CYTOLOGICAL STUDIES OF THE GENUS CLYPEOLA L. (BRASSICACEAE) IN IRAN

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The Karyotype and meiotic studies are investigated in 17 populations of four *Clypeola* species growing in Iran for the first time. The species studied showed 2n=2x=14 for *C. lappacea* and *C. dichotoma* while *C. aspera* and *C. jonthlaspi* were tetraploids. For *C. aspera* 2n=4x=26 & 24 and for *C. jonthlaspi* 2n=4x=32 were counted, supporting the published reports on *C. jonthlaspi*, *C. lappacea*, *C. dichotoma* and *C. aspera*. The karyotypic study was performed for *C. aspera* and *C. lappacea*. The chromosomes in these species were mainly metacentric and sub-metacentric. Mixoploidy and polysomaty has been observed in *C. aspera* populations. Meiosis studies were performed on 6 populations of three species of *Clypeola*. Chromosome number of n=16 for *C. jonthlaspi*, n=12 & 13 for *C. aspera* and n=7& 8 for *C. lappacea* were observed. Disploidy was observed in *C. aspera* and *C. lappacea*. Desynapsis and B chromosome are present in *C. lappacea*. Unreduced pollen grains were observed in three species.

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Key words: *Clypeola*; *Brassicaceae*; Karyotype; cytogenetic; meiosis; desynapsis; disploidy; B chromosome

مطالعات سیتولوژیکی جنس Clypeola L. از خانواده شب بو در ایران

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مطالعات کاریوتیپی و میوزی در ۱۷ جمعیت از چهار گونه از جنس Clypeola برای نخستین بار در ایران مورد بررسی واقع شده است. گونه‌های مورد بررسی از نظر وضعیت کروموزومی در دو گونه C. lappacea و C. dichotoma دیپلوئید با 2n=2x=14 است در حالی که دو گونه C. aspera و C. jonthlaspi تتراپلوئید بودند. نتایج 24 & 2n=4x=26 برای گونه C. aspera و 2n=4x=32 برای C. jonthlaspi گزارشات موجود در خصوص C. jonthlaspi, C. lappacea, C. dichotoma و C. aspera را حمایت می کنند. مطالعات کاریوتیپی بر روی C. aspera و C. lappacea انجام شد. در این دو گونه کروموزمها اساسا متاسانتریک و ساب متاسنتریک بودند. Mixoploidy و polysomaty در جمعیتهای C. aspera مشاهده شد. مطالعات میوزی بر روی ٦ جمعیت از ۳ گونه از Clypeola صورت پذیرفت. عدد کروموزومی n=16 در C. jonthlaspi، n=12, 13 برای C. aspera و n=7, 8 برای C. lappacea مشاهده شد. در گونه های C. aspera و C. lappacea دیسپلوئیدی مشاهده شد. دسیناپسیس و حضور کرووموزوم B در گونه C. lappacea دیده شد. دانه های گرده کاهش نیافته در هر سه گونه رویت شد.

INTRODUCTION

The species of the genus *Clypeola* L. are annual plants belonging to the tribe *Alysseae* of *Brassicaceae*. Distribution of this genus is limited to northern hemisphere. Rechinger (1968) noted five species for this genus in Iran including *C. aspera* Turrill, *C. lappacea* Boiss, *C. dichotoma* Boiss, *C. jonthlaspi* L. and *C. microcarpa* Boiss.

Al-Shehbaz et al. (2006) and Warwick et al. (2008) found the chromosome basic number x= 8 for *Alysseae*. They also confirmed the probability of presence of aneuploidy series below or more than eight in this tribe. Basic chromosome number in *Clypeola* was found x=7, 8 by Warwick et al. (2006 & 2008). They found n=7, 8, 14 & 16 in haploid and 2n= 14, 16 & 32 in diploid individuals. Warwick et al. (2008) found that *C. aspera* and *C. lappacea* (both taxa with n=7) formed a well-supported clade (98% bootstrap support), separate from *C. jonthlaspi* (an n=8 species).

Table 1. Locality and voucher information of studied species of *Clypeola.*

Species	Voucher no	Locality	Collector
C. jonthlaspi Turrill	18912-ALUH	Iran, Yazd, Eshqabad road, Ozbak Kuh	Abbasian
	18910-ALUH	Iran,South Khorasan, Boshrouyeh, Neyganan village	Abbasian
	1896-ALUH	Iran, Fars, 35 Km Neyriz, 1480 m	Abbasian
	18923-ALUH	Iran, Kermanshah, Taq-e-Bostan	Gholami
	190-ALUH	Iran,Tehran, Modiriyat bridge	Keshavarzi, Abbasian & Habibi
	1903-ALUH	Iran, Tehran, Boumehen	Abbasian
C. aspera Boiss.	18914-ALUH	Iran, yazd, Tabas, Neyzar village-1010 m	Abbasian
	18912-ALUH	Iran, Yazd, Eshqabad road, Ozbak Kuh	Abbasian
	1891-ALUH	Iran, Yazd, Tabas, Eshqabad road, Kalshane village, 1092 m	Abbasian
	36394-FUMA	Iran, Yazd, SE Tabas, NE Deyhuk, 1700m	Zanguee & Rafei
	1906-ALUH	Iran,Yazd, Tabas, Abid village	Abbasian
	5044-ALUH	Iran, Fars, 35 km Marvdasht	Rastipishe
	18917-ALUH	Iran-Qazvin- Abyek	Abbasian
	18910-ALUH	Iran, South Khorasan, Boshrouyeh, Neyganan village	Abbasian
C. lappacea L.	1901-ALUH	Iran, Lorestan, Malayer, Borujerd road, 60 km Borujerd	Abbasian
	1902-ALUH	Iran, Kermanshah, Bid Sorkh	Abbasian
C. dichotoma Boiss.	1907-ALUH	Iran, Azerbayjan-e-Sharqi, 48 Km NW Marand, salty hills, 1036 m	Akhani & Samadi

