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Cytological study the genus *Arenaria* L. (Caryophyllaceae)

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Abstract — Karyotype and meiotic studies were performed in 16 populations of seven *Arenaria* species growing in Iran. All the species studied showed 2n = 2x = 22 chromosome number supporting the earlier report on *A. persica*, *A. insignis* and *A. gypsophiloides* while the chromosome numbers of *A. polycnemifolia, A. zargariana, A. szowitsii and A. minutissima* are new to science. The chromosomes were mainly metacentric and submetacentric. 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 *Arenaria* species diversification. They also differed in their karyotype formulae indicating the occurrence of structural changes in their chromosomes. Meiotic analysis showed quadrivalent formation in Moorchegan population of *A. persica* and Kandovan population of *A. insignis* possibly 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. Cytomixis led to the formation of unreduced pollen grains in Mishoodagh population of *A. gypsophiloides* var. *glabra,* while B-chromosomes were observed in *A. insignis* and *A. polycnemifolia* species.

Key words: cytology, *Arenaria*.

INTRODUCTION

The family Caryophyllaceae includes worldwide 86 genera and approximately 2100 species (Trigas *et al*. 2007). The genus *Arenaria* L. (subfamily: Alsinoideae, family: Caryophyllaceae) consists of about 306 species mainly distributed in Eurasia, America and northern Africa (Williams 1897; Zhou 1996). According to Flora Iranica (Rechinger 1988), 17 annual and perennial species and two varieties of *Arenaria* comprises grow in Iran which have been placed in two subgenera of *Arenaria* and *Eremogone*. Some of the species are endemic for Iran. Most of the species found in high altitude in North, West, Northwest and Center of the country.

Several cytological studies exist on *Arenaria* species from other parts of the world (Hariman and Mccormick 1963; Favarger *et al*. 1979; Aryavand and Favarger 1980; Guardia *et al*. 1982; Contadriopoulos and Favarger 1983; Corrias 1983; Galland 1988; Celebioglu and Favarger 1989; 1993; Runemark 1996; Chambers *et al*. 1998; Nieto-Feliner 2000; Castro and Rossello 2005), indicating the occurrence of 2n = 14, 16, 20, 22, 24, 30, 34 and 36 in the genus. However, although recently some cytological studies have been reported on other Caryophyllacea species of Iran (Sheidai *et al*. 2008; Gholipour and Sheidai 2010), no report is available on *Arenaria* species. Therefore the present study reports karyotype and meiotic characteristics of *Arenaria* species and populations of Iran considering the species somatic chromosome number, karyotype symmetry and chromosome pairing for the first time.

MATERIALS AND METHODS

Plant material - Karyotype and meiotic studies were performed in sixteen populations of *Arenaria* species growing in Iran. The species studied are: 1 - *Arenaria gypsophiloides* L. var.

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gypsophiloides (one population); 2 - *A. gypsophiloides* L. var. *glabra* FENZEL. (one population); 3 - *A. insignis* LITW. (two populations); 4 - *A. minutissima* RECH. F. & ESFAND. (one population); 5 - *A. persica* BOISS. (five populations); 6 - *A. polycnemifolia* BOISS. (two populations); 7 - *A. szowitsii* Boiss. (two populations); 8 - *A. zargariana* Parsa. (one population).

Meiotic studies could be performed in *A. insignis, A. persica, A. polycnemifolia*, *A. gypsophiloides* var. *gypsophiloides* and *A. gypsophiloides* var. *glabra*. The voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU) and central herbarium of botanical garden of Iran (TARI).

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.) and fixed in ethanol: acetic acid (3:1) for 24 hrs. The fixed tips were then washed thoroughly in distilled water and macerated in 60°C 1N HCl for about 5 min. Squash technique was used for cytological studies with 2% aqueous aceto-orcein as the stain. The somatic chromosome number and karyotypic details were studied in at least 5 well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured by Image Tools3 software (SHEIDAI and RASHID 2007).

The chromosomes were identified according to LEVAN *et al*. (1964), karyotype symmetry was determined according to STEBBINS (1971), while other karyotype 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.

