

# Cytological studies into some species and populations of *Carduus* (Asteraceae) in Iran

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**Abstract.** Meiotic and chromosome number studies were carried out into 14 populations of five *Carduus* species, as well as a karyotype study into 15 populations of six *Carduus* species. The meiotic analysis has shown  $2n=16$  and 26 for populations of *C. hamulosus*,  $2n=32$  for *C. thoermeri*,  $2n=34$  for *C. transcaspicus*,  $2n=60$  for *C. pycnocephalus* subsp. *albidus* and *C. pycnocephalus* subsp. *Pycnocephalus*, and  $2n=60$  for *C. onopordioides*. The karyotype study has revealed the chromosome size differences in the species, as well as the karyotype formulae and Stebbins' class indication of the occurrence of both quantitative and qualitative changes of karyotypes during species diversification.

**Key words:** *Carduus*, chromosome number, karyotype, meiotic abnormalities

## Introduction

Tribe *Cardueae* of the subfamily *Cichorioideae*, (*Asteraceae*) comprises about 2500 species distributed into 83 genera (Bremer 1994; Susanna & al. 2006; Funk & al. 2009), with mainly Old World distribution in the northern hemisphere. About 1600 species and 36 genera have been mentioned in the subtribe *Carduinae* (Bremer 1994) within which falls *Carduus* with about 90 species native to Europe, Asia and Africa. The genus *Carduus* comprises annual or perennial Old-World prickly thistles, consumed by some Lepidoptera species (Bremer 1994; Häffner & Hellwig 1999).

Haploid chromosome numbers in *Asteraceae* range from 2 to 120, and the most common number is 9, regarded as plesiomorphic (Jeffrey 2007). Tribal delimitation of *Cardueae* is controversial and traditional classification into four subtribes (*Echinopsinae*, *Carlininae*, *Carduinae*, and *Centaureinae*) is uncertain (Garcia-Jacas & al. 2002). Molecular studies using DNA sequences of the internal transcribed spaces (ITS) of the

nuclear ribosomal DNA genes and the *matK* gene of the chloroplast DNA support the monophyly of *Cardueae*, including *Carlininae* and *Echinopsinae*. Subtribe *Centaureinae* is also a well supported clade, and its sister-clade contains the genera *Arctium*, *Cousinia*, *Jurinea*, and *Saussurea* from the *Carduinae*. However, the *Carduinae* are a paraphyletic assemblage, and the subtribal placement of *Berardia*, *Cardopatium*, *Cousiniopsis*, and *Staehelina* remains unresolved (Garcia-Jacas & al. 2002). The present classification into four subtribes is unsatisfactory, but still the only practical approach (Garcia-Jacas & al. 2002).

Taxonomy and generic delimitation of the genus *Carduus* are difficult and problematic (Dogan & al. 2007), due to morphological diversity and character variations in the genus, and also to the occurrence of hybridization and introgression between the species (Tutin & al. 1976; Desrochers & al. 1988a, b).

The genus *Carduus* shows close affinity to the genus *Cirsium* and it is unclear whether *Carduus* and *Cirsium* are sister-groups, or at least one of them is

paraphyletic or even polyphyletic. Their separation is often regarded as artificial (Bremer 1994; Häffner & Hellwig 1999).

Cytotaxonomical studies have been considered useful in showing the species relationships, taxonomic delimitation and genetic differences in several plant groups, including in the grass genera of *Aegilops* (Sheidai & al. 1999a, 2000a, 2002), *Bromus* (Stebbins 1981; Sheidai & Nouroozi 2005; Sheidai & Fadaei 2005; Sheidai & al. 2008), *Stipa* (Stebbins 1998, Sheidai & At-taei, 2005; Sheidai & al. 2006), *Festuca* (Sheidai & Bagheri-Shabestarei 2007), and *Hordeum* (Sheidai & Rashid 2007), as well as in other plant groups like *Asparagus* (Sheidai & Inamdar 1991), *Hyoscyamus* (Sheidai & al. 1999b) and *Echinops* (Sheidai & al. 2000b).

Cytological reports on *Carduus* species have shown the importance of cytological changes in the species diversification (for example, Van Loon & Snelders 1979; Kuzmanov & al. 1981; Zemskova & Ciklauri 1987; Lövkvist & Hultgård 1999). However, there are only two chromosome number reports from Iran (Ghaffari 1989, 1999). The present study considers for

the first time the ploidy level, chromosome pairing and meiotic characteristics of 14 populations of five *Carduus* species, as well as the karyotype analysis of 15 populations of six species growing wild in Iran.

## Material and methods

### Plant material

In order to provide plant material for the meiotic study, young flower buds were collected from 14 populations of five *Carduus* species growing in Iran (Table 1), namely: 1 – *C. hamulosus* Ehrh. subsp. *hystrix* (six populations), 2 – *C. transcaspicus* Gandog subsp. *macrocephalus* (three populations), 3 – *C. thoermeri* Weinm subsp. *armens*, 4 – *C. onopordioides* Fisch., 5 – *C. pycnocephalus* L. subsp. *albidus* (two populations), and *C. pycnocephalus* L. subsp. *pycnocephalus*. Karyotype analyses were performed on 15 populations of six species (Table 1), namely: 1 – *C. hamulosus* Ehrh. subsp. *hystrix* (nine populations), 2 – *C. transcaspicus* Gandog subsp. *macrocephalus*

Table 1. Cytological study of the *Carduus* species.

