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Cytogenetic studies in the *Centaurea aucheri* group (sect. Phaeopappus)

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Abstract. In this study, it was aimed to evaluation of the revision in *Centaurea aucheri* group by the cytogenetical studies and determines the chromosome number for *C. albonitens* species, to determine chromosome morphology and karyotype analysis. Results of meiotic behavior and karyomorphologycal parameters of *Centaurea aucheri* group indicated that raised this group in five distinct species are correct. Somatic and gametic chromosome numbers indicated that *C. albonitens, C. assadii, C. farsestanica, C. indistincta* and *C. phaeopappa* are diploid with n=9 and 2n=18 and *C. aucheri* is tetraploid with n=18 and 2n= 4x= 36+0-2B. Gametic chromosome number and karyomorphology of *C. aucheri* and *C. indistincta* species is reported here for the first time. Also, meiotic behavior and karyomorphology parameters of *C. albonites, C.assadi , C. farsestanica* and *C. phaeopappa* are newly reported here. Analysis of karyotype and behavior of meiosis indicated that *C. aucheri* is a natural allotetraploid species. Karyotype symmetry parameters showed that all the studied species were classified in the class 2A except of *C. aucheri* which was located in class 2B.

Keyword: Allotetraploid, *Centaurea aucheri*, Cytogenetic, Karyomorphology, Meiotic behavior.

INTRODUCTION

Centaurea, as the fourth largest genus among the genera in Asteraceae and also the second largest genus in the tribe Cardueae consist of 200–250 species placed in 40 sections (Wagenitz and Hellwig 1997; López et al. 2011 Hilpold et al. 2014). The present classification of *Centaurea* in 40 sections is highly problematic (Wagenitz 1975) and does not take into account all of the pollen, karyological and carpological evidence (Susanna et al. 1995). In Flora Iranica 88 species belong to 28 sections are reported (Wagenitz 1980). *Centaurea* section *Phaeopappus* (DC.) O. Hoffm.is one of the section comprising of five species in Flora Iranica. Of these, 3 taxa viz: *C. albonitens, C. aucheri* and *C. spectabilis* are distributed in Iran (Wagenitz 1980). Ranjbar and Heydari (2016) elevated this section to 14 species in the world, which 12 are belongs to Iran (10 species are endemic to the country). In flora of Iran 5 subspecies has been cited for *C. aucheri* viz: subsp. *aucheri*, subsp. *elbur-*

sensis, subsp. *farsistanica*, subsp. *indistincta* and subsp. *szowitsii* (Wagenitz 1980; Mozafarian 2018). Ranjbar and Negaresh (2013) raised these five subspecies to the *C. aucheri* (DC.) Wagenitz, *C. assadii* Ranjbar and Negaresh, *C. farsistanica* (Wagenitz) Ranjbar & Negaresh, *C. indisticta* (Wagenitz) and *C. phaeopappa* (DC.) Schultz Bipontinus respectively based on the morphological characteristics. The aims of this present paper is evaluation of the revision in *Centaurea aucheri* group by the cytogenetical studies.

MATERIALS AND METHODS

The origin of the plant material studied here is shown in Table 1. For meiotic studies floral buds of plants found in nature were collected and immediately fixed in Piennr's fluid containing ethanol 96%, chloroform, propionic acid, 6:3:2 (v/v/v)for 24 hours at room temperature. Anthers dissected out from the buds were squashed and stained with 2% acetocarmine. Chromosome counts obtained from a minimum of 50 pollen

Table 1. The species and origin of the material examined.