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 - In order to reveal significant difference in the size of chromosomes, chromosome arms and their ratios, the analysis of variance (ANOVA) followed by the least significant difference test (LSD) were performed among the species and populations studied (SHEIDAI and JALILIAN 2006). Moreover, principal components analysis (PCA) was performed to identify the most variable karyotypic characters. Karyotypic distinctness of the species studied was checked by using different clustering methods ordination plot of principal components analysis (PCA) (SHEIDAI and JALILIAN 2006). 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). Statistical analyses used SPSS ver. 9 (1998) and DARwin ver. 5.0.155 (2006) software.

RESULTS

Karyotype analysis - All the species studied possessed $2n = 2x = 22$ chromosome number (Table 1, Figs. 1 & 2.). The size of the longest chromosome varied from 3.24 µm in Kandovan population of *A. persica* to 5.55 µm in Moorchegan population in the same species (Table 1). The size of shortest chromosomes varied from 1.42 µm in Sorkhehesar population of *A. zargariana* to 3.04 µm in Moorchegan population of *A. persica*. The chromosomes were mainly metacentric (m) and sub-metacentric (sm) (Table 1).

The highest values for total haploid chromosome length and the mean chromosome length occurred in Moorchegan population of *A. persica* (46.20 & 4.20 µm respectively), while the lowest value of the same occurred in Kandovan population of *A. persica* (26.49 & 2.40 µm respectively). The highest value of chromosomes size variation (CV = 83.00) occurred in Moorchegan population of *A. persica* while the lowest CV (64.00) occurred in Sorkhehesar population of *A. zargariana*. The ANOVA and LSD tests revealed a significant difference ($p < 0.05$) for total size of the chromosomes, size of the short arms and the long arms as well as chromosomes arms ratios among the species and populations studied.

Pearson correlation determined showed a positive significant correlation between the mean chromosome length and the size of the short arms and the long arms of the chromosomes ($r > 0.80$, $p < 0.05$).

The *Arenaria* species studied differed in their karyotypic formulae (Table 1). Total form percentage (TF%) varied from 37 in Ghooshchy population of *A. szowitsii* to 44 in Chaharbagh population of *A. polycnemifolia*. The *Arenaria* species were placed in 1A, 2A and 1B, 2B classes of STEBBINS' karyotype symmetry while, Kallar

population of *A. minutissima* and Ghooshchy population of *A. szowitsii* occupied 2B class of STEBBINS' classification. Among the species placed in 1A class, *A. polycnemifolia* shows a higher value of A1 index (0.74) of ROMERO-ZARCO.

PCA analysis of karyotype data (data not given) shows that the first three components comprise about 71% of the total variation. In the first component with about 59% 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 ($r > 0.90$). Moreover the total lengths of chromosome pair numbers 6 and 8 possessed a high correlation ($r = 0.98$ & $r = 0.97$). In the second component with about 6.8% of total variance, L/S ratio of the chromosome pair numbers 7 and 10 possessed the highest correlation ($r > 0.50$).

Chromosome pairing and segregation - The species studied was diploid and possessed $2 = 2x = 22$ chromosome number (Table 2, Fig. 1) supporting the karyotype results. *A. persica* studied showed the highest value of total and intercalary among the species and population studied (1.62 & 0.21 respectively). Two populations of *A. polycnemifolia* studied differed significantly ($p < 0.05$) in the mean values of total and terminal chiasmata and the mean values of ring bivalents. Although *A. persica* and *A. insignis* populations studied are diploid and are expected to form only bivalents in metaphase of meiosis-I, quadrivalents were formed in Moorchegan population of *A. persica* and Kandovan population of *A. insignis* (Fig. 1, G).

B-chromosomes (Bs) of 0-2 were observed in *A. insignis* and *A. polycnemifolia* (Fig. 1, B, F & H). Due to the low number of meiocytes showing presence of B-chromosomes in *A. insignis*, their effects on chiasma frequency and chromosome associations could not be worked out.