Chromosome numbers of *C. microcarpa* and *C. jonthlaspi* were studied by Gorannova and Ancew (1997). They recorded 2n=4x=32 in diploid *C. jonthlaspi* with small chromosomes in their karyotype with inconspicuous centromer. Aryavand (1975) reported *C. aspera* with n=13 and 2n=26, *C. dichotoma*, n=7. He believed that n=7 belonged to *Bergeretia* and *Pseudoanastatica* sections which *C. lappacea*, *C. aspera* and *C. dichotoma* are nested respectively; N=8 belonged to *jonthlaspi* section.

Runemark (2002) pointed 2n=32 and 2n=16 for *C. jonthlaspi* subsp *jonthlaspi* and *C. jonthlaspi* subsp *microcarpa* respectively. All previous studies in this genus are limited to chromosome counting. In present study, karyotypes have been provided for two *Clypeola* species of Iran and meiotic behavior have been considered for the first time in Iran.

MATERIALS AND METHODS

Karyotypic and meiotic studies were performed in 17 populations of 4 *Clypeola* species in Iran. The species studied are: *C. jonthlaspi*, *C. aspera*, *C. lappacea* and *C. dichotoma* (table 1). Meiotic studies were performed in three populations of *C. jonthlaspi* and one population for *C. lappacea* and *C. aspera*. Voucher specimens (table 1) are deposited in Herbarium of AlZahra University.

For meiotic studies, young flower buds were collected from 10 randomly selected plants of each population and fixed in glacial acetic acid and ethanol (1:3) for 24 hrs. Flower buds were washed and preserved in 70% ethanol at 4°C until used (Sheidai *et al*. 1999). Cytological preparations used squash technique and 2% aceto-orcein as the stain. The best time for fixing is 11-13.

For karyotype study the seeds of studied species after sterilization, were kept in 4^0 C for about 7 days. Freshly grown root tips were collected from the germinated seeds of at least ten randomly selected plants in each population. They pretreated with 0.2 M 8-hydroxyquinolin for about 3-5 hours or with Ice water for about 2 hours and fixed in ethanol and acetic acid (3:1) for 24 hrs. The fixed tips were then washed thoroughly in distilled water and macerated in 60^0C in

HCL for about 3-5 min. Squash technique was used for cytological studies with 2% aqueous aceto-orcein as the stain. The somatic chromosome number and karyotype details were studied in at least five well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured by Image Tools3 software (Sheidai et al. 2000; Borgen 1987). The Chromosomes were identified according to Levan et al. (1964). Karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters like haploid total chromosome length, total form percentage (TF%=Sum of short arms of the chromosomes/Total chromosome length Huziwara (1962), coefficient of variation (CV; Verma 1980) of the chromosome size, Stebbins two ways system of karyotype symmetry (Stebbins 1971) as well as A1 and A2 indices of Romero-Zarco (1986) were determined (Sheidai & Jalilian 2008).

Table 2. Karyotypic features of the *Clypeola* species and populations studied : L= Total length of haploid chromatin (µm), L= Size of the longest chromosome (µm), S= Size of the shortest chromosome (µm), TF%=Totl form percentage, X= The mean chromatin length, S%= Relative length of the shortest chromosome, A_1=Intra-chromosomal symmetry index, C.V= Coefficient of variation of the chromosome size, D.R.L= Difference of range of relative length, St= Stebbins class, KF= Karyotype formulae, 2n= Diploid number of chromosome.