Taxon	Origin	Altitude(m)	Longitude and Latitude	H. Code
Centaurea albonitens Turrill	Azerbaijan: Tabriz, 20 Km towards Marand	1360	E:46°03'57"; N:38°15'48"	109080
	Azerbaijan: between Khoy and Salmas	1530	E: 44°54'15"; N: 38°24'39"	109081
	Zanjan, 10 Km towards Miyaneh	1500	E: 47°27'35"; N: 37°12'36"	109101
	Zanjan, 30 Km towards Miyaneh	1600	E: 47°30'53"; N: 37°14'41"	109102
Centaurea aucheri	Kordestan:Saqqez to Divandareh	2200	E: 46°54'15"; N: 36°24'39"	109091
	Kordestan: W Saqqez	2160	E: 46°16'13"; N: 36°15'39"	109092
	Qazvin: Avaj, 10 Km. to Hamadan	2400	E: 49°09'38"; N: 35°32'17"	109201
	Qazvin: Neck of Avaj	2320	E: 49°12'05"; N: 35°35'35"	109202
	Qazvin: Abgarm to Avaj	2300	E: 49°14'07"; N: 35°42'58"	109203
	Hamadan: 90 Km. to Qazvin	2070	E: 49°02'19"; N: 35°27'54"	109301
	Lorestan: Doroud, Gahar, Saravand	2110	E: 49°10'03"; N: 33°22'26"	109421
	Markazi: Tafresh, Noghre-kamar	2080	E: 50°08'13"; N: 34°41'53"	109501
Centaurea assadii	Azerbaijan:Miyaneh to Bostanabad	1720	E: 47°18'27"; N: 37°32'05"	109087
	Tehran: Kouhdashteh	1800	E: 51°20'13"; N: 35°44'23"	109701
	Tehran: Kouhdashteh	1950	E: 51°23'50"; N: 35°44'29"	109702
	Tehran: Sorkheh hesar	1500	E: 51°36'03"; N: 35°43'12"	109714
	Tahran: Sorkheh hesar	1700	E: 51°35'46"; N: 35°43'09"	109712
	Tehran: Jajroud	2200	E: 51°29'45"; N: 35°42'39"	109754
	Tehran: Abali	2400	E: 51°57'55"; N: 35°45'54"	109718
Centaurea farsistanica	Shiraz: Bamoo park	1750	E: 52°36'52"; N: 29°42'48"	109614
	Shiraz: Bamoo park	1820	E: 52°37'15"; N: 29°43'11"	109615
	Shiraz: Bamoo park	2000	E: 52°36'33"; N: 29°12'41"	109617
	Fars: Dasht arjan, Khers dareh	2400	E: 51°58'56"; N: 29°39'47"	109687
	Yasouj, Kakan	2300	E: 51°35'57"; N: 30°38'18"	109813
	Yasouj: Kamehr	2400	E: 51°35'38"; N: 30°39'55"	109815
Centaurea indistincta	Tehran: Kouhdashteh	1800	E: 51°20'13"; N: 35°44'23"	109706
	Tehran: Kouhdashteh	2300	E: 51°23'50"; N: 35°44'30"	109709
	Lorestan: Doroud, Gahar	2100	E: 49°10'03"; N: 33°22'25"	109432
	Zanjan	1630	E: 47°33'53"; N: 37°14'41"	109132
Centaurea phaeopappa	Karaj, Eshtehard, Dakin	1550	E: 50°27'36"; N: 35°37'18"	109745
	Qazvin: Abgarm to Avaj	2300	E: 49°14'07"; N: 35°42'58"	109208
	Tahran: Kohdashteh	1800	E: 51°20'13"; N: 35°44'24"	109784
	Karaj, Ziyaran	2000	E: 50°31'43"; N: 36°07'06"	109764
	Azerbaijan: Oroumiyeh to Mahabad	1330	E: 45°19'22"; N: 37°13'52"	109075
	Azarbaijan: Salmas to Oroumiyeh	1375	E: 45°36'08"; N: 37°34'46"	109094
	Azerbaijan: Mishodagh Mt.	2040	E: 45°38'23"; N: 38°17'50"	109049
	Qazvin: Takestan to Hamadan	2350	E: 49°01'44"; N: 35°20'46"	109257

mother cells within each collection. Actively growing root tips were used for mitotic analysis. Roots were pretreated with 0.002M, 8-hydroxyquinoline at 20˚C for 3 hr, and then fixed in 3:1 (ethanol: acetic acid). Staining was carried out with the Feulgen reaction enhanced by squashing in 2% acetocarmine. Nomenclature adopted by Levan et al. (1964) was followed for recognizing chromosome types. Both mitotic and meiotic slides were made permanent by the venetain turpentine (Wilsom 1945). Voucher specimens are preserved in the Herbarium of Research Institute of Forests and Rangelands (RIFR).

