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ARTICLE

Cytogenetic variability and new chromosome number reports in *Silene* L. species (Sect. Lasiostemones, Caryophyllaceae)

Masoud Sheidai*, Mehrnoosh Nikoo, Abbaas Gholipour

Faculty of Biological Sciences, GC, Shahid Beheshti University, Tehran, Iran

ABSTRACT Karyotype and meiotic studies were performed in 19 populations of five *Silene* species of the section *Lasiostemones* Boiss., growing in Iran. The species of *S. longipetala*, *S. tenella*, *S. claviformis* and *S. Marschallii* possessed 2n = 2x = 24 chromosome number, while *S. propinqua* populations were diploid and tetraploid with two different base number of x = 10 and 12 (2n = 4x = 40). The results obtained support the earlier report on *S. Marschallii* while the chromosome number of *S. longipetala*, *S. tenella*, *S. claviformis* and *S. propinqua* are new to science. The chromosomes were mainly metacentric and sub-metacentric. The species studied differed significantly in total size of the chromosomes, size of the short arms and the long arms, indicating the role of quantitative genomic changes in the *Silene* species diversification. They also differ in their karyotypic formulae indicating the occurrence of structural changes in their chromosomes. The *Silene* species were placed in 1A, 2A and 1B classes of Stebbins karyotype symmetry which are considered relatively primitive in this system. PCA ordination of the *Silene* species indicated karyotypic distinctness of the species studied. Meiotic analysis showed that Arak population of *S. Marschalii* forms quadrivalents due to the occurrence of heterozygote translocation between two pairs of chromosomes which in turn may increase the amount of genetic variability in the next generation. Unreduced pollen grains were formed in populations of *S. Marschalii* due to multipolar cell formation, while B-chromosomes were observed in some of the species studied.

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The genus *Silene* L. (Caryophyllaceae) is comprised of about 700 species which are distributed throughout the world. They are mostly hermaphrodite although a few species are diocious or gynodioceious (Bari 1973; Greuter 1995). *Silene* species are mostly distributed throughout the northern hemisphere, Europe, Asia and northern Africa. They are annual, biennial, or perennial herbs with the basic chromosome number *x* = (10) 12. The genus *Silene* includes several important weedy species, some very beautiful horticultural plants and some medicinal plants (Swank 1932; Vestal 1952; Oxelman and Lidén 1995).

Several cytogenetic studies have been performed on *Silene* from different parts of the world (Heaslip 1951; Bari 1973; Melzheimer 1978; Markova et al. 2006), while such studies are not available for the *Silene* species growing in Iran. About 110 *Silene* species grow in Iran out of which about 35 species are endemic with very limited geographical distribution (Melzheimer 1980).

Most of the *Silene* species are diploid having 2n = 2x = 24, or 2n = 2x = 20 (Bari 1973), *S. fortunei* is triploid (2n = 3x = 30, Heaslip 1951), some others are tetraploid (2n = 4x = 48) and hexaploid (2n = 6x = 72) and a few species show higher polyploidy level for e.g. 2n = *c.* 96, 120 and 192 (Bari 1973). Moreover 2n = 18 is reported for *S. conica* (Sopova and Sekovski 1982) as well as *S. lacera* (Gvinianidze and Avazneli 1982), 2n = 46 for *S. firma* (Zhang 1994), which make x= 9 and x = 23 along with x= 10 and 12, the known basic chromosome numbers for the *Silene*.

Chowdhuri (1957) placed the *Silene* species in 22 sections but recent molecular studies do not support such sectional classifications particularly for the endemic North American taxa (Oxelman et al. 1997, 2000; Burleigh and Holtsford 2003).

According to Flora Iranica (Melzheimer 1980) the section *Lasiostemones* (Boiss.) is comprised of 10 species, out of which 2 species are endemic. The members of the section *Lasiostemones* are caespitose mountainous plants with perfect flowers arranged in paniculate or racemose inflorescences, possessing usually glabrous calyx and hairy or densely ciliate filaments. The present study reports the karyotypic features of 15 populations of five *Silene* species belonging to the section *Lasiostemones* as well as meiotic analysis of 4 populations of two species growing in Iran for the first time.

