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Karyotype analysis and chromosome number for two *Cirsium* taxa (Asteraceae) in Iran

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Keywords: Asteraceae, B-chromosome, *Cirsium*, karyology, mitotic study.

Summary. *Cirsium* Mill. contains more than 250 species in the world mainly distributed in the Northern hemisphere. Different chromosome numbers with different ploidy levels were reported in this genus. In this study, karyotype details and chromosome numbers were established for two *Cirsium* taxa in Iran. *C. ciliatum* subsp. *szovitsii* and *C. echinus* had the mitotic chromosome numbers of $2n = 2x = 34$. Karyotype analyses showed that chromosomes were generally metacentric and sub-metacentric. In *C. echinus,* Lowshan population had the longest chromosome (19.10 μm) and Heyran Canyon population (4.73 μm) the shortest one while in *C. ciliatum*, the longest chromosome was observed in Urmia to Salmas population (14.67 μm) and the shortest one (4.71 μm) in Doshanlu population. Total haploid chromosome length ranged from 275.29 to 376.42 μm in populations studied. Both taxa were grouped in 2B class. B-chromosomes were recorded for two taxa studied too. Chromosome type, mitotic chromosome numbers and occurrence of B-chromosomes were in agreement with previous results (Albers, Pröbsting, 1998; Lövkvist, Hultgård, 1999; Yüksel et al., 2013; Yildiz et al., 2016).

Анализ кариотипа и число хромосом двух видов *Cirsium* (Asteraceae) флоры Ирана

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Ключевые слова: В-хромосома, кариология, митотическое исследование, Asteraceae, *Cirsium*.

Аннотация. В мировой флоре род *Cirsium* Mill. содержит более 250 видов, в основном распространенных в Северном полушарии. Для видов этого рода характерно варьирование числа хромосом и кариотипы с разным уровнем плоидности. Для *Cirsium ciliatum* subsp. *szovitsii* и *C. echinus*, произрастающих в Иране, установлено

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митотическое число хромосом $2n = 2x = 34$. Кариотип обоих видов представлен в основном метацентрическими и субметацентрическими хромосомами. У *C. echinus* наибольшая длина хромосомы (19,10 мкм) отмечена в популяция из окр. г. Ловшан, наименьшая (4,73 мкм) – в популяции из каньона Хейран. Для *C. ciliatum* эти значения составили, соответственно, 14,67 (популяция из окг. г. Сельмас) и 4,71 мкм (популяция из окр. дер. Дошанлу). В изученных популяциях длина гаплоидных хромосом варьирует от 275,29 до 376,42 мкм. Оба таксона отнесены к классу 2B, у обоих зарегистрированы B-хромосомы. Выявленные особенности и значения (тип хромосом, число митотических хромосом и встречаемость B-хромосом) согласуются с результатами предыдущих исследований (Albers, Pröbsting, 1998; Lövkvist, Hultgård, 1999; Yüksel et al., 2013; Yildiz et al., 2016).

Introduction

Cirsium Mill. (Asteraceae) with more than 250 taxa is a large genus that is distributed in holarctic with several diversification centers (Zomlefer, 1994). These taxa show high diversity from the northern Mediterranean area over to Caucasus (Häffner, Hellwig, 1999). *Cirsium* species are classified in five sections, as *Pseudepitrachys* Petrak, *Echenias* (Cass.) Petrak, *Cephalonoplos* (Neck.) DC., *Cirsium* and *Epitrachys* DC. in Iran (Petrak, 1979).

The first chromosome counting for *Cirsium* dated back to Aishima (1934) as $x = 17$ and two ploidy levels (diploid and tetraploid) were defined. Frankton and Moor (1963) proposed $x = 17$ for the elements of tribe *Cardueae*. The presence of aneuploidy in *Cirsium* of the new world was recorded by Ownbey and Olson (1969). Some of the cytological studies in this genus related to the definition between *Cirsium* and *Carduus* L., based on the basic chromosome number (Frankton, Moore, 1961; Hedberg I., Hedberg O., 1977). Some researches illustrated that the lower chromosome number was correlated with the more assymetrical chromosomes. Bureš et al. (2004) studied the chromosomes of 17 *Cirsium* species and stated that B-chromosome presence had significant effect on the increase or decrease of chiasmata frequency and the obtained genetic variation in gametes.