RESULTS

Centaurea albonitens *Turrill*

Centaurea albonitens is widely distributed in western Iran, especially Zanjan, west and east Azerbaijan Provinces. Five samples of this taxon showed chromosome numbers of n=9 and 2n=18 in both meiosis and mitotic respectively. Meiosis in this taxon showed 9 bivalents at diakinesis which ring tetravalent in some cells were observed (Figure 1A). Also, two bivalents were associated with the nucleolus at diakinesis, which is confirmed to presence of two satellite chromosomes in this species. First metaphase indicated 9 bivalents, which most of them were in rod shape (Figure 1B). Anaphase I showed (9-9) chromosomes segregation (Figure 1C). Somatic chromosome counts in 50 root tips disclosed a chromosome number of 2n=18 (Figure 1D, E). The representatives of chromosome set at mitotic metaphase are shown in Figure 1F and Table 2. The karyotype of *C. albonitens* consists of one pair of metacentric chromosome and 8 pairs of sub-metacentric chromosomes. The total length of the chromosomes varied from 5.18 µm to 2.96 µm (Table 2). This count (2n=18) agrees with previous reports by Garcia-Jacas et al. (1998) and Ranjbar and Negaresh (2013). Karyotype analysis and meiotic count for this taxon is reported here for the first time.

C. aucheri *(DC.) Wagenitz*

Eight samples of this taxon showed chromosome numbers of n=18 and 2n=36 in both meiosis and mitotic respectively. Meiosis showed some clamping of chromosomes at first metaphase. The bivalents at metaphase I were usually in the shape of rod with one terminal chiasmata (Figure 2A). Many cells were observed in order to ascertain the correct chromosome count. The number