Chromatin/chromosome migration occurred in different directions from early prophase to telophase-II in almost all *Arenaria* species studied (Table. 3, Fig. 1, J). The highest percentage of cytomixis occurred in Kandovan population of *A. polycnemifoli.* Cytomixis led to the formation of aneuploid cells both with extra chromosomes and lost chromosomes (Fig. 1, J & K), which in turn might be responsible for infertile pollen grains obsserved in these species.

Almost in all the populations studied, chromosomes stickiness and laggard chromosomes were observed during metaphase, anaphase I and telophase I (Table 3, Fig. 1, I). The sticky chromosomes were observed from early stages of prophase to the final stages of meiosis. The number of chromosomes involved in stickiness varied from two to many, often forming a complete clumping of the chromosomes. The highest percentage of metaphase stickiness occurred in Kandovan population of *A. insignis* (12.70), while Shahmirzad population of the same species showed no metaphase stickiness. Anaphase-I stickiness occurred only in Shahmirzad population of *A. polycnemifolia*. χ^2 test showed significant difference ($p<0.05$) for cytomixis and stickiness among the species and populations studied indicating their genetic differences (BAPTISTA-GIACOMELLI *et al.* 2000; SHEIDAI *et al.* 2003).

The highest percentage of anaphase-I laggards occurred in Mishoodagh population of *A. persica* (9.09), while the lowest value of the same occurred in Kandovan population of *A. insignis* (1.40). A high percentage in telophase I laggards

TABLE 1 — Karyotype features of the *Arenaria* species and population studied.

Species	Locality	2n	TL	L	S	TF%	ST	A1	A2
A. minutissima	Kallar	22	2.53	3.58	1.65	43.31	2B	0.76	0.72
A. persica	Kallar	22	2.77	3.35	1.87	42.98	2A	0.70	0.82
A. persica	Alvand	22	3.83	4.99	2.42	41.86	1B	0.73	0.80
A. persica	Kandovan	22	2.41	3.24	1.79	42.60	2A	0.75	0.82
A. persica	Moorchegan	22	4.19	5.55	3.03	41.88	1A	0.72	0.83
A. polycnemifolia	Chaharbagh	22	3.23	4.51	2.18	44.66	1A	0.73	0.82
A. szowitsii	Ghooshchy	22	2.76	3.88	1.78	37.38	2B	0.64	0.71
A. szowitsiI	Mishoodagh	22	2.63	3.95	1.65	39.58	1B	0.67	0.71
A.zargariana	Sorkhehasar	22	2.26	3.53	1.41	39.80	1B	0.67	0.63

Abbreviations: TL = Total chromatin length (µm), L = Size of the longest chromosome pair (µm), S = Size of the shortest chromosome pair (µm), X = Mean chromosome length (µm), TF = Total form percentage, KF = Karyotype formulae, ST = STEBBINS' symmetry class, A1 & A2 = ROMERO-ZARCO indices.