Species	Voucher No	Locality
<i>C. hamulosus</i> subsp. <i>hystrix</i> * ♦	HSBU 8700100	West Azarbaijan: Bazargan, Kalisakandy, 1412 m, Azizi
<i>C. hamulosus</i> subsp. <i>hystrix</i> * ♦	HSBU 8700101	Ardabil: Khalkhal to Ardabil, 1801 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> * ♦	HSBU 8700102	East Azarbaijan: Ahar, 1581 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> * ♦	HSBU 8700103	East Azarbaijan: Kaleibar, Ghasre Babak, 1139 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> ♦	HSBU 8700114	Ardabil: Khalkhal to Asalem, 1789 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> ♦	HSBU 8700115	Ardabil: ahrod, 1800 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> ♦	HSBU 8700116	Ardabil: Namin road of Fandoghloo, 1500 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> ♦	HSBU 8700117	Mazandaran: Noor, Yoosh, 1348 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> * ♦	HSBU 8700104	Ardabil: Sabalan, 3000 m, Nouroozi
<i>C. hamulosus</i> subsp. <i>hystrix</i> *	HSBU 8700141	Ardabil: Meshkin shahr, 1742 m, Nouroozi
<i>C. thoermeri</i> *	HSBU 8700106	West Azarbaijan: Bazargan, Kalisakandy, 1412 m, Azizi
<i>C. thoermeri</i> ♦	HSBU 8700118	East Azarbaijan: Kaleibar, Ghasre Babak, 1139 m, Nouroozi
<i>C. onopordioides</i> *	HSBU 8700142	Ardabil: Asalem to Khalkhal, 1886 m, Nouroozi
<i>C. onopordioides</i> ♦	HSBU 8700119	West Azarbaijan: Khoye, Qutore, 1939 m, Azizi
<i>C. pycnocephalus</i> subsp. <i>albidus</i> ♦	HSBU 8700120	Zanjan: Zanjan, 1886 m, Nouroozi
<i>C. pycnocephalus</i> subsp. <i>albidus</i> *	HSBU 8700108	Tehran: Evin, 1998 m, Azizi
<i>C. pycnocephalus</i> subsp. <i>albidus</i> *	HSBU 8700109	Tehran: Jajrood, 1887 m, Azizi
<i>C. pycnocephalus</i> subsp. <i>pycnocephalus</i>	HSBU 8700110	Tehran: Sorkhe hesar, 1987 m, Azizi
<i>C. arabicus</i> subsp. <i>arabicus</i> ♦	HSBU 8700121	Esfahan: Kashan, 1775 m, Nouroozi
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i> ♦	HSBU 8700122	Ardabil: Meshkin shahr, Qutore sue, 1976 m, Nouroozi
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i> * ♦	HSBU 8700112	Mazandaran: Pole zanguleh, 1898 m, Azizi
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i> *	HSBU 8700111	Mazandaran: Valy abad, 1997 m, Azizi
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i> *	HSBU 8700143	Ardabil: Khalkhal, 1801 m, Nouroozi

Abbreviations: \* – species examined in meiotic study, ♦ – species examined in karyotype study.

*alus* (two populations), 3 – *C. thoermeri* Weinm subsp. *armens*, 4 – *C. onopordioides* Fisch., 5 – *C. pycnocephalus* L. subsp. *albidus*, 6 – *C. arabicus* subsp. *arabicus*. The voucher specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU).

### Cytological studies

**Meiotic analyses.** Meiotic studies were carried out on young flower buds collected from at least 10 randomly selected plants from each species and population. Minimum 100 metaphase/diakinesis pollen mother cells (PMCs) and 500 anaphase and telophase cells were analyzed for data collection (Sheidai & Rashid 2007). Pollen tonality, as measure of fertility, was determined by staining minimum 1000 pollen grains with 2% acetocarmine : 50% glycerin (1:1) for about 30 minutes. The round complete pollen grains which were stained were taken as fertile, while the incomplete, shrunken pollen grains with no stain were considered as infertile (Sheidai & Rashid 2007). Analysis of variance (ANOVA) followed by Least Significant Test (LSD) were applied to detect any significant difference in the chiasma frequency and chromosome pairing, and a  $\chi^2$  test was performed for meiotic abnormalities among the studied species (Sheidai & Rashid 2007). SPSS ver. 9 (1998) software was used for the statistical analyses.

**Karyotype analyses.** For karyotype studies, freshly grown root tips were collected from the seeds of at least ten randomly selected plants in each species, pre-treated with 0.002 M 8-hydroxyquinolin (1–2 hr) and fixed in ethanol : acetic acid (3:1) for 24 hr. The fixed tips were then washed thoroughly in distilled water and macerated in 60°C 1N HCl for about 5 min. For cytological studies, the squash technique was used with 2% aqueous aceto-orcein. 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 Tools 3 software.

The chromosomes were identified according to Levan & al. (1964), karyotype symmetry was determined according to Stebbins (1971), as well as other karyotype parameters like the total form percentage (TF%), coefficient of variation (CV) of the chromosome size, and A1 and A2 indices of Romero-Zarco (1986). Pearson coefficient of correlation among the karyotype features was established.

