Mabberley, D. J. 1997. The plant-book. A portable dictionary of the higher plants. Cambridge University Press, Cambridge, U.K.
- Matoba, H., Nagano, K. and Hoshi, Y. 2007. The Tendency of Chromosomal Evolution in Some Japanese *Artemisia* using Numerical analysis of Karyotypes. Cytologia **72**: 181–188.
- McArthur, E. D., Pope, C. L. and Freeman, D. C. 1981. Chromosomal studies of the subgenus *Tridentatae* of *Artemisia*: evidence for autopolyploidy. American Journal of Botany **68**: 589–605.
- and Sanderson, S. C. 1999. Cyto geography and chromosome evolution of subgenus *Tridentatae* of *Artemisia* (Asteraceae). Am. J. Bot. **86**: 1775–1754.
- Moore, D. M. 1973. Index to plant chromosome numbers for 1967–1971. Regnum Veg. **90**: 1–539.
- 1982. Flora Europaea. Check-list and chromosome index. Cambridge University Press, Cambridge, U.K.
- Mozaffarian, V. 1998. A Dictionary of Iranian plant names, Farhang Moaser Publishing.
- Nagl, W. and Ehrendorfer, F. 1974. DNA content, heterochromatin, mitotic index, and grown in perennial and annual *Anthemidea* (Asteraceae). Plant Syst. Evol. **123**: 737–740.
- Naseri, H. R., Azarnivand, H. Sefidkon, F. and Asri, Y. 2006. Essential oil composition of *Aretmisia absinthium*, Procciding of Information system in sustainable agriculture, agroenvironment and food technology, Volos, Greece. pp. 672–676.
- Nazarova, E. A. and Pogolian, A. I. 1970. Chisla khromosom ne-Comkotorykh kavkazskikh vidov rastenii. Biologicheskii Zhurnal Armenii **23**: 96–98.
- Oliva M. and Valle's J. 1994. Karyological studies in some taxa of the genus *Artemisia* (Asteraceae). Canadian Journal of Botany **72**: 1126–1135.
- Ornduff, R. 1969. Index to plant chromosome number for 1967. Regnum Veg. **59**: 1–129.
- Otto, S. P. and Whitton, J. 2000. Polyploid incidence and evolution. Annual Review of Genetics **34**: 401–437.
- Peccenini, S. 1994. Distribuzione di *Artemisia chamaemelifolia* Vill. sulle Alpi. Revue Valdôtaine d'Histoire Naturelle. **48** (suppl.): 421–423.
- Podlech, D. and Bader, O. 1974. Chromosomenstudien an Afghanischen Pflanzen II. Mitteilungen der Botanischen Staatssammlung München **11**: 457–488.
- Poljakov, P. P. 1961. Systematic studies in the genus *Artemisia* L. Trudy Ins. Bot. Akad. Nauk. Kazakh. SSR. Alma Acta. **11**: 134–177.
- Qiao, Y. M., Yan, X. X. and Zhang, S. Z. 1990. A study on the chromosomes of 20 species of the genus *Artemisia*. Grassland China **1990**: 24–31.
- Rabiee, M. 2001. Botanical and ecological studies of the species of *Artemisia* in Gillan province, Iran, Ms.C thesis, University of Tehran.
- Rees, H. and Narayan, R. K. J. 1981. Chromosomal DNA in higher plants. Philos. Trans. R. Soc. London B Biol. Sci. **292**: 569–578.
- Romero, Z. C. 1986. A new method for estimating karyotype asymmetry. Taxon **35**: 526–530.

- Saedi, K. 2003. Botanical and ecological studies of the species of *Artemisia* in Azerbaijan province, Ms.C thesis, University of Tehran.
- Schweizer, D. and Ehrendorfer, F. 1983. Evolution of C-band patterns in Asteraceae–Anthemideae. *Biologisches Zentralblatt* **102**: 637–655.
- Siljak-Yakovlev, S. 1996. La dysploidie et l'évolution du caryotype. *Bocconea* **5**: 211–220.
- Solbrig, O. T. 1977. Chromosomal cytology and evolution in the family Compositae. In: Heywood V. H., Harborne, J. B., Turner, B. L., eds. *The Biology and Chemistry of the Compositae*, vol. I. London, New York, San Francisco: Academic Press, 269–281.
- Stahevich, A. E. and Wojats, A. J. 1988. Chromosome numbers of some North American species of *Artemisia* (Asteraceae). *Can. J. Bot.* **66**: 672–676.
- Stebbins, G. L. 1971. Chromosomal evolution in higher plants. E Arnold, London.
- Tavassoli, A. and Derakhshandeh-Peikar, P. 1993. Chromosome numbers of some *Artemisia* L. species from Iran. *Iranian Journal of Botany* **6**: 169–175.
- Torrel, M., Vallès, J., Garcia-Jacas, N., Mozaffarian, V. and Gabrielian, E. 2001. New or rare chromosome counts in the genus *Artemisia* L. (Asteraceae, Anthemideae) from Armenia and Iran. *Botanical Journal of the Linnean Society* **135**: 51–60.
- , Garcia-Jacas, N., Susanna, A. and Vallès, J. 1999. Phylogeny in *Artemisia* (Asteraceae, Anthemideae) inferred from nuclear ribosomal DNA (ITS) sequences. *Taxon* **48**: 721–736.
- Vallès, J. and McArthur, E. D. 2001. *Artemisia* systematics and phylogeny: cytogenetic and molecular insights. In *Proceedings: Shrubland Ecosystem Genetics and Biodiversity*, June 13–15, 2000, Provo, Utah. Proceedings RMRS-P-000. Edited by E. D. McArthur and D. J. Fairbanks. United States Department of Agriculture Forest Service, Rocky Mountain Research Station, Ogden, Utah. In press.
- , Garnatje, T., Garcia, S., Sanz, M. and Korobow, A. 2005. Chromosome numbers in the tribes Anthemideae and Inuleae (Asteraceae). *Botanical Journal of the Linnean Society* **148**: 77–85.
- and Siljak-Yakovlev, S. 1997. Cytogenetic studies in the genus *Artemisia* L. (Asteraceae): fluorochrome-banded karyotypes of five-taxa, including the Iberian endemic species *Artemisia barrelieri* Besser. *Can. J. Bot.* **75**: 595–606.
- and Torrel M. 1995. Reports (552–558). In: Kamari, G., Felber, F. and Garbari, F. (eds.), *Mediterranean chromosome number reports–5*. *Fl. Medit.* **5**: 357–363.
- Volkova, S. A. and Boyko, E. V. 1986. Chisla khromosom nekotorykh vidov semeistva Asteraceae iz iuzhnoi chasti Sovetskogo dalnego vostoka. [Chromosome numbers in some species of Asteraceae family from the southern part of the Soviet far east. *Botanicheskii Zhurnal* **71**: 1693.
- Watanabe, W. 2002. Index to chromosome numbers in Asteraceae. <http://www-asteraceae.cla.kobe-u.ac.jp/index.html>.
- Yan, X. X., Zhang, S. Z., Yan, J. F., Fu, X. Q. and Wang, L. Y. 1989. Chromosome numbers and geographical distribution of 68 species of forage plants. *Grassland China* **1989**: 53–60.
-