The cytological studies of *Cirsium* in Turkey showed different chromosome numbers of $2n = 32$, 34, 60 and 68 (Melahat et al., 2008). The most common chromosome number was $2n = 34$ (diploids) and $2n = 68$ (tetraploids). Nourouzi et al. (2010) studied meiosis in 17 *Cirsium* species of Iran. They recorded 0–2 B-chromosomes. Meiotic abnormalities were recorded in some species. Yüksel et al. (2013) studied the karyotype properties of 10 *Cirsium* species of sect. *Epitrachys* in Turkey.

Despite much attention to the taxonomy and morphology of *Cirsium* species, karyological records for the genus are scarce. Karyology of Iranian *Cirsium* species has been studied to clarify their taxonomy and make contributions to other multidisciplinary studies on the genus (Nouroozi et al., 2010, 2011; Sheidai et al., 2012). In this study karyotype analyses and chromosome numbers of two *Cirsium* taxa are presented.

Materials and Methods

Karyotype study was done on two *Cirsium* taxa: *C. echinus* (M. Bieb.) Hand.-Mazz. and *C. ciliatum* subsp. *szovitsii* (K. Koch) Petr. To achieve the chromosome numbers of taxa studied, six accessions including four accessions of *C. echinus* and two accessions of *C. ciliatum* subsp. *szovitsii* were gathered from nature (Table 1). Cypselas were sterilized by 50 % H_2O_2 for 10–15 min., washed by sterile water for 5 min., then put in Petri dishes and kept at room temperature until germination. Growing root tips with 1–2 cm in length were pretreated with

Table 1

Voucher details of *Cirsium* taxons studied

Locality, collector and voucher number	Pop no.	Taxon
Gilan prov., Asalem to Khalkhal, Asbdavani village, 2000 m, Norouzi, Babaee (ALUH 910)	1	*C. echinus* (M. Bieb.) Hand.-Mazz.
Gilan prov., Rudbar, Lowshan, 353 m, Norouzi, Babaee (ALUH 912)	2	
Gilan prov., Astara, Heyran Canyon, 860 m, Norouzi, Babaee (ALUH 908)	3	
East Azerbaijan prov., Kiamaky Canyon, 1999 m, Norouzi, Babaee (ALUH 909)	4	
Ardebil prov., Meshgin Shahr, Doshanlu, 1170 m, Norouzi, Babaee (ALUH 902)	5	*C. ciliatum* subsp. *szovitsii* (K. Koch) Petr.
West Azerbaijan prov., Urmia to Salmas, 1282 m, Norouzi, Babaee (ALUH 904)	6	

0.002 m 8-hydroxyquinoline for 3 hours at 4 °C (7–9 AM). Then they were fixed with Carnoy solution (1 : 3 glacial acetic acid/absolute ethanol) for 24 h. at 4 °C. Materials were hydrolyzed in 1 N HCl for 20 min. at 60 °C bath and washed by sterile water.

Meristematic regions were stained with 1 % aqueous Aceto-orcein for 60 min. at 60 °C bath and squashed on slides with one drop of 45 % glacial acetic acid. At least 5–10 well prepared metaphase plates for each population were photographed with Olympus BX-51 microscope and measured by IdeoKar 1.0 software.

The chromosomes were identified based on Levan et al. (1964). Karyotype asymmetry indices as variation of chromosome length (CV_{CL}) (Paszko, 2006), coefficient variation of centromeric index (CV_{CI}), mean centromeric asymmetry (M_{CA}) and mean centromeric index (M_{CI}) (Paszko, 2006) and intra and inter-chromosomal asymmetry (A1 and A2 respectively) (Zarco, 1986) were determined.

Results

In present study, karyotype analyses were done for two taxa (*C. echinus* and *C. ciliatum* subsp. *szovitsii*). Chromosome counts were $2n = 2x = 34$ with the basic chromosome number $n = 17$ (Table 2). Based on Stebbins symmetry class, populations of two taxa studied were grouped in 2B class. The taxa studied differed in karyotype formula. They had metacentric (m) and sub-metacentric (sm) chromosomes (Figs 1, 2). In studied populations of *C. echinus,* the longest chromosome was observed in Lowshan population and the smallest in Heyran Canyon one. Among populations studied of *C. ciliatum* subsp. *szovitsii*, the longest chromosome (14.67 µm) was observed in Urmia to Salmas population and the shortest one (4.71 µm) was observed in Doshanlu population.