## Results

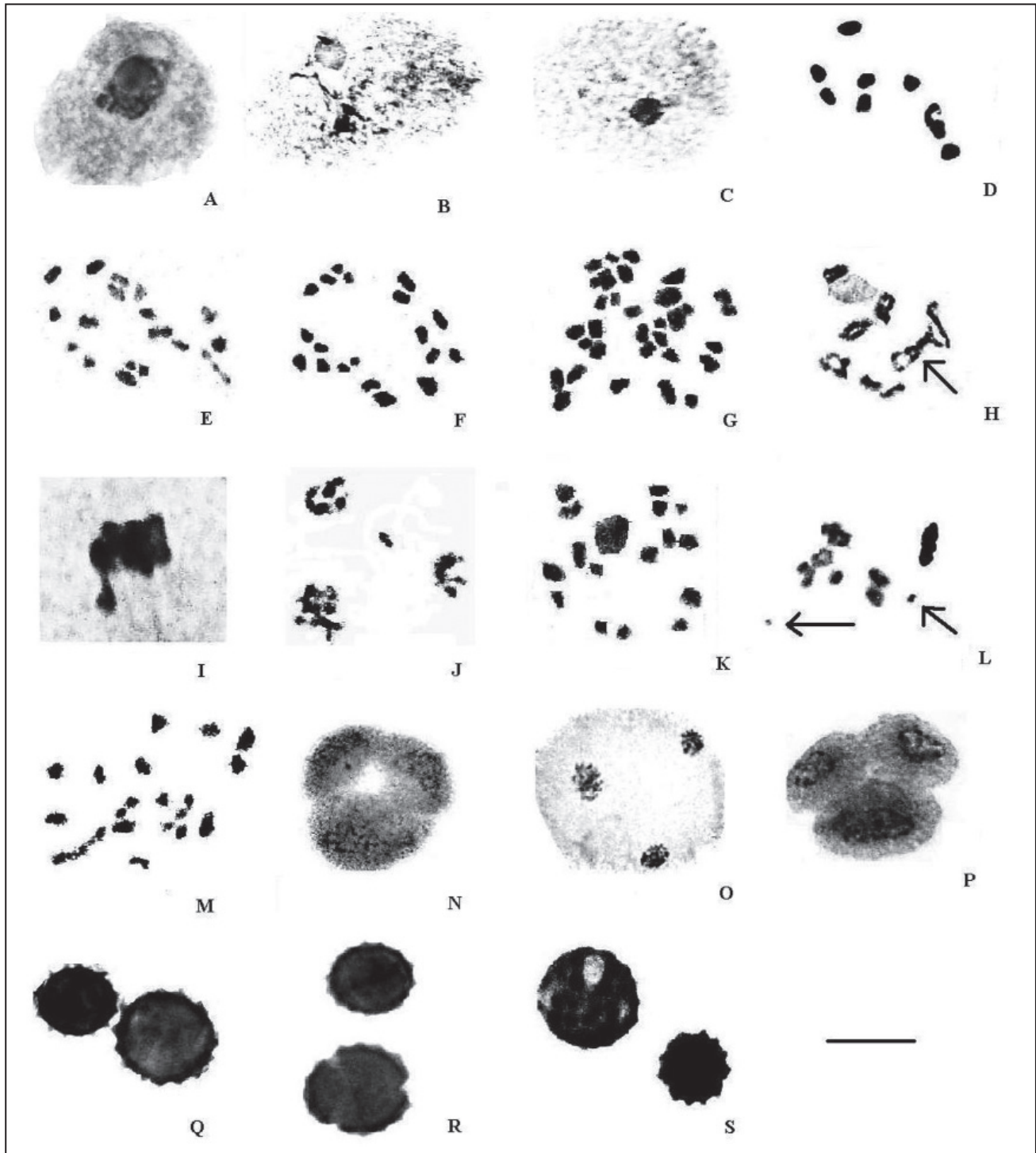
Analysis of the studied *Carduus* species has shown occurrence of the post-pachytene diffuse stage (Fig. 1, A-C). Despiralization of chromosomes occurred after pachytene, marking the commencement of the diffuse stage. The occurrence of diffuse stage has been reported in several plant species (Sybenga 1992). Diffuse stage may be complete, when the whole chromosomes decondense, or it may be partial when some parts of the genome show decondensation. The present study showed the occurrence of partial/complete diffuse stage in the *Carduus* species.

### Chromosome number and chiasma frequency

Details of the chromosome number, chiasma frequency and chromosome pairing in *Carduus* species are reported (Table 2 & 3) (Fig. 1, A-S). The Kalisakandi, Khalkhal, Ahaar and Ghasre Babak populations of *C. hamulosus* showed a chromosome number  $2n=2x=16$ , while Sabalan and Meshkinshahr populations of this species showed a chromosome number  $2n=2x=26$ .

The other chromosome numbers were as follows: Khalkhal, Valiabad and Polezanguleh populations of *C. transcaspicus*  $2n=2x=34$ ; Kalisakandi population of *C. thoermeri*  $2n=4x=32$ , and Asalem population of *C. onopordioides*  $2n=4x=60$ . Two populations of *C. pycnocephalus* subsp. *albidus* and one population of *C. pycnocephalus* subsp. *pycnocephalus* showed  $2n=4x=60$ . Among *C. hamulosus* populations with chromosome number  $2n=16$ , Ghasre Babak population showed the highest value of the total and terminal chiasmata (15.60 and 14.60 respectively), while the lowest values of the same parameter occurred in the Ahar population (9.18 and 8.33 respectively). Ghasre Babak population also had the highest values of the relative total and terminal chiasmata (1.95 and 1.82 respectively), as compared to other populations of *C. hamulosus* with chromosome numbers  $2n=16$  and 26.