Species	Locality	T.L(µm)	L(µm)	S(µm)	L/S	TF%	X	S%	A1	A2	C.V	D.R.L	St	KF	2n
C. aspera,	Neyzar village	34.79	5.05	1.9	2.65	38.28%	2.89	37.62%	0.36	0.3	33.32%	0.09	1B	9m+3Sm	24
C .aspera,	Neyzar village	40.547	5.49	2.15	2.55	37.28%	3.12	39.16%	0.39	0.28	28.44%	0.08	2B	7 m+6 Sm	26
C. aspera,	Ozbak kuh	35.21	5.35	1.41	3.79	39.32%	2.7	26.35%	0.34	0.35	35.72%	0.11	1B	10 m+3 Sm	26
C. aspera,	Ozbak kuh	39.34	5.95	2.05	2.9	41.03%	3.27	34.40%	0.31	0.31	31.34%	0.09	1B	11 m+1 Sm	24
C. aspera,	Kalshane village	29.41	4.72	1.47	3.21	40.87%	2.45	31.14%	0.31	0.37	37.09%	0.11	1B	12 m	24
C. aspera,	Yazd-Deyhuk	43.52	5.48	2.08	2.63	39.24%	3.34	37.95%	0.34	0.26	26.68%	0.07	2B	11 m+2 Sm	26
C. aspera,	Yazd-Deyhuk	38.42	5.75	1.84	3.12	40.13%	3.2	32%	0.33	0.32	32.57%	0.1	1B	11 m+1 Sm	24
C. aspera,	Fars- 35 km Shiraz	36.76	5.81	1.4	4.15	40.70%	3.06	24.09%	0.31	0.37	37.44%	0.11	1C	10 m+2 Sm	24
C. aspera,	Fars- 35 km Shiraz	49.03	6.18	2.58	2.39	37.93%	3.77	41.74%	0.38	0.31	25.56%	0.07	2B	7 m+6 Sm	26
C. aspera,	Qazvin- Abyek	47.87	6.48	2.48	2.61	36.89%	3.68	38.27%	0.37	0.28	28.29%	0.08	2B	7 m+6 Sm	26
C. aspera,	Neyganan village	35.79	5.34	1.67	3.19	39.82%	2.75	31.27%	0.33	0.34	34.80%	0.1	1B	11 m+2 Sm	26
C. lappacea	Malayer-Borujerd	16.09	3.27	2.85	1.14	37.69%	2.29	87.15%	0.38	0.28	28.68%	0.02	1B	5 m+2 Sm	14
C. lappacea	Bid Sorkh- gauth	21.47	4.13	2.26	1.82	37.21%	3.06	54.72%	0.4	0.21	21.07%	0.08	2A	4 m+ 3 Sm	14

Table 3. Meiotic characters of the studied *Clypeola* species.
(Abbreviations: ROD= mean number of rod bivalents, RB =mean number of ring bivalents, I =mean number of univalents, IV =mean number of quadrivalents, IX =mean number of intercalary chiasmata, TX =mean number of terminal chiasmata, IX= mean number of intercalary chiasmata, TX= mean number of terminal chiasmata, TOX =mean, number of total chiasmata, B=B chromosome, RODN =mean number of rod bivalents/ haploid chromosome number, RBN =mean number of ring bivalents/ haploid chromosome number, IN =mean number of univalents/ haploid chromosome number, IVN: mean number of quadrivalents / chromosome number, IXN = mean number of intercalary chiasmata/ chromosome number, TXN = mean number of terminal chiasmata/ chromosome number, TOXN = mean number of total chiasmata/ chromosome number).

Species	Locality	N	Voucher No	ROD	RB	I	IV	IX	TX	TOX	B	RODN	RBN	IN	IVN	IXN	TXN	TOXN
C.jounthlaspi	Bumehen	16	1903	13.25	1.91	0.13	0	13.25	3.83	17.08	0	0.82	0.11	0.01	0.00	0.82	0.23	1.06
C.aspera	Abid	12	1904	9.33	1.83	0.5	0.16	9.33	4.33	13.66	0	0.77	0.15	0.04	0.01	0.77	0.36	1.13
C.aspera	Abid	13	1904	9	3.66	0.33	0	0.9	7.44	16.44	0	0.70	0.28	0.02	0.00	0.06	0.57	1.26
C.lappacea	brugerd-kermanshah	7	1901	5.7	0.52	0.88	0	5.7	1.05	6.76	0.05	0.81	0.07	0.13	0.00	0.81	0.15	0.97
C.lappacea	brugerd-kermanshah	8	1901	5.58	1.58	0.58	0.16	5.58	3.66	8.83	0.08	0.70	0.20	0.07	0.02	0.70	0.46	1.10

RESULTS

Karyotypic features

Details of karyotypic features of studied *Clypeola* species are provided in table 2 and figs. 1 & 2. The karyotype for *C. jonthalspi* was not prepared due to the small size of chromosomes with inconspicuous centromer**.**

Clypeola jonthlaspi. In this study five populations of *C. jonthlaspi* (Neyzar, Neyriz, Modiriat bridge, Taq-Bostan and Marand) were studied. *C. jonthlaspi* showed 2n=4x=32 which agrees with former publications (Aryavand 1975; Warwick et al. 2006 & 2008; Al-Shehbaz 2006).

Clypeola aspera. In this study 7 populations were studied. Polysomatic was observed in this species which were two chromosome numbers 2n=24 and 2n = 26 .The number of 2n=26 agrees with former studies (Aryavand 1975), the number of 2n=24 is new for this species.

Clypeola lappacea. In this study 2 populations were studied, 2n=2x=14 which agrees with previous literatures with reported n=7 (Maassoumi 1980; Al-Shehbaz & Al-Omar 1982 & 1983; Warwick *et al* 2008).