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*Corresponding author. E-mail: msheidai@yahoo.com

Table 1. Karyotypic features of the Silene species and population studied.

Sp	Locality	2n	Ploidy level	TL	L	S	L/S	X	TF	KF	A1	A2	ST
1-*Silene longipetala*	Ilam	24	2x	32.18	3.22	1.99	1.62	2.68	42	11m+1sm	0.25	0.14	1A
2-*S. tenella*	Gadook	24	2x	32.18	3.29	1.99	1.65	2.68	42	11m+1sm	0.24	0.14	1B
3- *S. tenella*	Sabalan	24	2x	32.42	3.60	1.70	2.12	2.70	42	12m	0.28	0.20	2A
4- *S. tenella*	Chashm	24	2x	37.10	3.75	2.24	1.67	3.08	42	11m+1sm	0.27	0.18	1A
5- *S. tenella*	Shahdej	24	2x	32.59	3.54	2.00	1.77	2.70	43	12m	0.23	0.15	1A
6-*S. tenella*	Neor	24	2x	26.33	2.53	1.47	1.72	2.19	42	12m	0.26	0.13	1A
7- *S. claviformis*	Soodkooh	24	2x	40.90	4.68	2.70	1.73	3.40	42	11m+1sm	0.31	0.16	1A
8- *S. claviformis*	Payesib	24	2x	40.77	4.48	2.73	1.64	3.39	39	8m+4sm	0.37	0.15	2A
9- *S. Marschallii*	Gadook	24	2x	35.80	3.66	2.35	1.56	2.98	43	12m	0.24	0.24	1A
10-*S. Marschallii*	Damavand	24	2x	38.17	4.28	2.33	1.84	3.10	40	11m+1sm	0.32	0.17	1A
11- *S. Marschallii*	Lavasanat	24	2x	47.26	4.76	2.97	1.60	3.90	39	11m+1sm	0.35	0.14	1A
12- *S. Marschallii*	Bakhtiari	24	2x	36.15	3.91	2.31	1.69	2.95	42	12m	0.26	0.14	1A
13- *S. Marschallii*	Tonekabon	24	2x	37.27	3.92	2.10	1.87	3.10	41	11m+1sm	0.29	0.19	1A
14-*S. Marschallii*	Manjil	24	2x	42.92	4.63	2.16	2.14	3.57	42	12m	0.25	0.19	1B
15- *S. propinqua*	Shajoo	48	4x	--	--	--	--	--	--	--	--	--	--

Abbreviations: TL = Total chromatin length (μm), L = Size of the longest chromosome pair (μm), S = Size of the shortest chromosome pair (μm), X = Mean chromatin length (μm),, TF = Total form percentage, KF = Karyotypic formulae, A1 & A2 = Romero-Zarco indices, ST = Stebbins· symmetry class.

Materials and Methods

Plant material

Karyotype and meiotic studies were performed in fifteen populations of five *Silene* species of the section *Lasiostemones* Boiss., growing in Iran. The species studied are: 1- *Silene longipetala* Vent, 2- *S. tenella* C. A. Mey. (five populations), 3- *S. claviformis* (two populations), 4- *S. Marschallii* C. A. Mey. (eight populations), 5- *S. propinqua* SCHISCHK. (two populations). Meiotic studies could be performed in four populations of *S. Marschallii* and one population of *S. propinqua*. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU).

Cytological studies

For karyotypic studies freshly grown root tips were collected from the seeds of at least ten randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (1-2 hrs.). Squash technique was used for cytological studies and karyotypic details were studied in at least 5 well-prepared metaphase plates as reported earlier (Sheidai and Rashid 2007).