Lowshan population of *C. echinus* had the longest chromosome length (19.10 µm) and Doshanlu population of *C. ciliatum* subsp. *szovitsii* had the shortest (4.71 µm). Doshanlu population of *C. ciliatum* subsp. *szovitsii* had the shortest total haploid length (275.29 µm) while Heyran canyon population of *C. echinus* had the longest (376.42 µm). A1 index varied from 0.28 in Doshanlu population of *C. ciliatum* subsp. *szovitsii* to 0.36 in Lowshan population of *C. echinus*. A2 index ranged from 0.27 (Doshanlu population of *C. ciliatum* subsp. *szovitsii*) to 0.40 (Kiamaky Canyon population of *C. echinus*). The highest value of coefficient variation of centromeric index (CV_{CI}) was observed in Urmia to Salmas population of *C. ciliatum* subsp. *szovitsii* (18.35) and the lowest value of CV_{CI} was observed in Lowshan population of *C. echinus* (14.91). Kiamaky Canyon population of *C. echinus* showed the highest value of coefficient variation of chromosome length (CV_{CL}) (39.82) and Doshanlu population of *C. ciliatum* subsp. *szovitsii* showed the lowest (26.77).

Table 2

Summary of karyotype features in *Cirsium* populations studied

KF	ST	TF%	M_{CI}	M_{CA}	CV_{CL}	CV_{CI}	THL	L/S	Range S–L	A2	A1	Pop no.	Taxon
12m + 5sm	2B	39.53	0.40	20.86	28.52	14.95	355.81	2.25	6.95–15.62	0.29	0.33	1	*C. echinus*
14m + 3sm	2B	38.32	0.38	23.20	29.65	14.91	328.71	2.76	6.92–19.10	0.30	0.36	2	
12m + 5sm	2B	39.86	0.39	21.26	32.54	17.77	376.42	3.82	4.73–18.06	0.33	0.33	3	
13m + 4sm	2B	39.60	0.40	20.41	39.82	17.58	322.03	2.86	5.46–15.61	0.40	0.32	4	
12m + 5sm	2B	41.39	0.41	17.88	26.77	16.90	275.29	2.72	4.71–12.83	0.27	0.28	5	*C. ciliatum* subsp. *szovitsii*
12m + 5sm	2B	40.21	0.40	19.32	28.36	18.35	304.40	2.80	5.25–14.67	0.28	0.30	6	

Abbreviations: A1: intrachromosomal asymmetry, A2: interchromosomal asymmetry, S: size of the shortest chromosome pair, L: size of the longest chromosome pair, THL: total haploid length, CV_{CI}: coefficient variation of centromeric index, CV_{CL}: variation of chromosome length, M_{CA}: mean centromeric asymmetry, M_{CI}: mean centromeric index, TF: total form percentage, ST: Stebbins' symmetry class, KF: karyotype formulae.

Lowshan population of *C. echinus* was characterized by the lowest value of mean centromeric index (M_{CI}) (0.38) and total form percentage (TF %) (38.32) while Doshanlu population of

C. ciliatum subsp. *szovitsii* was characterized by the highest value of M_{CI} (0.41) and TF % (41.39). These two population had the highest (23.20) and the lowest (17.88) value of mean centromeric asymmetry (M_{CA}) vice versa.