Clypeola dichotoma. One population for this species was studied and the number of 2n=2x=14 was obtained. This report is according to previous studies with n=7 (Aryavand 1975; Maassoumi 1980).

The *Clypeola* species were placed in 2A, 1B, 2B and 1C classes of Stebbins Karyotype symmetry, which are considered relatively primitive in this system except for *C. aspera*, population of Fars, 35 Km to Shiraz with 2n=24 that is stated in 1C class. Therefore, it seems that the studied *Clypeola* species have symmetrical karyotypes.

Conclusion

Clypeola microcarpa has been variously treated as distinct subspecies or variety of *C. jonthalaspi* (Chaytor & Turill 1935; Breistroffer 1936; Runemark 2002; Bush 1939). In Flora Iranica (Rechinger 1981) it was considered as a distinct species and recorded from Khorassan province. Runemark (2002) recorded 2n=16 for this taxon, but the specimens fitted to *C. microcarpa* from Khorassan province showed 2n=4x=32. Therefore, it may be that this species do not occur in Iran.

Mandakova and Lysak (2008) pointed that simultaneous morphological variation in *Brassicaceae* is not necessarily along with modifications in Karyotype. It means that morphologically distinct taxa in *Brassicaceae* may have similar karyotypes. This is in accordance with the *C. jonthalspi* subspecies in Iran. Observed chromosome number is consistence with basic chromosome number for this genus (Warwick et al. 2006 & 2008). The polysomy and mixoploidy (mixoploidy is a condition in which the tissue is composed of cells with different ploidy levels) is observed in *C. aspera*. In similar studies, Borgen (1987) pointed to mixoploidy in *Lobularia* (previously considered as an *Alyssese* element). Bolourian (2009) recorded two chromosome set in some cells of *Alyssum*, beside polysomatic phenomenon in most of *Alyssum* populations.

Meiotic studies

The meiotic behavior of chromosomes in chiasmata formation, frequency and chromosomes different conjunctions and genetic abnormalities are recorded. The meiotic configurations are listed in table 3.

Clypeola jonthalspi. The number n=16 was obtained for *C. jonthalspi* (fig. 3). This is in concordance with its previously recorded sporophyte number (Aryavand 1975; Ancev and Goranova 1997; Warwick et al. 2006 & 2008; AL-Shehbaz 2006). In *C. jonthlaspi* abnormalities as synozytic node, unreduced and sterile pollen grains were observed. In this population most cells were in diakinesis-metaphase I and there were only few cells in telophase II.

Clypeola aspera. Two different chromosome numbers n=12 and n=13 (fig. 3) were observed in different individuals of *C. aspera* from Abid village population (Disploidy). In meiotic study of this species, abnormalities such as, anaphase bridge (fig. 4 B), disordered divisions in anaphase II (fig. 4A), chromosome stickiness, laggard chromosome, and unreduced pollen grains (fig. 4 C) were observed.

Clypeola lappacea. Obtained chromosome number in this study for *C. lappacea* were n=8 and n=7 in different individuals of Borujerd-Kermanshah populations (Disploidy). Variation in meiosis steps in this species as synozitic knot and diffuse (fig. 4 G) were observed. Laggard chromosome in anaphase II, telophase II (fig. 4 E), desynaptic (fig. 4 D), anaphase bridge in anaphase I (fig. 4F), multipolar and tripolar cells (fig. 4 I-J), micronucleus (fig. 4 H) were observed. There were unreduced and sterile pollen grains (fig. 4 K). Presence of B chromosome (fig. 4 L) and polyploid cell (fig. 3 I) is recorded here for the first time in *C. lappacea*.