Among the species and populations with chromosome numbers  $2n=60$ , Jajrood population of *C. pycnocephalus* subsp. *albidus* showed the highest values of the total and intercalary chiasmata (45.74 and 10.51 respectively). Similarly, among the species with a chromosome number  $2n=34$ , Valiabad population of *C. transcaspicus* subsp. *Macrocephalus* had the highest values of the total, terminal and intercalary chiasmata (20.72, 16.74 and 3.98, respectively).



**Fig. 1.** Representative meiotic cells in the *Carduus* species.

**Legend:** A = syzyzium stage in *C. transcaspicus*. B and C = post pachytene diffuse stage in *C. hamulosus*. D = metaphase I cell showing bivalents in Kalisa Kandi population of *C. hamulosus*. E = metaphase I cell showing bivalents in *C. thoermeri*. F = metaphase I cell showing bivalents in Valiabad population of *C. transcaspicus* subsp. *Macrocephalus*. G = metaphase I cell showing bivalents in *C. pycnocephalus* subsp. *albidus*. H = diakinesis cell showing quadrivalent (arrow) in Meshkin-Shahr population of *C. hamulosus*. I = metaphase cell showing complete chromosome clumping in Kalisa Kandi population of *C. hamulosus*. J = tripolar cell showing laggard chromosome (arrow) in Ahar population of *C. hamulosus*. K = metaphase cell showing double chromosome number (unreduced) in *C. hamulosus*. L = metaphase cell showing B-chromosomes (arrows) in *C. hamulosus*. M = metaphase cell showing bivalents in *C. transcaspicus* subsp. *macrocephalus*. N-P = tripolar cells in *C. hamulosus*, *C. pycnocephalus* subsp. *albidus* and *C. transcaspicus* subsp. *macrocephalus* respectively. Q-S = potential unreduced pollen grains (bigger-size pollen grains) in *C. hamulosus*, *C. pycnocephalus* subsp. *albidus* and *C. transcaspicus* subsp. *macrocephalus*, respectively. Scale bar = 10  $\mu$ m.

**Table 2.** Meiotic characteristics of the *Carduus* species.

Species	Locality	2n	ROD	RB	I	IV	IX	TX	TOX
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Kalisa kandi	16	3.18	4.54	0.50	0.00	0.06	12.29	12.35
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Khalkhal to Ardabil	16	5.06	2.32	0.32	0.22	0.80	9.45	10.25
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Ahar	16	5.60	1.66	0.84	0.15	0.84	8.33	9.18
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Ghase Babak	16	1.20	6.80	0.00	0.00	1.00	14.60	15.60
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Sabalan	26	9.91	2.31	0.93	0.14	0.89	14.00	14.89
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Meshkin-Shahr	26	6.07	3.39	1.78	1.32	1.42	14.46	15.89
<i>C. thoermeri</i>	Kalisakandi	32	9.32	5.38	2.23	0.08	0.61	19.58	20.20
<i>C. onopordioides</i>	Asalem to Khalkhal	60	16.86	8.16	6.66	1.00	4.43	31.56	36.00
<i>C. pycnocephalus</i> subsp. <i>albidus</i>	Tehran, Evin	60	17.36	11.00	1.13	0.53	6.43	36.20	42.70
<i>C. pycnocephalus</i> subsp. <i>albidus</i>	Jajrood	60	11.96	13.74	6.25	0.58	10.51	35.22	45.74
<i>C. pycnocephalus</i> subsp. <i>pycnocephalus</i>	Sorkkeh-Hesar	60	14.26	12.73	1.86	0.86	9.90	34.66	44.56
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	Valyabad	34	7.26	5.42	8.24	0.01	3.98	16.74	20.72
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	Pole-Zanguleh	34	6.00	4.40	12.9	0.07	2.87	13.85	16.72
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	Khalkhal	34	9.91	3.93	5.20	0.27	2.54	16.45	19.00

**Abbreviations:** ROD = mean number of rod bivalents, RB = mean number of ring bivalents, I = mean number of uni-valents, IV = mean number of quadric-valents, IX = mean number of intercalary chiasmata, TX = mean number of terminal chiasmata, TOX = mean number of total chiasmata.