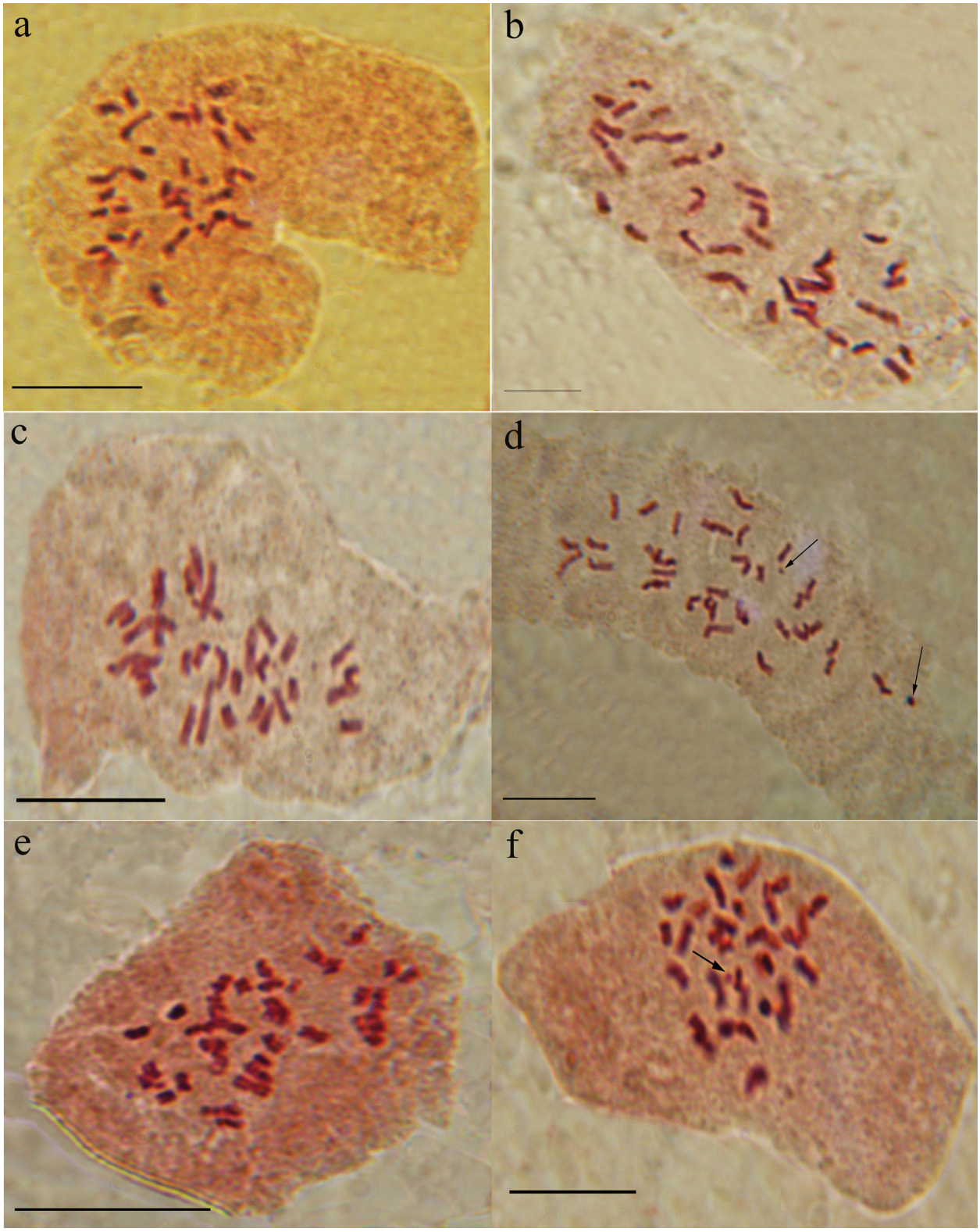

Fig. 1. Somatic chromosomes of *Cirsium* taxa studied: a – Asbdavani village population; b – Lowshan population; c – Heyran Canyon population; d – Kiamaky Canyon population in *C. echinus*; e – Doshanlu population; f – Urmia to Salmas population of *C. ciliatum* subsp. *szovitsii* (scale bar: 50 micrometers; Arrows show the B-chromosome).