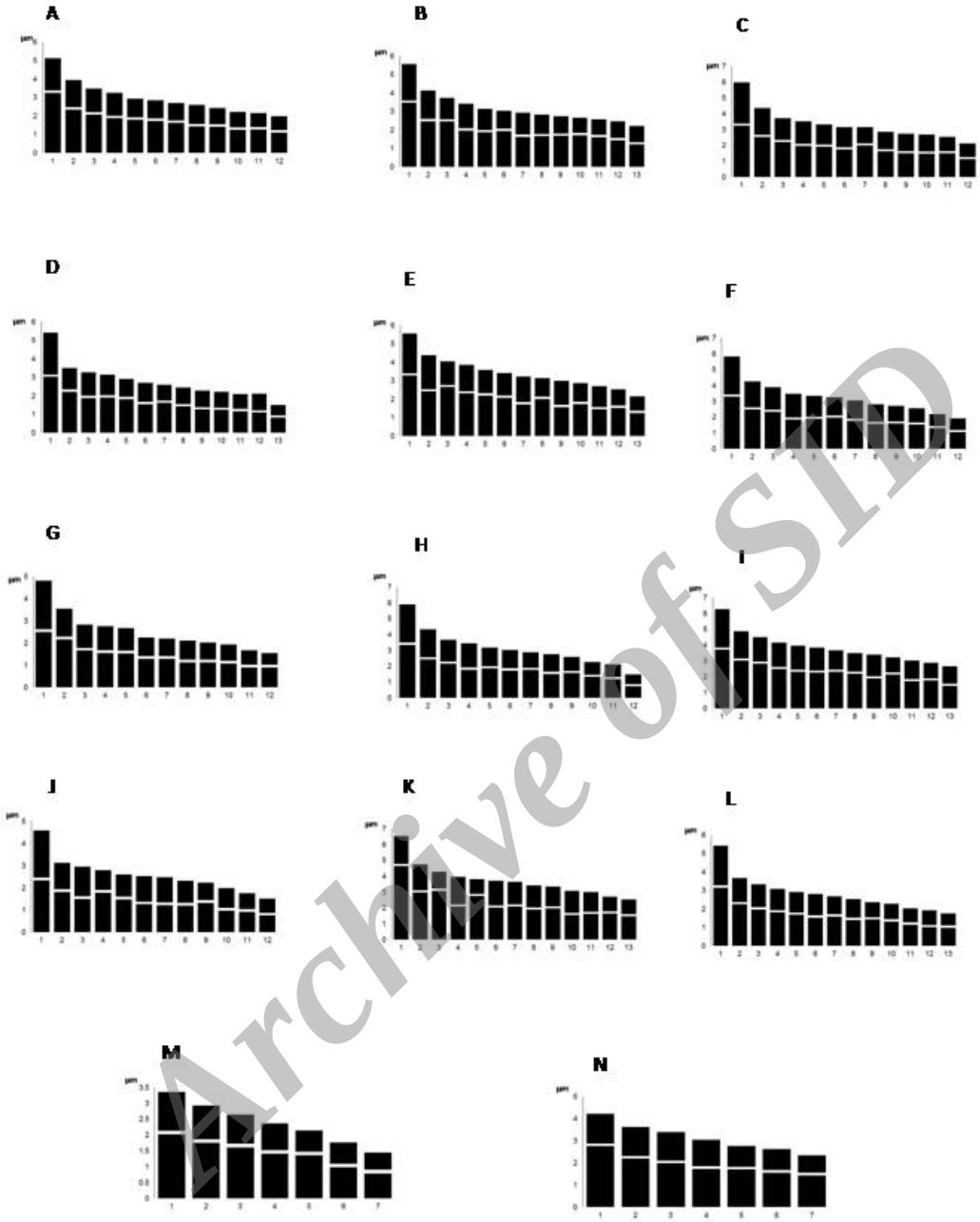

Fig. 1. Representative ideograms of two species of *Clypeola*. A-L, *C. aspera*, A-B: P-1, Tabas-Neyzar village, C-D: P-3,Yazd-Eshgabad road-Ozbak kuh, E-F: P-5 Yazd, 40 Km Deyhuk, G: P-4,Tabas-Eshqabad road Kalshane villag, H-I: P-6 Fars, 35 Km Shiraz, K: P-7 Qazvin- Abyek, L: P-9, South Khorasan, Boshrouyeh, Neyganan village; M-N, *C. lappacea*, M: P-11, Malayer-borujerd road, 60 km Borujerd, N: P-12, Kermanshah, Bid Sorkh. Scale = µm.