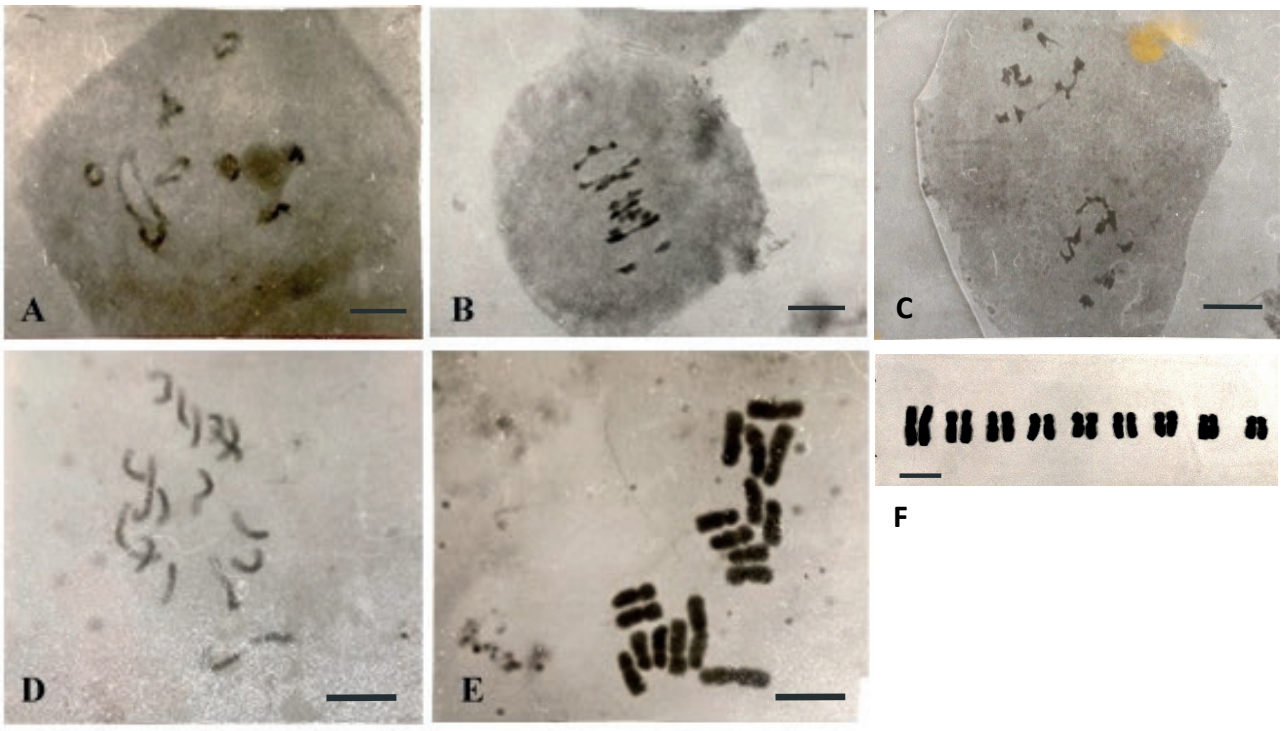

Figure 1. Meiosis and mitotic micrographs of *Centaurea albonitens*. A; Diakinesis, showing ring tetravalent (arrow). B: Metaphase I (n=9). C: Anaphase I (9-9). D: Late prophase (2n=18). E: Metaphase (2n=18). F: Karyotype showing nine pairs of chromosomes. Scale= 5µm.

Table 2. Measurement of somatic chromosomes in a diploid *C. albonitens* (Obtained from 50 cells).

No. of chromosome	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio L/S = r
1	5.18	2.59	2.59	1
2	4.44	2.59	1.85	1.4
3	3.89	2.22	1.67	1.33
4	3.33	2.22	1.11	2
5	3.33	2.04	1.29	1.85
6	3.33	2.04	1.29	1.85
7	3.14	1.85	1.29	1.43
8	2.96	1.85	1.11	1.67
9	2.96	1.85	1.11	1.67

n=18 observed at diakinesis (Figure 2B) and (18-18) segregation at first anaphase (Figure 2C). In the first meiosis stage, we did not observe any tetravalents at metaphase I and diakinesis. Also we did not found any abnormality at first and second anaphase of meiosis, which is prevalent in autopolyploids species. These results indicated that this taxon is natural allotetraploid species. Somatic chromosome counts of 50 root tips disclosed a chromosome number of 2n= 36+0-2B (Figure 2D,E). These B-chromosomes were not found at meiosis stage. The karyotype consisted of 15 metacentric pairs, and 3 submetacentric pairs (Figure 3A). The total length of the chromosomes varied from 6.11 µm to 2.85 µm (Table 3). Different chromosome number of this taxon (2n=36) with others subspecies (2n=18) and allotetraploid behav-