The chromosomes were identified according to Levan et al. (1964), karyotype symmetry was determined according to Stebbins (1971), while other karyotypic parameters like total form percentage (TF %), coefficient of variation (CV) of the chromosome size as well as A1 and A2 indices of Romero-Zarco (1986) were determined (Sheidai and Jalilian 2008).

Meiotic studies were performed on young flower buds collected using minimum 100 metaphase/ diakinesis pollen mother cells (PMCs) and 500 anaphase and telophase cells for data collection (Sheidai and Rashid 2007). Pollen satiability as a measure of fertility was determined by staining minimum 1000 pollen grains with 2% acetocarmine: 50% glycerin (1:1) for about ½ hr. Round. Complete pollens which were stained were taken as fertile, while incomplete, shrunken pollens with no stain were considered as infertile (Sheidai and Rashid 2007).

Statistical analyses

For karyotype analyses, in order to reveal significant difference, the analysis of variance (ANOVA) followed by the least significant difference test (LSD) were performed on the size of chromosomes, size of the long arms and size of the short arms as well as arms ratio among the species and populations studied (Sheidai and Jalilian 2008). Moreover, principal components analysis (PCA) was performed to identify the most variable karyotypic characters. Karyotypic distinctness of the species studied was checked by using ordination plot of principal components analysis (PCA) (Sheidai and Jalilian 2008).

For meiotic analyses, χ^2 test was performed to detect a significant difference in chiasma frequency and chromosome pairing as well as meiotic abnormalities (Sheidai and Rashid 2007). In order to detect significant difference between poten-

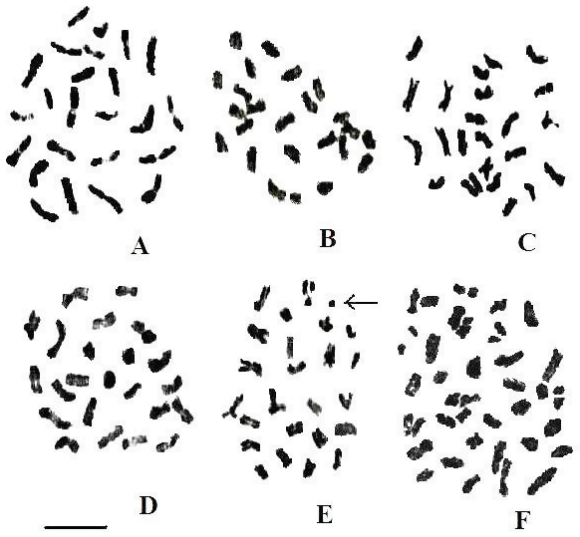

Figure 1. Representative metaphase somatic cells in the *Silene* species studied. A = *Silene Marschalii* Manjil population, B = *S. longipetala* Ilam population. C = *S. tenella* Shahdej population, D = *S. claviformis* Soodkooh population. E = *S. claviformis* Payesib population. F = *S. propinqua* Hamedan population.

tial unreduced pollen grains and the normal (reduced pollens), t-test was performed. Statistical analyses used SPSS ver. 9 (1998) and DARwin ver. 5.0.155 (2006) software.

Results and Discussion

Karyotypic features

Details of karyotypic analyses in the *Silene* species studied are presented in Table 1, Figs. 1-3. The species of *S. longipetala*, *S. tenella*, *S. claviformis* and *S. Marschallii* possessed 2n = 2x = 24 chromosome number, while *S. propinqua* was tetraploid with 2n = 4x = 48 chromosome.

The results obtained support the earlier report on *S. Marschallii* (Nersesian and Goukasian 1995), while the chromosome number of *S. longipetala*, *S. tenella*, *S. claviformis* and *S. propinqua* are new to science.