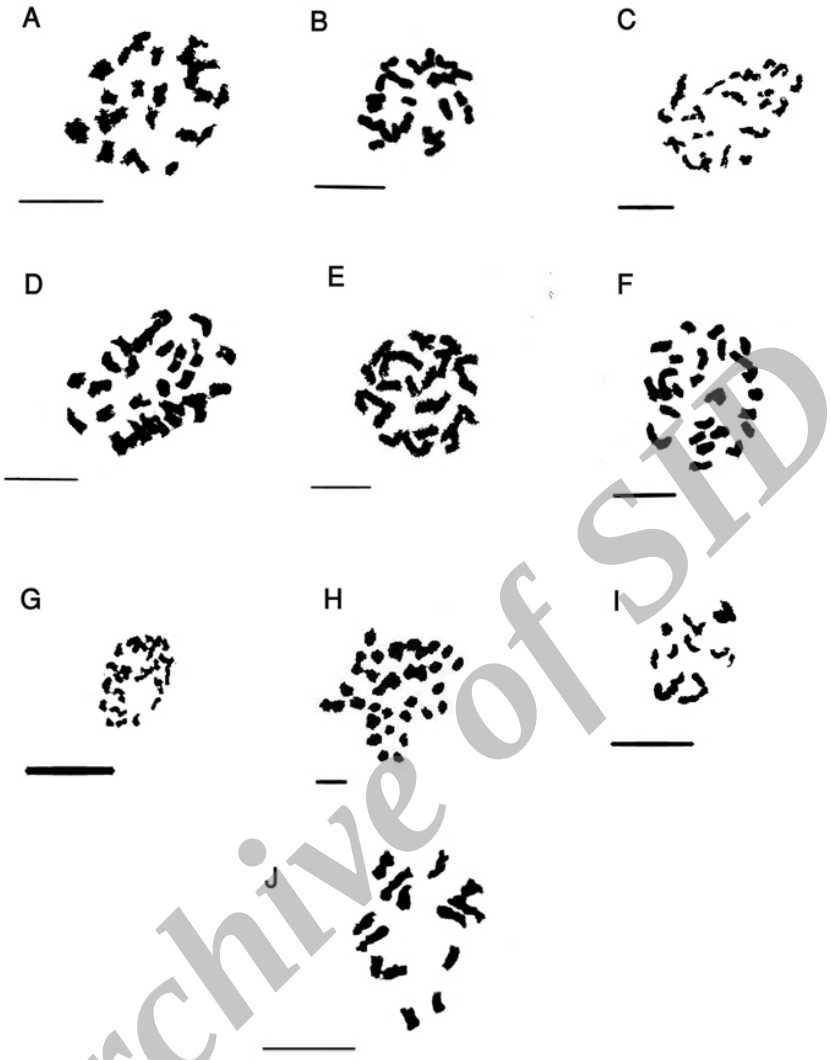

Fig. 2. Representative somatic cells in *Clypeola* species. A-C, *Clypeola aspera* 2n=24; D-F, *C. aspera* 2n=26; G-H, *C. jonthlaspi* 2n=32; I, *C. dichotoma* 2n=14; J, *C. lappacea* 2n=14. Scale= 10µm.

Conclusion

Desynapsis which was observed in *C. lappacea* could result in meiotic abnormalities which reduce the fertility of species. Desynapsis formation is effective in micronucleus formation and effect pollen fertility (Enss and Larter 1960). Pagliarini (2000) pointed that presence of univalent chromosomes (due to low chiasmata numbers or presence of asynapsis or desynapsis genes) is capable of causing irregularity in cell division in metaphase I or laggard in anaphase I, both of which are effective in micronucleus formation and pollen fertility. Due to the high presence of sterile pollen grains and desynapsis and micronucleus formation in *C. lappacea*, the results of present study confirm the Pagliarini (2000) and Enss and Larter (1960) results. The most observed meiotic abnormalities in *C. lappacea* were desynapsis, laggared chromosome and stickiness. Sheidai et al. (2008) pointed to multi-polar as an effective factor in unreduced pollen formation through irregularity in chromosome separation in anaphase I in *Silene* species. It seems that it could be the case in *C. aspera* too. According to the chromosome number of *C. lappacea* from western parts of Iran with 2n=14, we will find that this diploid species is distributed in eastern parts, but *C. jonthalsppi* and *C. aspera* which are tetraploids have vast distribution area.