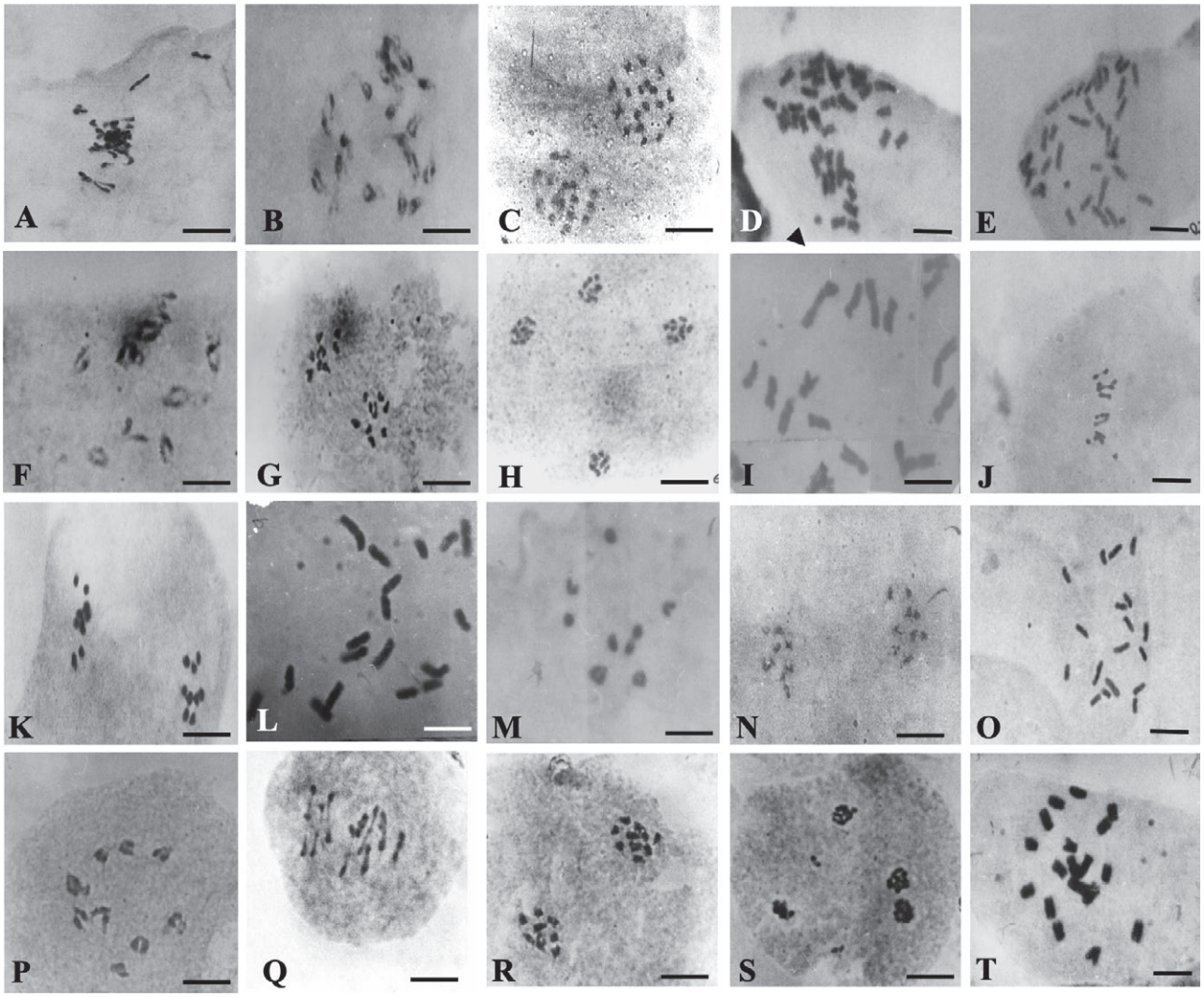

Figure 2. Meiosis and mitotic micrographs of *Centaurea aucheri* group A- E. *C. aucheri*, A: metaphase I. B: diakinesis. C: anaphase I. D: mitotic metaphase, showing B-chromosome (arrow). E: mitotic metaphase, showing 2B-chromosomes (arrows). Scale = 5µm.