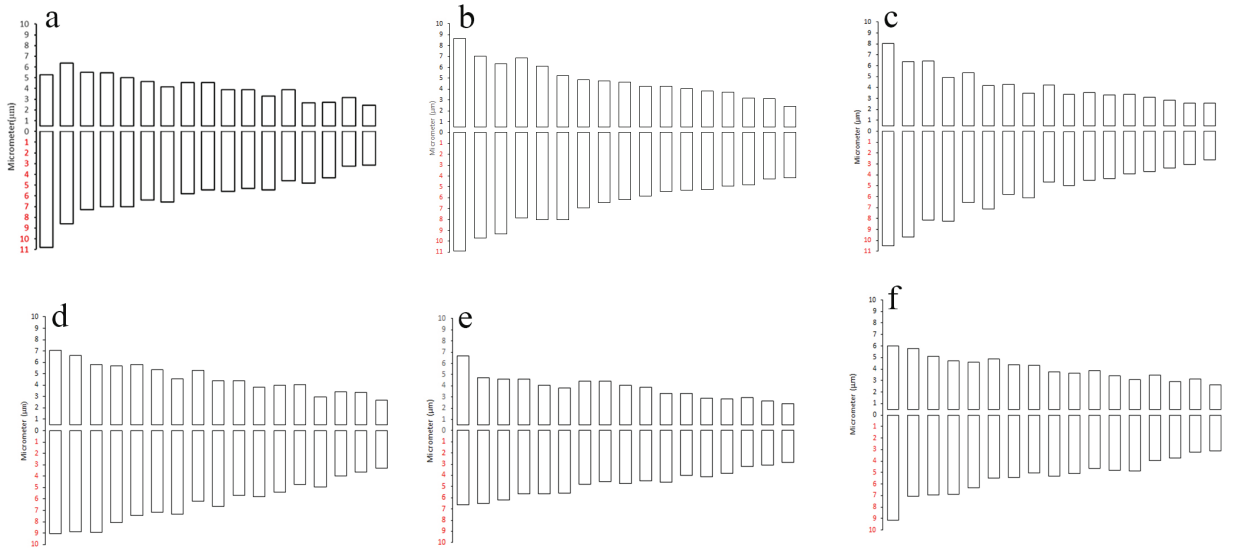

Fig. 2. Haploid ideograms of *Cirsium* taxa studied: a – Asbdavani village population; b – Lowshan population; c – Heyran Canyon population; d – Kiamaky Canyon population of *C. echinus*; e – Doshanlu population; f – Urmia to Salmas population of *C. ciliatum* subsp. *szovitsii*.

Discussion

Obtained chromosome counts for *C. ciliatum* subsp. *szovitsii* and *C. echinus* ($2n = 2x = 34$) were in concordant with the previous findings of Tonian (1981, 1982), Yüksel et al. (2013) and Yildiz et al. (2016). Most *Cirsium* species are diploid ($2n = 34$) (Ownbey et al., 1975). In Armenia, chromosome count for *C. ciliatum* was recorded as $2n = 4x = 68$ (Tonian, 1982) while in Turkey Melahat et al. (2008) recorded $2n = 2x = 34$. Nouroozi et al. (2010) recorded miotic chromosome number in 17 species of *Cirsium* with chromosome numbers of $2n = 2x = 34$ and $2n = 4x = 68$. There is no report on mitotic number of *Cirsium* species in Iran.

In present study, chromosome length varies between 4.71 to 19.10 micrometer. The ratio of longest to shortest chromosome varies between 2.25 to 3.82. Yüksel et al. (2013) analyzed the karyotype features of ten *Cirsium* species of sect. *Epitrachys* in Turkey. They stated that generally the chromosome number for these species is $2n = 2x = 34$ and the chromosome length is of taxonomic use to separate species.

Karyotype analyses showed that *Cirsium* species are of m and sm types that is in agreement with Yüksel et al. (2013). Based on karyotype asymmetry parameters, Doshanlu population of *C. ciliatum* subsp. *szovitsii* had higher value of TF % and represented more symmetrical karyotype. Lowshan population of *C. echinus* with the highest value of A1 index showed more asymmetrical karyotype.

In present study, B-chromosome are recorded for the two taxa studied for the first time in Iran. B-chromosomes were 0 to 2 in species studied, rounded and very smaller than A-chromosomes. B-chromosomes were recorded for some *Cirsium* species as *C. acaule* (L.) Scop. (0–2) (Lövkvist, Hultgård, 1999), *C. arvense* (L.) Scop. (0–1) (Lövkvist, Hultgård, 1999) and *C. oleraceum* Scop. (0-2) (Albers, Pröbsting, 1998). B-chromosomes are present in both diploid and tetraploid *Cirsium* species so it seems that there is no advantage for their occurrence due to ploidy levels. Further studies of the karyotype properties will have implications in the systematics of the genus *Cirsium* in Iran.

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