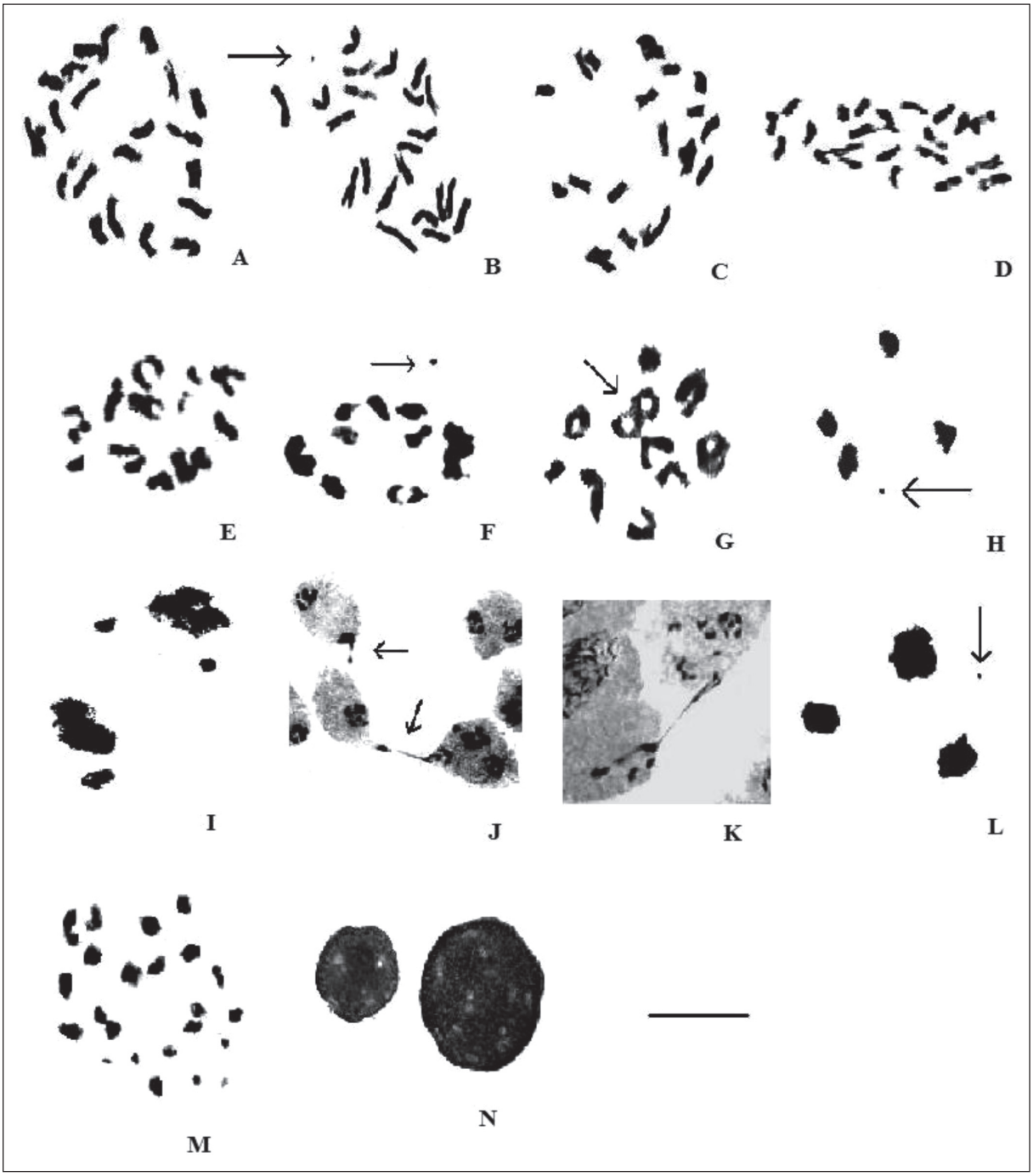

Fig. 1 — Representative somatic and meiotic cells in the *Arenaria* species studied. A-D = Somatic cells showing 2n = 22 in Kallar population of *Arenaria persica*, Chaharbagh population of *A. polycnemifolia*, Ghooshchy population of *szowitsii* and Kallar population of *A. minutissima* respectively. E = Metaphase cell showing bivalents in Shahmirzad population of *A. insignis*, F = Metaphase cell showing B-chromosome (arrow) in Chaharbagh population of *A. polycnemifolia*, G = Metaphase cell showing quadrivalent (arrow) in Kandovan population of *A. insignis*, H = Telophase-II cell showing B-chromosome (arrow) in Moorchegan population of *A. persica*, I = Laggard chromosomes in Moorchegan population of *A. persica*, J & K = cytomixis (arrow indicates chromosome migration) in *A. gypsophyloides*, L = Tripolar cell in Chaharbagh population of *A. polycnemifolia*, M & N = Unreduced meiocyte and unreduced pollen grain (larger sized pollen) in *A. gypsophyloides* respectively. Scale bar = 10 µm.

occurred in Moorchegan population of *A. persica* (10.0).

Multipolar cells were observed in Shahmirzad population of *A. polycnemifolia* and Kandovan population of *A. insignis* (Table 3, Fig. 1, L). Pollen fertility ranged from 97.30-99.00% in the populations studied (Table 3). χ^2 test showed a significant difference ($p < 0.05$) for the laggard chromosomes and multipolar cells among the species and populations studied.