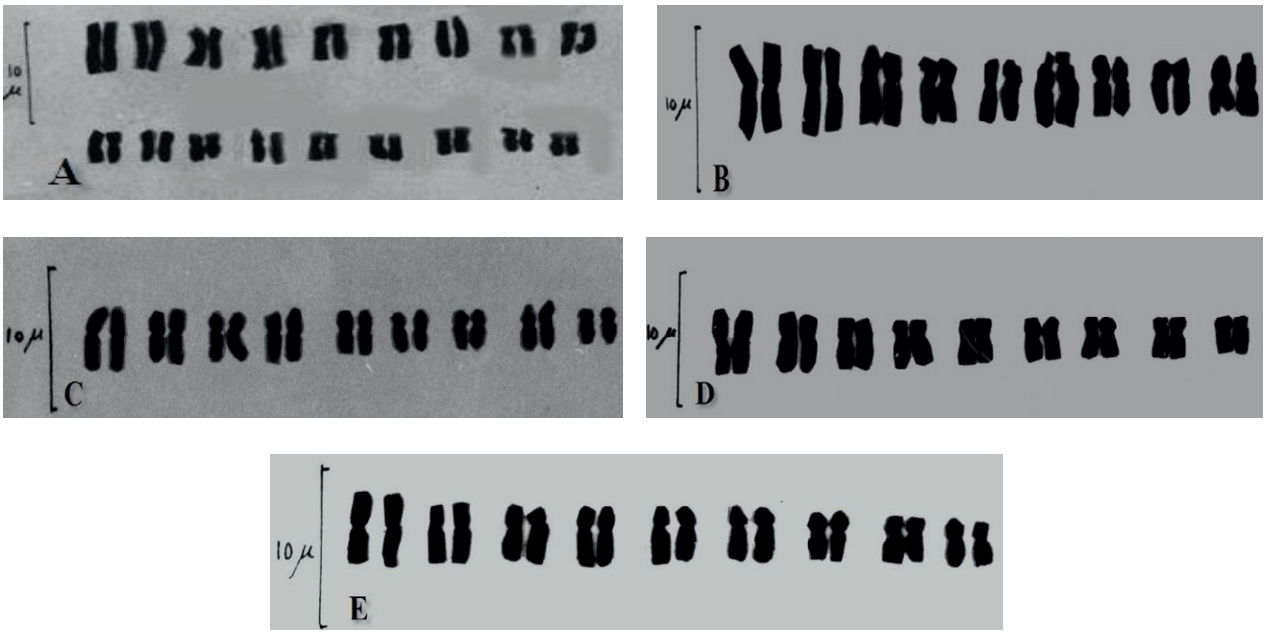

Figure 3. A: Karyotype of *C. aucheri*. B: Karyotype of *C. assadii*. C: Karyotype of *C. farsestanica*. D: Karyotype of *C. indistincta*. E: Karyotype of *C. phaeopappa* Scale bar = 10 µm.

Table 3. Measurement of somatic chromosomes in a tetraploid *C. aucheri* (Obtained from 12 cells).

No. of chromosome	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio L/S = r
1	6.11	3.67	2.44	1.50
2	5.61	3.35	2.26	1.48
3	5.11	3.02	2.08	1.45
4	4.76	2.49	2.26	1.10
5	4.41	2.49	1.91	1.30
6	4.38	2.47	1.91	1.29
7	4.23	2.44	1.79	1.36
8	4.20	2.61	1.58	1.64
9	4.16	2.84	1.32	2.14
10	4.06	2.44	1.61	1.51
11	3.91	1.97	1.94	1.01
12	3.55	2.14	1.41	1.52
13	3.52	2.23	1.29	1.72
14	3.50	2.17	1.32	1.64
15	3.32	2.11	1.20	1.75
16	3.23	1.82	1.41	1.29
17	2.91	1.76	1.14	1.53
18	2.85	1.61	1.23	1.30

ior indicated that upgrade to species rank (*C. aucheri* (DC.) Wagenitz) by Ranjbar and Negaresh (2013) is correct. In addition, we found some specimens of this taxon together with *C. aucheri* subsp. *szowitsii* (*C. phaeopappa*) in one place (Abgarm to Avaj, Table 1), that chromosome examination showed independence in chromosome number for each species (subsp. *aucheri* , n=18, 2n=36: subsp. *szowitsii* n=9, 2n=18) and did not show any hybrid adjective between this two taxon.

C. assadii *Ranjbar & Negaresh*

Wagenitz (1980) is introduced the *C. aucheri* subsp. *elbursensis* (*C. assadii*) as a new endemic subspecies to Iran for the first time. Previous chromosome number report for this taxon is n=9 by Ghaffari (1986). The results obtained in this study showed nine bivalents in pollen mother cells at diakinesis (Figure 2F). Another stages of meiosis showed chromosome segregation (9-9) at anaphase I and 9 chromatid segregation at anaphase II (Figure 2G,H). Chromosome complement at metaphase of mitotic was 2n=18 (Figure 2I). Karyotype consisted of seven pairs of metacentric and two pairs of submetacentric chromosomes (Figure 3B). The total length of the chromosomes varied from 4.74 µm to 2.79 µm (Table 4). This taxon distributed in Azerbaijan, Mazandaran, Qazvin, and Tehran provinces. We found overlapping of this species with *C. indistincta* and *C. phaeopappa* in one place (Tehran, kouhdashteh, see Table 1) and did not see any hybrid between them. Lack of geographical distribution independence, joint with other subspecies, morpho-

logical characteristics and difference in chromosomal characteristics with *C. aucheri*, indicated that this taxon is a distinct species.