**Table 3.** Relative meiotic characteristics of the *Carduus* species.

Species	RODN	RBN	IN	IVN	IXN	TXN	TOXN
<i>C. hamulosus</i> subsp. <i>hystrix</i>	0.39	0.56	0.06	0.00	0.01	1.53	1.54
<i>C. hamulosus</i> subsp. <i>hystrix</i>	0.63	0.29	0.04	0.02	0.10	1.18	1.28
<i>C. hamulosus</i> subsp. <i>hystrix</i>	0.70	0.20	0.10	0.01	0.10	1.04	1.14
<i>C. hamulosus</i> subsp. <i>hystrix</i>	0.15	0.85	0.00	0.00	0.12	1.82	1.95
<i>C. hamulosus</i> subsp. <i>hystrix</i>	0.76	0.17	0.07	0.01	0.06	1.07	1.14
<i>C. hamulosus</i> subsp. <i>hystrix</i>	0.46	0.25	0.13	0.10	0.10	1.11	1.20
<i>C. thoermeri</i>	0.58	0.33	0.13	0.01	0.03	1.22	1.26
<i>C. onopordioides</i>	0.56	0.27	0.22	0.03	0.14	1.05	1.21
<i>C. pycnocephalus</i> subsp. <i>albidus</i>	0.57	0.36	0.03	0.02	0.21	1.20	1.42
<i>C. pycnocephalus</i> subsp. <i>albidus</i>	0.39	0.45	0.20	0.02	0.35	1.17	1.52
<i>C. pycnocephalus</i> subsp. <i>pycnocephalus</i>	0.47	0.42	0.06	0.03	0.33	1.15	1.48
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	0.42	0.31	0.48	0.01	0.23	0.98	1.21
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	0.35	0.25	0.75	0.01	0.16	0.81	0.98
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	0.58	0.23	0.30	0.01	0.14	0.96	1.11

**Abbreviations:** RODN = mean number of rod bivalents/cell, RBN = mean number of ring bivalents/cell, IN = mean number of univalents/cell, IVN = mean number of quadric-valents/cell, IXN = mean number of intercalary chiasmata/bi-valent, TXN = mean number of terminal chiasmata/bivalent, TOXN = mean number of total chiasmata/bivalent.

The chromosomes formed bivalents and univalents in the metaphase of meiosis-I, while a low value of quadrivalents occurred in all populations, except in the Kalisakandi and Ghasre Babak populations of *C. hamulosus*. Quadrivalent formation in diploid *Carduus* species indicates the occurrence of heterozygotic translocation which, in turn, brings about more genetic variation in the next generation to be used by these taxa. The ANOVA test showed no significant difference in the relative chiasma frequency and distribution, as well as in chromosomes association among the studied *Carduus* species (data not shown).

### Meiotic abnormalities

Almost in all studied species, laggard chromosomes were observed during anaphase I and II, which could form micronuclei in telophase I and II (Fig. 1, J). Chromosome stickiness also occurred from the early stages of prophase to late telophase. The number of chromosomes involved in stickiness varied from one to many, forming a complete clump in the metaphase (Fig. 1, I). Some meiocytes with a double chromosome number were also observed, possibly due to desynapsis (Fig. 1, K). Owing to failure of anaphase I and II, tripolar cells were formed in the studied species (Fig. 1, N-P).

Larger-size pollen grains (potential, unreduced pollen grains) were observed, along with smaller-size pollen grains (normal, reduced pollen grains) in the studied *Carduus* species (Fig. 1, Q-S).

### B-chromosomes

B-chromosomes (Bs) of (0–2) were observed in most studied species and populations (Fig. 1, L). They were smaller than A-chromosomes and did not pair with them. Being included in the telophase nucleus, Bs could be arranged along the equator and move to the poles. B-chromosomes have been reported in *Carduus crispus* L. 0–2 (Krogulevich & Rostovtseva 1984), *C. crispus* subsp. *crispus* 0–4 (Májovský & Váchová 1986), *C. defloratus* L. 0–4 (Lippert & Heubl 1988), *C. defloratus* subsp. *deflarus* L. 0–5 (Gremaud 1981), and *C. defloratus* subsp. *rhaeticus* Rothm. 0–2 (Gremaud 1981). Therefore, this is the first report of the occurrence of B-chromosomes in *C. hamulosus* subsp. *hystrix*, *C. thoermeri*, *C. onopordioides*, *C. pycnocephalus* subsp. *albidus*, *C. pycnocephalus* subsp. *Pycnocephalus*, and *C. transcaspicus* subsp. *macrocephalus*.

### Karyotype

The somatic chromosome numbers and details of karyotypes of the studied *Carduus* species are presented (Table 4) (Figs 2 & 3). *C. hamulosus* subsp. *hystrix*

populations of Kalisakandi, Ardabil, Ahaar, Ghasre Babak, Sabalan, Lahrood, Fandoghloo, and Khalkhal had chromosome numbers  $2n=2x=16$ , while Yoosh population of this species had  $2n=4x=32$ .

The other values of the chromosome numbers were as follows: *C. thoermeri*  $2n=16$ , *C. onopordioides*  $2n=30$ , *C. pycnocephalus* subsp. *albidus*  $2n=60$ , *C. arabicus* subsp. *arabicus*  $2n=34$ , Qoturesue population of *C. transcaspicus* subsp. *macrocephalus*  $2n=14$ , and Polezanguleh population of this species with  $2n=34$ .

The size of the longest chromosome varied from 0.98  $\mu\text{m}$  in Yoosh population of *C. hamulosus* subsp. *hystrix* to 2.66  $\mu\text{m}$  in *C. thoermeri*, while the size of the shortest chromosomes varied from 0.58  $\mu\text{m}$  in Yoosh population of *C. hamulosus* subsp. *hystrix* to 1.69  $\mu\text{m}$  in Qoturesue population of *C. transcaspicus* subsp. *macrocephalus* (Table 4). Similarly, the lowest haploid and mean chromosome length occurred in Yoosh population of *C. hamulosus* subsp. *hystrix* (1.55  $\mu\text{m}$ ), while the highest value occurred in Qoturesue population of *C. transcaspicus* subsp. *macrocephalus* (Table 4).