Among diploid species studied, the size of the longest chromosome varied from 2.53 µm in Neor population of *S. tenella* to 4.76 µm in Lavasanak population of *S. Marschallii* (Table 1), while the size of shortest chromosomes varied from 1.70 µm in Neor population of *S. tenella* to 2.73 µm in Payesib population of *S. claviformis*. The chromosomes were mainly metacentric (m) and sub-metacentric (sm; Table 1).

The highest haploid total chromatin length as well as mean chromosome length occurred in Lavasanat population of *S. Marschallii* (47.26 and 3.90 µm respectively), while the lowest value of the same occurred in Neor population (East Azarbayejan) of *S. tenella* (26.33 and 2.19 µm respectively).

The highest value of chromosomes size variation (CV= 24.00) occured in Gadook population of *S. Marschallii* while the lowest CV (13.00) occured in Neor population of *S. tenella*. The ANOVA and LSD tests revealed a significant differences ($p < 0.05$) for total size of the chromosomes, size of the short arms and the long arms among the species and populations studied, indicating the role of quantitative genomic changes in the *Silene* species diversification.

The *Silene* species studied differ in their karyotypic formulae indicating the occurrence of structural changes in their chromosomes (Table 1). Total form percentage (TF%) varied from 39 in Payesib population of *S. claviformis* to 43 in Shahdej population of *S. tenella* and Gadook population of *S. Marschallii* (Table 1); a higher value of TF% indicates the presence of relatively more symmetrical karyotype. The *Silene* species were placed in 1A, 2A and 1B classes of Stebbins karyotype symmetry which are considered relatively primitive in this system.

PCA analysis (data not given) shows that the first three components comprise about 79% of the total variation. In the first component with about 64% of total variance, the size of the short arms and long arms as well as total length of the chromosomes are the most variable characters and possessed the highest correlation with this component ($r > 0.80$). Moreover the ratio of long arm to short arm (L/S) of the chromosome pair number 6 possessed a high correlation ($r = 0.65$). In the second component with about 10% of total variance, L/S ratio of the chromosome pair numbers 11 and 12 possessed the highest correlation ($r > 0.50$). Therefore it seems that along with significant changes in the size of the chromosomes arms, the L/S ratio of 3 pairs of chromosomes (*i.e.* chromosome pair numbers 6, 11 and 12) have changed during the karyotype differentiation in the *Silene* species studied. Such a result supports the results of ANOVA stated earlier.

PCA ordination of the *Silene* species (Fig. 3) shows that almost the populations of each species form a distinct group. This is particularly true for *S. claviformis* as two populations of this species are placed separate from the other species. The populations of *S. Marschallii* are placed in the lower left corner of the PCA plot and the populations of *S. tenella* occupy the lower and upper part of the right side of the plot (Fig. 3), indicating karyotypic distinctness of the *Silene* species.

Chromosome pairing and segregation

Data with regard to chiasma frequency and distribution as well as chromosome pairing are provided in Table 2, Fig. 4. The populations of *S. Marschalii* showed 2n = 2x = 24 chromosome number while (Fig. 4A-D) while, Orumiyeh population of *S. propinqua* showed 2n = 40 chromosome number (Fig. 4E). Considering 2n = 4x = 48 chromosome number obtained in karyotypic study of *S. propinqua*, this species possesses two polyploidy levels of 2x and 4x and the occurrence two different basic number of x = 10 and 12. There are also other