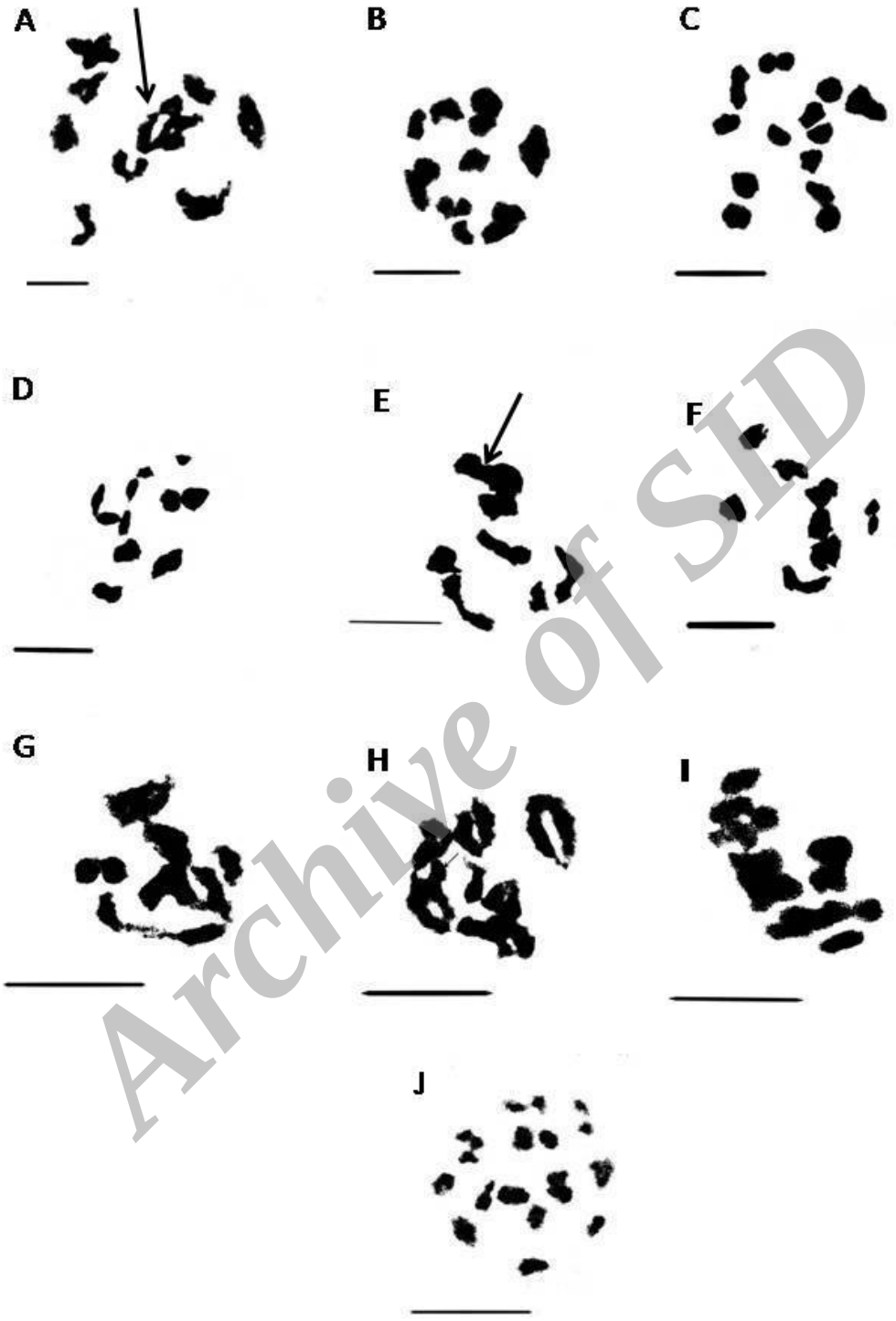

Fig. 3. Representative pollen mother cells of *Clypeola* species showing gametic chromosome number in diakinesis-metaphase 1. A-B, *C.aspera,* n=12; C, *C. aspera*, n=13; D-F, *C. lappacea*, n=8; G-H, *C. lappacea*, n=7. Polyploid cells. I, *C. lappacea*; J, *C. jonthlaspi*, n=16. Arrows in A and E show quadrivalent. Sacale= 10 µm.