The highest value of Coefficient of Variation (CV) (39.00) for chromosome size was recorded in *C. pycnocephalus* subsp. *Albidus*, indicating the highest degree of size variation among its chromosomes, while the least CV value (14.00) was registered in Kalisakandi population of *C. hamulosus* subsp. *hystrix*.

**Table 4.** Karyotype features of the *Carduus* species.

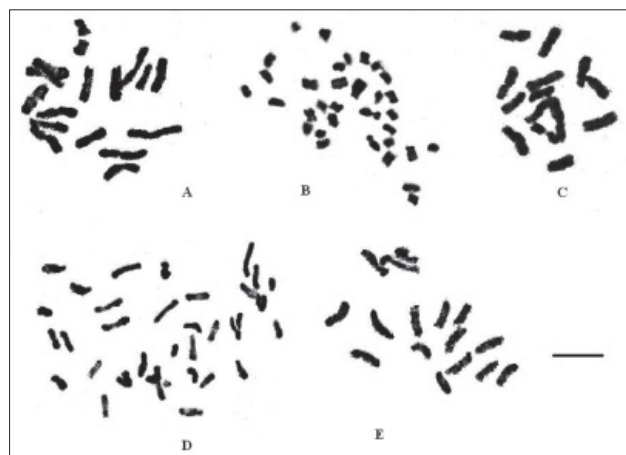
Species	Locality	2n	2x	L	S	L/S	X	ST	A1	A2	TF%	CV	Karyotype formulae
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Klisa kandi	16	2x	1.58	1.39	1.14	2.97	1A	0.89	0.14	47	14	7m+1M
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Ardabil	16	2x	2.81	1.44	1.95	4.26	2B	0.53	0.28	34	28	3m+5sm
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Ahar	16	2x	2.61	1.64	1.59	4.25	1A	0.64	0.20	38	20	5m+3sm
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Ghasre Babak	16	2x	2.35	1.58	1.49	3.92	1A	0.69	0.20	40	20	7m+1sm
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Khalkhal	16	2x	2.09	1.27	1.65	3.36	2B	0.62	0.26	38	26	6m+2sm
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Lahrod	16	2x	2.54	1.55	1.63	4.09	2A	0.62	0.20	38	20	5m+3sm
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Fandoghloo	16	2x	2.42	1.38	1.75	3.81	2B	0.60	0.33	36	33	4m+4sm
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Yoosh	32	4x	0.98	0.58	1.69	1.55	2B	0.59	0.23	37	23	8m+7sm+1st
<i>C. hamulosus</i> subsp. <i>hystrix</i>	Sabalan	16	2x	1.39	0.94	1.48	2.33	1B	0.67	0.33	40%	33	12m+1sm
<i>C. thoermeri</i>	Ghasre Babak	16	2x	2.66	1.42	1.87	4.08	2B	0.57	0.24	35	24	3m+5sm
<i>C. onopordioides</i>	Qoture	30	2x	1.29	0.80	1.62	2.09	1B	0.63	0.28	38	28	12m+2sm+1t
<i>C. pycnocephalus</i> subsp. <i>albidus</i>	Zanjan	60	2x	1.57	1.05	1.50	2.62	2C	0.67	0.39	40	39	23m+6sm+1st
<i>C. arabicus</i> subsp. <i>arabicus</i>	Kashan	34	2x	2.29	1.42	1.62	3.71	2B	0.62	0.28	38	28	11m+6sm
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	Qoturesue	14	2x	2.34	1.69	1.39	4.02	1A	0.73	0.17	42	17	6m+1sm
<i>C. transcaspicus</i> subsp. <i>macrocephalus</i>	Polezanguleh	34	2x	1.27	0.79	1.61	2.05	2B	0.63	0.23	38	23	11m+5sm+1t

**Abbreviations:** L = size of the longest chromosome ( $\mu\text{m}$ ), S = size of the shortest chromosome ( $\mu\text{m}$ ), L/S = ratio of the longest/shortest chromosome, X = mean chromosome length ( $\mu\text{m}$ ), ST = Stebbins' class, A1 and A2 = Romero-Zarco indices, TF% = total form percentage, and CV = Coefficient of Variation.