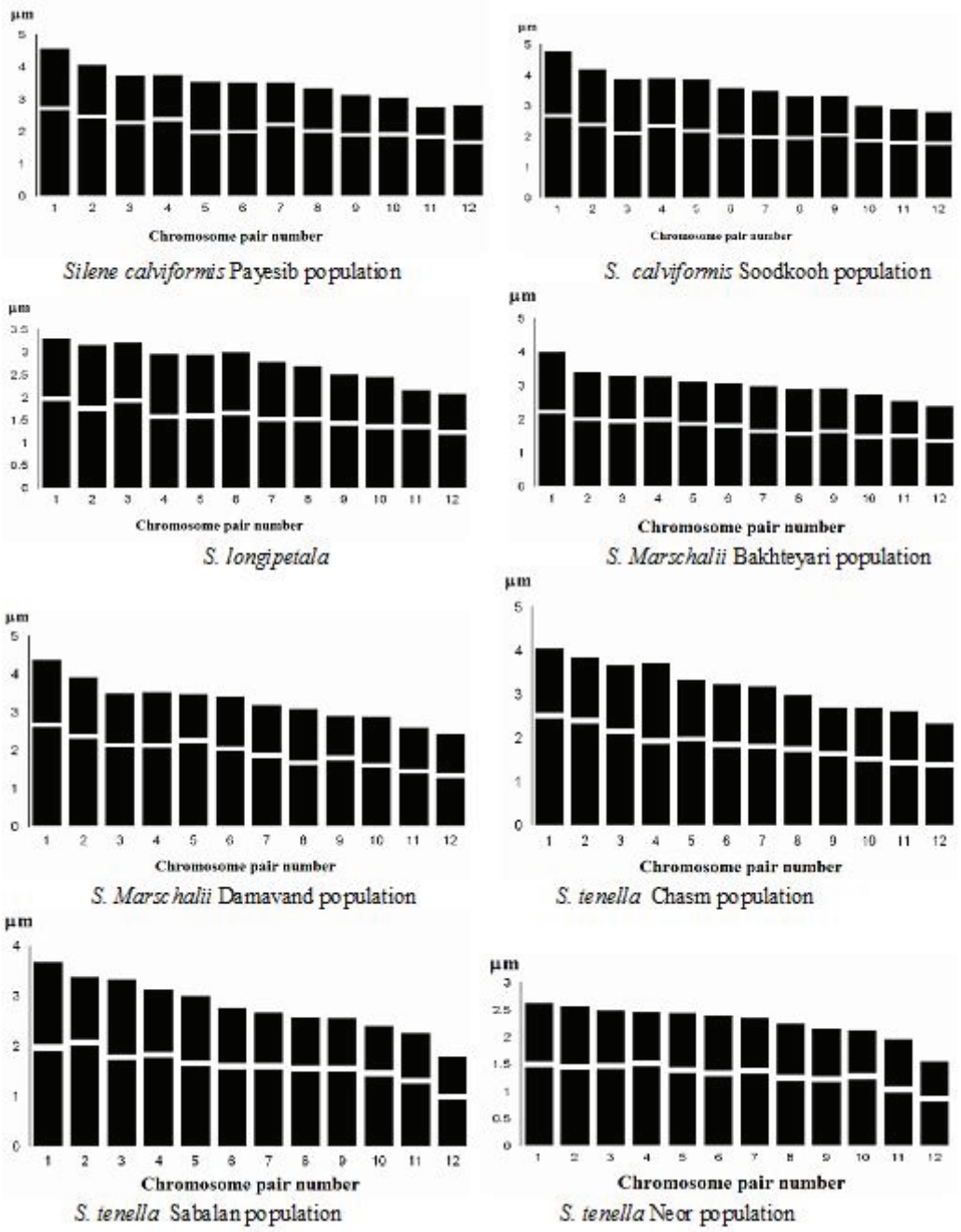

Figure 2. Representative idiograms of the *Silene* species.

Silene species with two or more different base numbers like *S. conica* with 2n = 2x = 18, 20 and 24 (Van Loon and Snelders 1979; Sopova and Sekovski 1982; Van Loon 1982) and *Silene lacera* with 2n = 2x = 18 and 24 (Gagnidze and Gviniashvili 1997; Gvinianidze and Avazneli 1982).

Among three populations of *S. Marschalii* studied, the highest value of total and intercalary chiasmata occurred in Arak population (22.50 and 3.43 respectively), while the lowest value of the same occurred in Dashtelar population (17.26 and 1.33). Arak population of *S. Marschalii* also possessed the highest mean value of ring bivalents (8.87), while Dashtelar population possessed the highest mean value of rod bivalents (5.41).