The occurrence of large pollen grains (possibly 2n pollen grains) was observed along with smaller (normal) pollen grains in Mishoodagh population of *A. gypsophiloides* var. *glabra* (Fig. 1, N). The large pollen grains comprised about 5% of pollen grains in this population. Several meiocytes showed the presence of double chromosome number in *A. gypsophiloides* (Fig. 1, M).

DISCUSSION

The somatic chromosome numbers reported here support the earlier report on *A. persica* (ARYAVAND and FAVARGER 1980), and report the chromosome number of *A. minutissima*, *A. polycnemifolia*, *A. szowitsii* and *A. zargariana* for the first time. Significant differences obtained for the total size of the chromosomes, size of the short arms and the long arms as well as chromosomes arms ratios among the species and populations studied, indicate the role of quantitative genomic changes in the *Arenaria* species diversification, while the positive significant correlations obtained between the mean chromatin length and the size of the short arms and the long arms of the chromosomes indicate the occurrence of quantitative changes in the chromatin material during *Arenaria* species diversification and that such changes have been accompanied with the changes in the size of both long arms and short arms of the chromosomes. This is also supported by differences in the karyotype formulae of the species studied.

A higher value of TF% in Chaharbagh population of *A. polycnemifolia* indicates the presence of relatively more symmetrical karyotype in this species. The *Arenaria* species seems to have a relatively symmetrical karyotypes as they occupy 1A, 2A and 1B, 2B classes of STEBBINS', these karyotype classes are considered relatively primitive in this system.

The results of PCA analysis of karyotype data indicates that along with significant changes in the size of the chromosomes arms, the L/S ratio of chromosome pair numbers 7 and 10 have changed during the karyotype differentiation in the *Arenaria* species studied, supporting the results of ANOVA stated earlier. All these results indicate the role of both quantitative and qualitative changes in the genome during *Arenaria* species diversification.

Variation in chiasma frequency and localization observed among the species and populations studied is suggested to be 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).

Quadrivalent formation in metaphase of meiosis-I in two species of *A. persica* and *A. insig-*

TABLE 2 — Chiasma frequency and chromosomes pairing in *Arenaria* species studied.

Species	Locality	2n	TX	IX	TOX	RB	RD	I	IV
A. gypsophyloides var. *gypsophiloides*	Sahand	22	27.06	3.30	30.58	7.04	9.46	5.50	0.00
A. gypsophyloides var. *glabra*	Mishoodagh	22	31.68	0. .44	32.12	7.92	8.80	5.28	0.00
A. polycnemifolia	Kandovan	22	20.46	1.32	22.00	0.66	8.58	12.76	0.00
A. polycnemifolia	Shahmirzad	22	34.10	0.00	34.10	10.56	8.80	1.54	0.00
A. insignis	Kandovan	22	33.00	0.00	33.00	10.56	10.34	0.44	0.22
A. insignis	Shahmirzad	22	29.92	0.22	30.14	7.70	13.64	5.94	0.00
A. persica	Moorchegan	22	30.80	4.62	35.64	7.92	8.80	4.40	0.44

Abbreviations: TXN = Mean number of terminal chiasmata, IX = Mean number of intercalary chiasmata, TOX = Mean number of total chiasmata, RB = Mean number of ring bivalents, RD = Mean number of rod bivalents, IN = Mean number of univalents, IV = Mean number of quadrivalents.