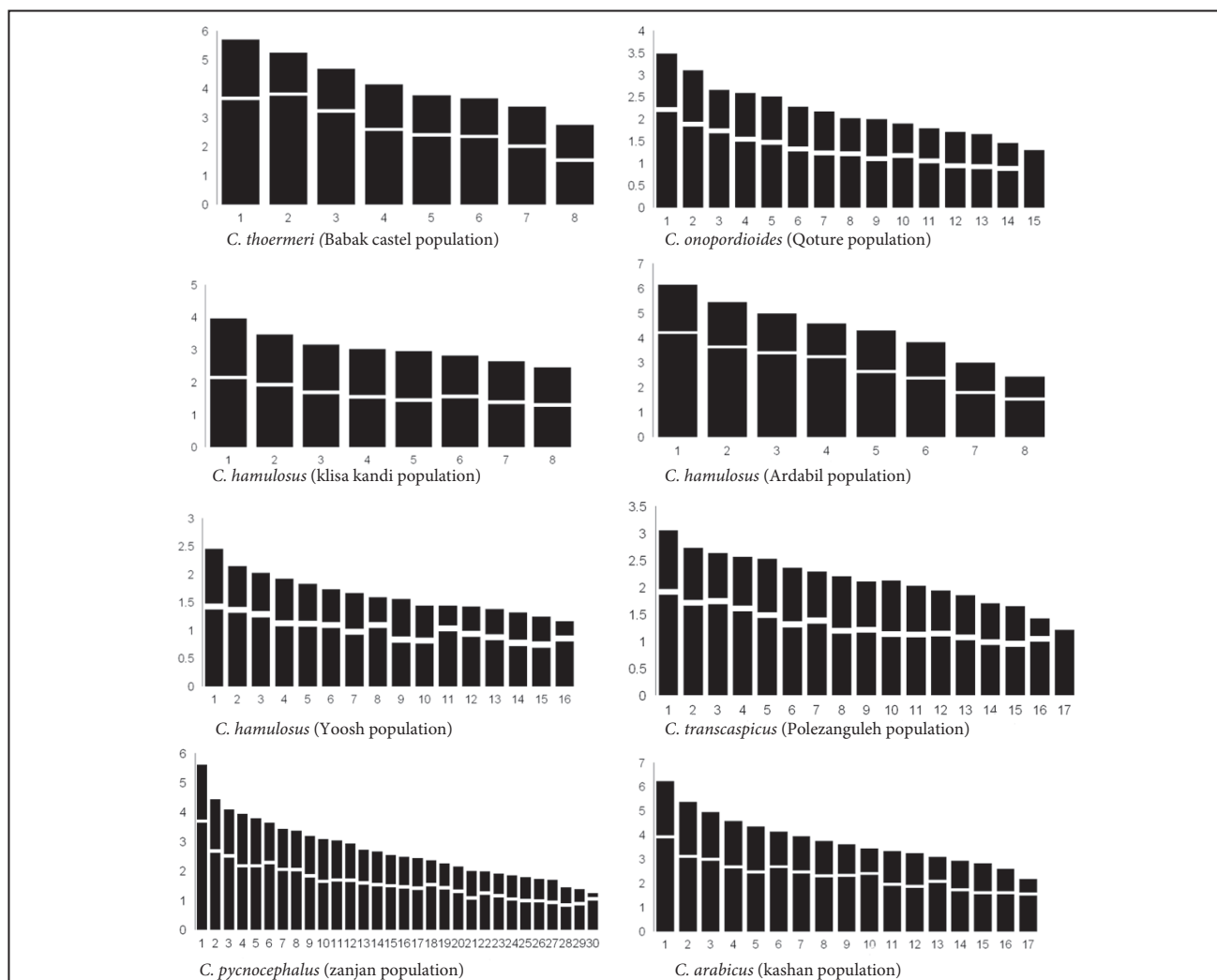
The total form percentage value (TF%) varied from 34 in Ardabil population of *C. hamulosus* subsp. *hystrix* to 47 in Kalisakandi population of the same species, a higher value of TF% indicates the presence of a relatively more symmetrical karyotype. The studied species also differ in their karyotype formulae (Table 4).

The studied *Carduus* species fell into 1A, 2A, 1B, 2B and 2C classes, according to Stebbins' karyotype classification. Zanjan population of *C. pycnocephalus* subsp. *albidus* showed the most asymmetrical karyotype (2C class) among the studied species.

Pearson correlation of the karyotype features showed a positive significant correlation between the somatic chromosome number, A2 index and CV ( $r=0.62$ ,  $p<0.01$ ), and significant negative correlation with the size of the longest chromosome, size of the shortest chromosome and the mean chromosome length ( $r=-0.60$ ,  $p<0.01$ ).



**Fig. 2.** Representative somatic cells in the *Carduus* species. Legend: A = somatic cell showing  $2n = 16$  in Kalisakandi population of *C. hamulosus*. B = somatic cell showing  $2n = 32$  in Yoosh *C. hamulosus*. C = somatic cell showing  $2n = 14$  in Qoturesue population of *C. transcaspicus* subsp. *macrocephalus*. D = somatic cell showing  $2n = 34$  in *C. arabicus* subsp. *arabicus*. E = somatic cell showing  $2n = 16$  in Ghasre Babak population of *C. thoermeri*. Scale bar = 10  $\mu\text{m}$ .



**Fig. 3.** Idiograms of the studied *Carduus* species.

## Discussion

### Diffuse stage

Various reasons have been suggested for occurrence of the diffuse stage. These are: high synthetic activity analogous to the lampbrush stage in amphibian oocyte, shedding of the lateral elements in the synaptonemal complex, postpachytene elimination or modification of histone proteins, and meiotic arrest to withstand the adverse environmental conditions (Sheidai & Inamdar 1991). As *Amaranthus* species grow wild under different environmental conditions, the occurrence of diffuse stage may be considered as adaptation to such variable conditions. However, the exact reason for occurrence of the diffuse stage in *Carduus* species remains presently unknown. Occurrence of the diffuse stage has been reported in several plant species (Sybenga 1992). Diffuse stage may be complete, when the whole chromosomes are decondensed, or it may be partial when some parts of the genome show decondensation. The present study showed occurrence of the partial/complete diffuse stage in the *Carduus* species.