Although S. Marschalii populatins studied are diploid and are expected to form only bivalents in metaphse of meiosis-I, Arak population formed 1-2 quadrivalents (Table 2, Fig. 4D),

Table 2. Chiasma frequency and chromosomes pairing in *Silene* species studied.

Sp	Locality	2n	TX	IX	TOX	RB	ROD	I	IV
S. Marschallii	Dashtelar	24	15.83	1.33	17.26	6.00	5.41	1.16	0.00
S. Marschallii	Zanjan	24	19.33	1.33	20.66	8.33	3.20	0.93	0.00
S. Marschallii	Khansar	24	18.13	1.97	20.22	8.00	3.72	0.36	0.00
S. Marschallii	Arak	24	19.12	3.43	22.55	8.87	3.11	0.00	0.01
S. propinqua	Orumiyeh	40	26.66	4.83	31.49	13.64	6.14	0.33	0.02

Abbreviations: TX = Mean number of terminal chiasmata, IX = Mean intercalary chiasmata, TOX = Mean total chiasmata, RB = Mean number of ring bivalents, ROD = Mean number of rod bivalents, I = Mean number of univalents, IV = Mean number of quadrivalents.

Table 3. Meiotic abnormalities, pollen fertility and size of pollen grains in *Silene* species studied.

Sp	Locality	L1%	L2%	MST%	AST%	PF%	NP (µm)	2NP (µm)
S. Marschallii	Dashtelar	2.2	4.2	0.0	0.0	98.9	19.78	29.10
S. Marschallii	Khansar	5.0	6.0	0.0	4.0	98.0	27.80	48.28
S. Marschallii	Zanjan	3.0	0.0	5.0	5.0	98.0	20.10	30.75
S. propinqua	Orumiyeh	1.0	1.0	0.0	0.0	99.0	--	--

Abbreviations: L1 & L2 = Laggard chromosomes in anaphase-I & II, MST = Metaphase cells showing stickiness, AST = Anaphase cells showing stickiness, PF = Pollen fertility, NP = Mean value of the size of normal (reduced) pollen grains, 2NP = Mean value of the size of potential unreduced pollen grains.

indicating the occurrence of heterozygote translocations between two pairs of chromosomes. Such chromsomal structural changes may increase the amount of genetic variability in the gametes by forming new genetic linkage groups which may be used for adaptation to adverse environmental conditions.

Tetraploid population (Orumiyeh) of *S. propinqua* formed 1-2 quadrivalents (Table 2, Fig. 4E) as expected which may be to its autotetraploid nature. χ^2 test did not showed a significant difference for chiasma frequency and chromosome pairing among *Silene* species and poopulations studied indicating that no significant change has occurred in the number genes controlling chromosome pairing.

Variation in chiasma frequency and localization is genetically controlled (Quicke 1993) and has been reported in populations of different species (Rees and Jones 1977). Such a variation in the species and populations with the same chromosome number is considered as a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (Rees and Jones 1977).