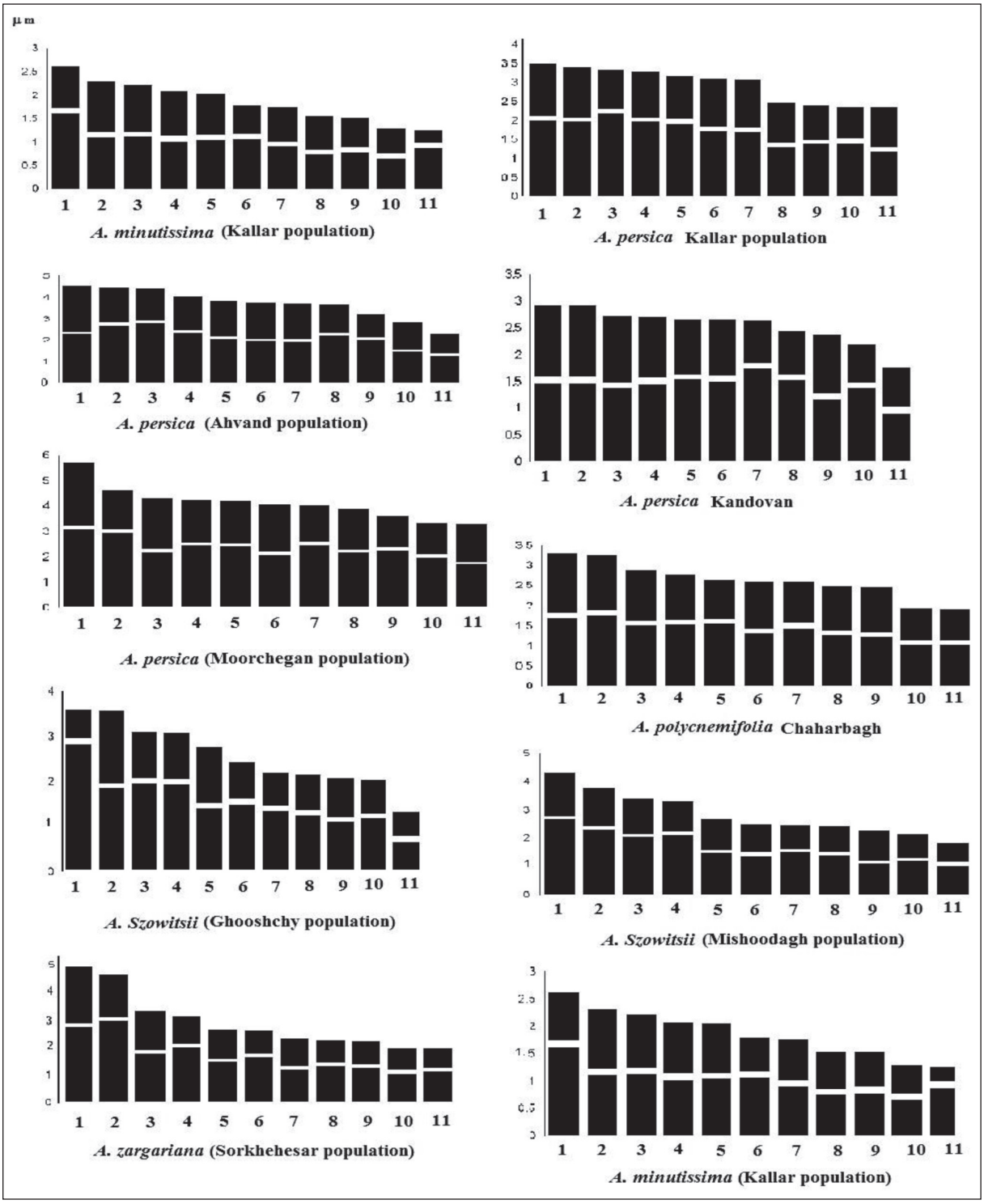

Fig. 2 — Idiograms of the studied *Arenaria* species.

nis may indicate the occurrence of heterozygote translocations between two pairs of chromosomes. Such chromosomes 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. The occurrence of heterozygote translocation in these species

further support our karyotype analysis showing the change in the arm ratios of the chromosomes in *Arenaria* species studied.

It seems that B-chromosomes are of limitted occurrence in the genus *Arenaria* and have been reported in *A. rotundifolia* (ARYAVAND and FAVARGER 1993). B-chromosomes are accessory chromosomes reported in more than 1300 species of Plants and almost 500 species of animals (CAMACHO *et al.* 2000). The Bs observed were much smaller than the A-chromosomes, round in shape and did not form any association with them. These 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.