### Chromosome number

Kuzmanov & al. (1981) reported  $2n=2x=22$  for *C. hamulosus*, while we are reporting  $2n=16$  and  $26$ , which are new chromosome numbers, indicating the role of aneuploidy in cytological evolution of *C. hamulosus*. The chromosome number  $2n=2x=34$  reported here for *C. transcaspicus* supports the earlier report of Ghaffari (1989), but in the present study  $2n=2x=14$  is recorded and indicates the occurrence of two basic chromosome numbers of 7 and 17 in this species. Similarly, in the present study  $2n=4x=32$  was found for *C. thoermeri*, while Kuzmanov & al. (1986) and Krasnikov (1991) reported  $2n=2x=16$ , and Kuzmanov & al. (1991) reported  $2n=2x=22$  for this species. Therefore, in the present study a new polyploidy level is recorded for this species.

The present study supports an earlier report of Zemskova & Ciklauri (1987) for *C. onopordioides* ( $2n=4x=30$ ) and of Díaz Lifante & al. (1992) for *C. pycnocephalus* subsp. *albidus* and subsp. *pycnocephalus* ( $2n=60$ ). However, other authors reported  $2n=32$ ,  $64$ ,  $62$ ,  $60$ ,  $54$  for this species (Kliphuis & Barkoudah 1977; Devesa 1981; Ghaffari 1989; Kuzmanov & al. 1991).

Occurrence of different chromosome and base numbers has been reported in some of the *Carduus* species, including *C. acanthoides*  $2n=2x=16$  and  $2n=2x=22$  (Wentworth & al. 1991; Lövkvist & Hultgård 1999), *C. thoermeri*  $2n=2x=16$  and  $2n=2x=22$  (Kuzmanov & al. 1986; Krasnikov 1991; Kuzmanov & al. 1991), *C. pycnocephalus*  $2n=2x=32$ ,  $2n=4x=64$ ,  $62$ ,  $60$ ,  $54$  (Kliphuis & Barkoudah 1977; Devesa 1981; Ghaffari 1989; Díaz-Lifante & al. 1992; Kuzmanov & al. 1991), and *C. seminudus*  $2n=2x=32$ ,  $28$  (Zemskova & Ciklauri 1987; Ghaffari 1999). These data indicate the role of chromosome number changes and aneuploidy in the evolution of the genus *Carduus*.

### Chromosome pairing, meiotic abnormalities and karyotype analyses

The ANOVA test showed no significant difference in the relative chiasma frequency and distribution, nor in the chromosomes association among the studied *Carduus* species (data not shown), indicating the presence of a sort of control mechanism over the relative values of chiasma formation per chromosome in the genus. Such changes and control mechanisms are of adaptive nature (Quicke 1993), as is also reported in other plant species (Rees & Jones 1977).

Meiotic abnormalities observed in the studied *Carduus* species may cause certain degree of pollen sterility. The  $\chi^2$  test for meiotic abnormalities has shown significant difference among the studied species and populations, indicating the species genetic differences.

The presence of giant pollen grains has been used as an indication of the production of  $2n$  pollen (Bretagnolle & Thomson 1995). Unreduced gametes are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Bretagnolle & Thomson 1995), which has been considered the major route to formation of naturally occurring polyploids. Different cytological mechanisms are responsible for the production of  $2n$  gametes (Bretagnolle & Thomson 1995). Detailed cytological study of the *Carduus* species indicates the possible role of anaphase failure and tripolar formation, as well as of unreduced meiocyte formation (possibly due to desynapsis) as the main mechanisms for unreduced gamete formation. Polyploidy is of wide occurrence in *Asteraceae* (Semple



& Watanabe 2009) and has been noted in 570 genera, with polyploidy level ranging from 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 12x, 14, 15x, 16x, 18x, 20x, 22x, 24x, 32, 36 and 38x (Semple & Watanabe 2009). Dysploidy and reduction in the base chromosome number is known to occur in *Asteraceae* and is considered one of the main cytological mechanisms in this family (Semple & Watanabe 2009). However, the present study reports the occurrence of unreduced (2n) pollen grains and its possible role in polyploidy production as another cytological mechanism operating in this family.

B-chromosomes are accessory chromosomes reported in about 1300 plant species (Camacho & al. 2000) and, when present in high number, may affect negatively the growth and vigor of the plants; in low number they can benefit them. These chromosomes are known to change the chiasma frequency and genetic recombination in the cells where they are present. However, we did not encounter many cells possessing B-chromosomes, so as to analyze statistically the effect of B-chromosomes on the chiasma frequency. We could not find any references dealing with the effect of B-chromosomes in the *Carduus* species, but in the genus *Cirsium*, which is closely related to *Carduus*, they have been considered to play a role in the genome size variation (Bureš & al. 2004) and also in changing the chiasma frequency (Nouroozi & al. 2010). Occurrence of B-chromosomes has been reported in 45 genera of *Asteraceae* (Semple & Watanabe 2009).

Variations in the size of chromosomes, type of chromosomes, TF%, CV and Stebbins' class, all indicate the role played by the structural changes of chromosomes in diversification of the *Carduus* species. Pearson correlation determined among the karyotype features shows that with the increase in somatic chromosome number, the degree of asymmetry and change in chromosomes size also increases bringing about more variations in the karyotype of these species, while the mean chromosome size decreases significantly, which is due to loss of chromatin.

Therefore, apparently the different cytological mechanisms, including change in the chromosome number, occurrence of B-chromosomes and unreduced pollen grain formation, structural changes of chromosomes, along with hybridization, may have played a role in diversification of the *Carduus* species.

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