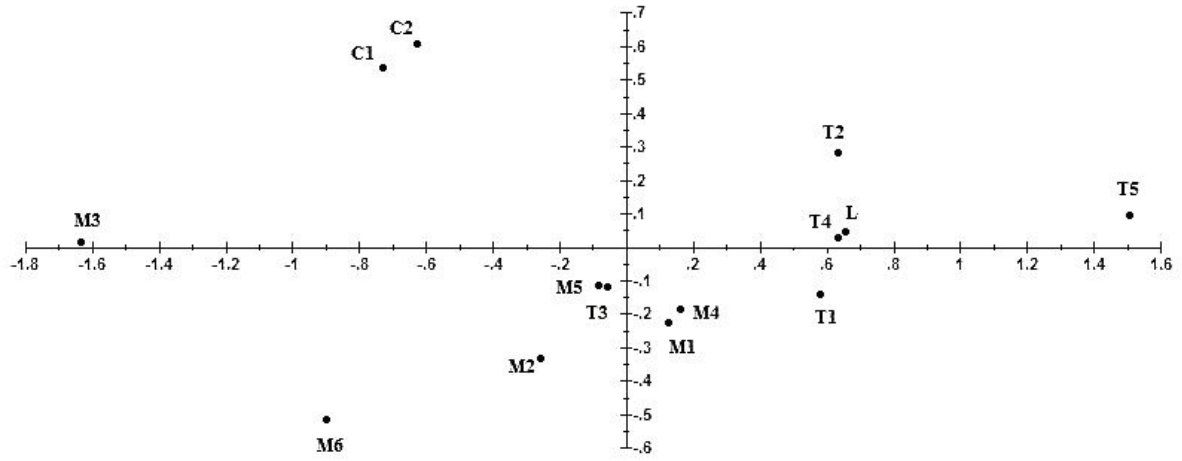

Figure 3. PCA ordination of *Silene* species based on karyotypic data. Species and populations abbreviations: C1 & C2 = Soodkooh and Payesib populations of *S. claviformis*, T1-T5 = Gadook, Sabalan, Chashm, Shahdej and Neor populations of *S. tenella*, M1-M6 = Damavand, Lavasanat, Bakhtiari, Tonekabon and Manjil populations of *S. Marschalii*, L = Ilam population of *S. longipetala*.

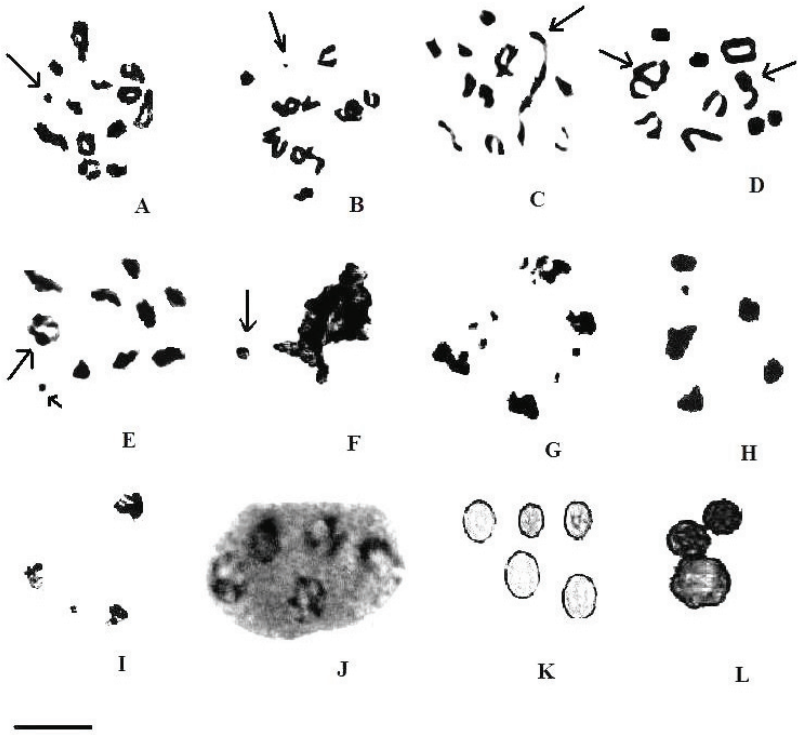

Figure 4. Representative meiotic cells in the *Silene* species studied. A & B = Meiocytes in Khansar and Arak populations of *Silene Marschalii* showing B-chromosome (arrow) respectively. C & D = Meiocyte in Arak population of *S. Marschalii* showing heterozygote translocation (arrow). E = Meiocyte in *S. Propinqua* showing quadrivalent (bigger arrow) and B-chromosome (smaller arrow). F = Meiocyte in Dashtelor population of *S. Marschalii* showing chromosomes clumping and B-chromosome (arrow). G = Telophase-II laggards in meiocyte of Khansar population of *S. Marschalii*. H = A pentaploar cell showing Laggard chromosome in Arak population of *S. Marschalii*. I = A triploar cell showing laggard chromosome in Khansar population of *S. Marschalii*. J = A pentaploar cell in Zanjan population of *S. Marschalii*. K & L = Potential unreduced (2n) pollen grain (bigger size pollen) in Zanjan and Arak populations of *S. Marschalii* respectively. Scale bar = 10 μm.