Cytomixis and chromosome migration observed in the *Arenaria* species studied may be considered for reduction in pollen fertility observed. Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originated from the pre-existing system of plasmodesmata formed within the tissues of the anther. The plasmodesmata become completery obstructed by the deposition of callose, but in some cases they still persist during meiosis and increase in size forming conspicuous inter-meiocytic connections or cytomictic channels that permit the transfer of chromosomes. Chromosome migration may also occur through cell wall dissolution among the neighboring meiocytes and forming syncyte (FALISTOCCO *et al.* 1995).

Cytomixis is not considered to be of great evolutionary importance, but it may lead to production of aneuploid plants (SHEIDAI *et al.* 2003; SHEIDAI and FADAEI 2005), or result in the production of unreduced gametes, as reported in several grass species (FALISTOCCO *et al.* 1995; SHEIDAI and NOUROOZI 2005; SHEIDAI and BAGHERI-SHABASTARI 2007). Unreduced gamete formation is of evolutionary importance as it can lead to the production of plants with higher ploidy levels.

χ^2 test showed significant difference ($p<0.05$) for cytomixis, chromosome stickiness, laggard chromosomes and multipolar cell formation among the species and populations studied indicating their genetic differences (BAPTISTA-GIACOMELLI *et al.* 2000; SHEIDAI *et al.* 2003). Genetic and environmental factors (NIRMALA and RAO 1996), as well as genomic-environmental interaction (BAPTISTA-GIACOMELLI *et al.* 2000), have been considered as the reason for chromosome stickiness in different plant species, and this may be true for the *Arenaria* species studied here as well. Such meiotic abnormalities may lead to the formation of abnormal tetrads and pollen grains and the occurrence of aneuploidy condition as well as unreduced (2n) pollen formation (VILLEUX 1985; NIRMALA and RAO 1996). Pollen fertility ranged from 97.30-99.00% in the populations studied (Table 3). A little reduction in pollen fertility observed may be due to meiotic abnormalities

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). Infact the occurrence of meiocytes with double the chromosome number in *A. gypsophiloides* further supports such suggestion. The occurrence of cytomixis and migration of chromosomes from one meiocyte to the neighboring cell and failure of chromosome movement to the poles may be considered as the possible mechanisms of unreduced meiocyte formation in this species.

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

TABLE 3 — Meiotic abnormalities, pollen fertility and size of pollen grains in the studied *Arenaria* species.

Species	Locality	PCY	MCY	AL1	TL	MST	AST	TR	PF
A. gypsophyloides var. glabra	Mishoodagh	0.00	7.57	5.88	0.00	4.86	0.00	0.00	98.9
A. polycnemifolia	Kandovan	7.69	12.00	0.00	0.00	8.00	0.00	0.00	98.0
A. polycnemifolia	Shahmirzad	3.44	11.11	8.33	0.00	5.55	81.66	14.28	98.8
A. insignis	Kandovan	0.00	5.00	1.40	1.38	36.70	0.00	12.90	97.3
A. insignis	Shahmirzad	0.00	3.39	0.00	0.00	0.00	0.00	0.00	98.9
A. persica	Moorchegan	0.00	0.00	9.09	10.00	5.00	0.00	0.00	99.0

Abbreviations: PCY = Cytomixis in Pachytene, MCY = Cytomixis in metaphase, AL1 = Laggard chromosomes in anaphase-I, TL1 = Laggard chromosomes in telophase-I, MST = Metaphase cells showings Stickiness, TR = Tripolar cell, AST = Anaphase cells showing stickiness, PF = Pollen fertility (all values in percentage).

major route to the formation of naturally occurring polyploids. Different cytological mechanisms are responsible for the production of 2n gametes (BERTAGNOLLE and THOMSON 1995). Cytomixis as well as the occurrence of multipolar cells might be considered as the possible mechanisms of unreduced meiocytes and pollen grain formation in the *A. gypsophiloides*. To our knowledge this is the first report on the occurrence of unreduced pollen grains in the *Arenaria*.

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