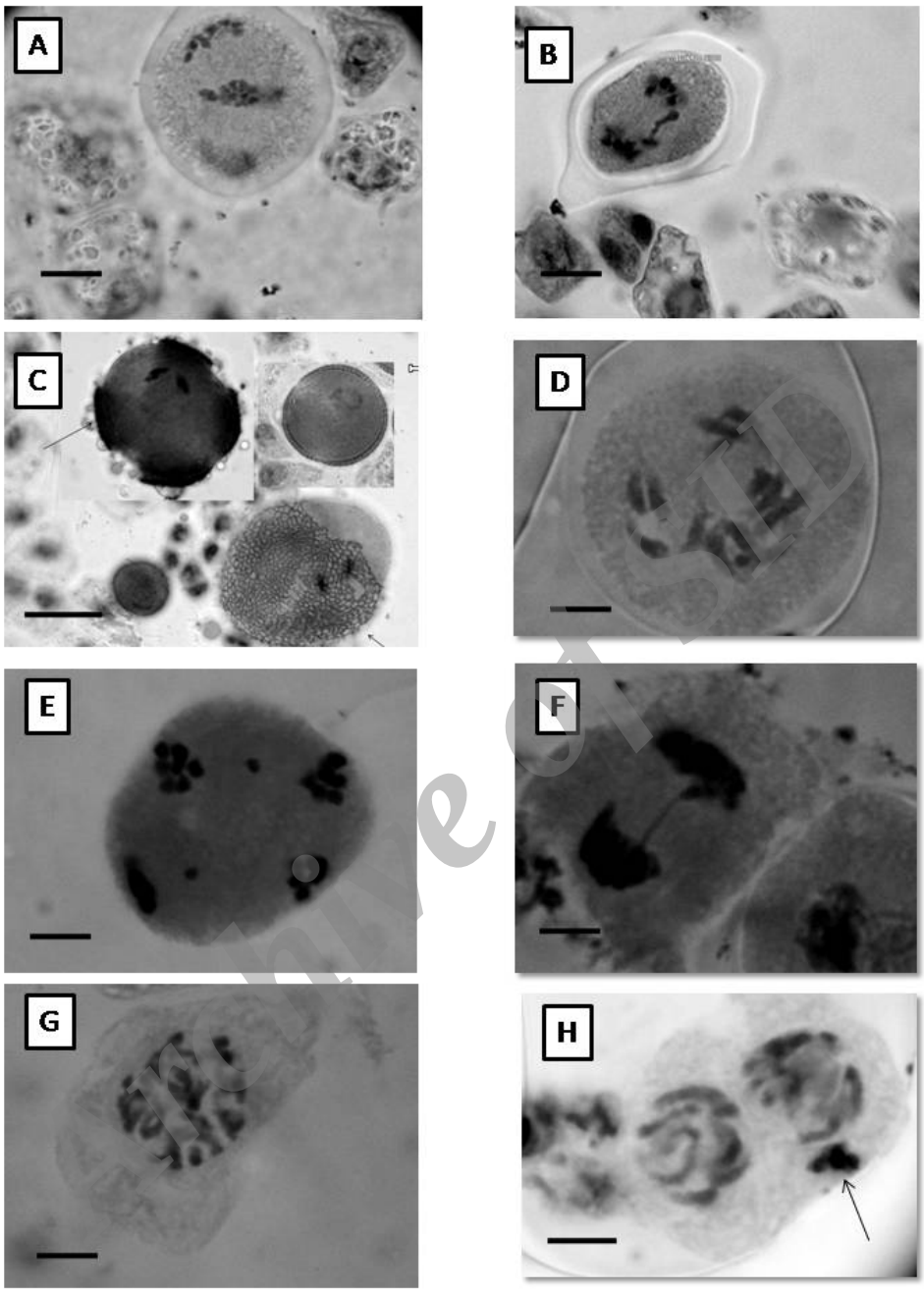

Fig. 4. Meiosis in *Clypeola lappacea* and *C. aspera*. A-C, *C. aspera,* A, disordered division in anaphase II, B, anaphasic bridge, C, unreduced normal and sterile pollen; D-K, *C. lappacea*, D, desynapsis, E, laggard chromosome in telophase II, F, anaphase I bridge, G, diffuse, H, micronucleus.

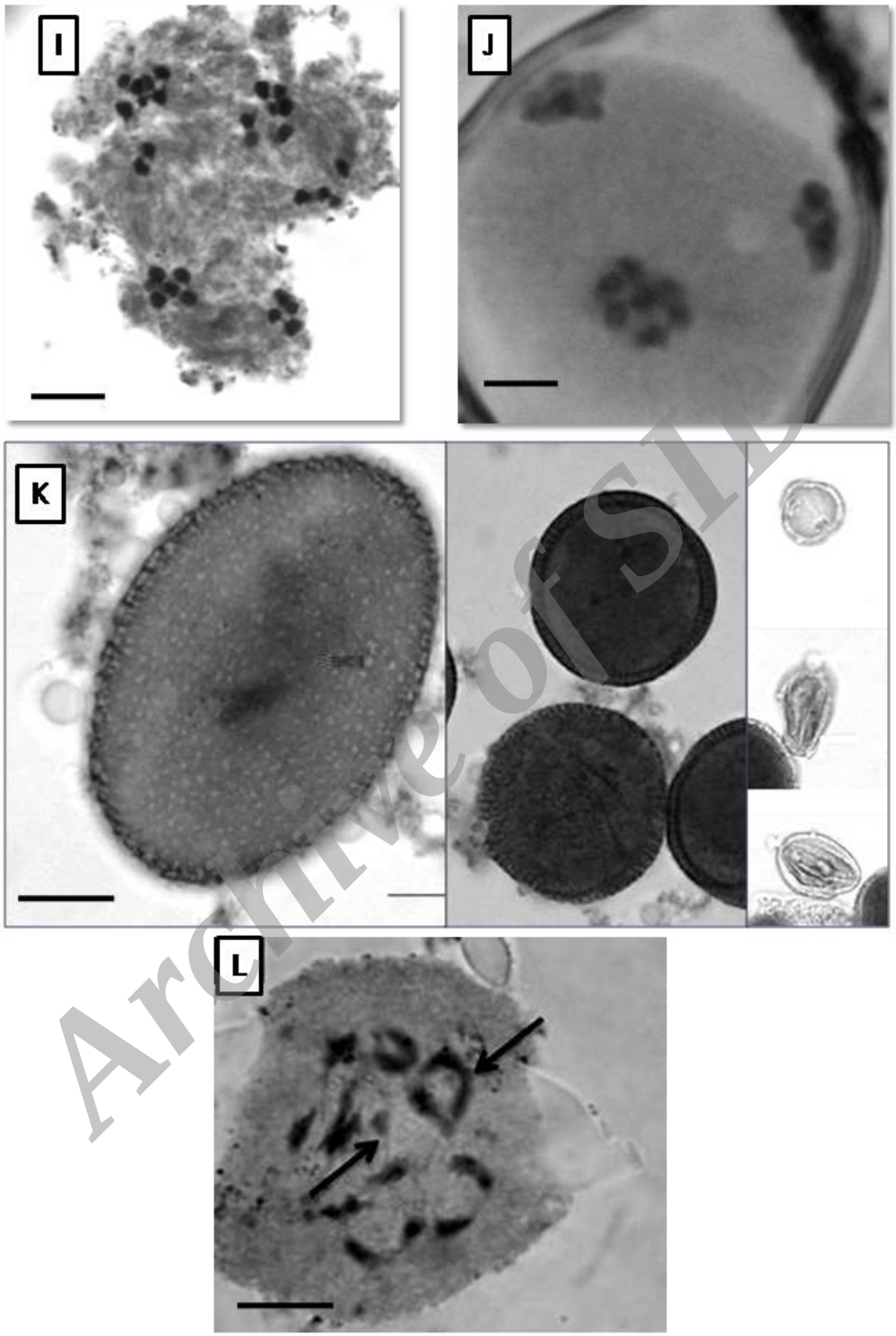

Fig. 4. continued. I, disordered chromosome, multi-polar cell; J, three polar cell; K, unreduced, normal and sterile pollen grain; L, arrow shows quadrivalent and B chromosome. Scale= 10 µm.

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