Almost in all the populations studied, laggard chromosomes and chromosomes stickiness were observed during anaphase I and II (Table 3, Fig. 4F-I). The sticky chromosomes occurred from early stages of prophase to the final stages of meiosis. The number of chromosomes involved in stickiness varied from two to many forming a complete clumping of the chromosomes (Table 3, Fig. 4F). The highest percentage of anaphase-I laggards occurred in Khansar population of *S. Marschallii* (5.00), while the highest value of the same occurred in Orumiyeh population of *S. propinqua* (1.0).

χ^2 test showed a significant difference for the percentage of chromosome stickiness and laggards among the species and populations studied. Genetic and environmental factors as well as genomic-environmental interaction have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao 1996; Baptista-Giacomelli et al. 2000).

Multipolar cells were observed (Fig. 4, H-J) in populations of *S. Marschalii* which may be due to spindle abnormalities. Such meiotic abnormalities may lead to the formation of abnormal tetrads and pollen grains, the occurrence of aneuploidy condition as well as unreduced (2n) pollen formation (Villeux 1985; Nirmala and Rao 1996). Pollen fertility ranged from 98.00-99.00% in the populations studied (Table 3). A little reduction in pollen fertility observed may be due to meiotic abnormalities obtained.

Unreduced pollen grain formation

The occurrence of large pollen grains (possibly 2n pollen grains) was observed along with smaller (normal) pollen grains in 3 populations of *S. Marschallii* (Fig. 4K, L). The large pollen grains comprised about 1% of pollen grains in these populations.

The mean diameter of normal (reduced) pollen grains ranged from 19.78 μm to 27.80 μm while, the mean diameter of unreduced pollen grains ranged from 29.10 μm to 48.28 μm. T-test analysis revealed a significant difference ($p < 0.001$) for the size between the larger sized pollen grains and smaller sized pollen grains. The presence of giant pollen grains has been used as an indication of the production of 2n pollen (Vorsa and Bingham 1979, Bertagnolle and Thomson 1995).

Unreduced gametes are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Villeux 1985), which has been considered as the major route to the formation of naturally occurring polyploids. Different cytological mechanisms are responsible for the production of 2n gametes (Bertagnolle and Thomson 1995). The occurrence of multipolar cells and irregularities in anaphase segregation of the chromosomes might be considered as the possible mechanisms of unreduced pollen grain formation in the *S. Marschalii* populations studied. To our knowledge this is the first report on the occurrence of unreduced pollen grains in the genus *Silene*.

B-chromosomes

B-chromosomes (Bs) of 0-1 were observed in Payesib population of *S. claviformis* (Fig. 1E), Arak, Dashtelor and Khansar populations of *S. Marschallii* (Figs. 4A-B, F) as well as Orumiyeh population of *S. propinqua* (Fig. 4F). The Bs observed were much smaller than the A-chromosomes, round in shape and did not pair with the A-chromosomes. B-chromosomes are accessory chromosomes occurring in more than 1300 species of Plants and almost 500 species of animals (Camacho et al. 2000). It seems that B-chromosomes are of limitted occurrence in the *Silene* and have been reported in *Silene ciliata* (0-2, Vuillemin 1992) and *S. maritime* (0-15, Cobon 1976; Cobon and Murray 1983). The B-chromosomes when present in high number affect negatively the growth and vigor of the plants, while in low number may benefit the plant possessing them.

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