C. farsistanica *(Wagenitz) Ranjbar & Negaresh*

Meiosis in this taxon was regular and showed nine bivalents at metaphase I which more of them were in rod shape (Figure 2J). Anaphase I indicated (9-9) chromosomes segregation (Figure 2K). Mitotic study showed 2n=18 chromosomes at metaphase (Figure 2L). This diploid species has a symmetrical karyotype with six pairs of metacentric and three pairs of submetacentric chromosomes (Figure 3C). The total length of the chromosomes varied from 3.98 µm to 2.25 µm (Table 5). This taxon is completely different with others subspecies in morphological characteristic and pattern of distribution (Ranjbar and Negaresh 2013), which are introduced by Wagenitz (1980) and Mozaffarian (2018).

Centaurea indistincta (Wagenitz) Ranjbar & Negaresh

This taxon is reported by Wagenitz (1980) as a new endemic subspecies species (*C. aucheri* subsp. *indistincta*) for flora of Iran, which was introduced by Ranjbar and Negaresh (2013) as a distinct species. Meiosis in this species showed nine bivalents at metaphase I and (9-9) univalents segregation at first anaphase (Figure 2M,N). Chromosome complement in this species was 2n=18 (Figure 2O). Karyotype consisted of five pairs of metacentric and four pairs of submetacentric chromosomes (Figure 3E). The total length of the chromosomes varied from 4.08 µm to 2.52 µm (Table 6).

Centaurea phaeopappa *(DC.) Schulpz & Bipontinus*

Meiotic and mitotic divisions were examined on eight samples of this species (Table 1). Meiosis showed nine bivalents at diakinesis and metaphase I (Figure 2P,Q). Nondisjunction of (8-10) segregation at anaphase I was observed (Figure 2R). Also, in some cells laggard chromosomes at anaphase II were observed (Figure 2S). Mitotic stages in this species indicated chromosome complement of 2n= 18 (Figure 2T) which is agrees with the previous report by Ghaffari (1988). The karyotype consisted of six pairs of metacentric and three pairs of submetacentric chromosomes (Figure 3F). The total length of the chromosomes varied from 4.21 µm to 2.70 µm (Table 7).

Table 4. Measurement of somatic chromosomes in a diploid *C. assadii* (Obtained from 9 cells).

Chromosome No	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio L/S = r
1	4.74	2.43	2.30	1.05
2	4.67	2.51	2.15	1.16
3	4.10	2.45	1.65	1.48
4	3.87	2.43	1.43	1.69
5	3.43	1.94	1.48	1.31
6	3.23	2.28	0.95	2.40
7	3.19	1.84	1.35	1.36
8	3.02	1.80	1.22	1.47
9	2.79	1.54	1.24	1.23

Table 5. Measurement of somatic chromosomes in a diploid *C. farsistanica* (obtained from 14 cells).

Chromosome No	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio L/S = r
1	3.08	2.24	1.73	1.29
2	3.34	1.95	1.39	1.39
3	3.09	1.82	1.27	1.43
4	2.71	1.90	0.80	2.36
5	2.54	1.31	1.23	1.06
6	2.46	1.39	1.06	1.31
7	2.38	1.27	1.11	1.14
8	2.29	1.44	0.85	1.69
9	2.25	1.49	0.76	1.94

Table 6. Measurement of somatic chromosomes in a diploid *C. indistincta* (obtained from 8 cells).

Chromosome No	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio L/S = r
1	4.08	2.21	1.86	1.19
2	3.72	2.30	1.42	1.62
3	3.15	1.95	1.37	1.41
4	2.92	2.08	1.06	1.96
5	2.92	1.59	1.33	1.20
6	2.75	1.55	1.37	1.12
7	2.57	1.73	1.02	1.69
8	2.13	1.64	0.93	1.76
9	2.52	1.59	0.93	1.71

DISCUSSION

The study has detected the somatic and gametic chromosome number and karyomorphology of *C.*

Table 7. Measurement of somatic chromosomes in a diploid *C. phaeopappa* (obtained from 23 cells).

Chromosome No	Total length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio L/S = r
1	4.21	2.13	2.08	1.02
2	3.72	2.21	1.50	1.47
3	3.59	2.04	1.55	1.31
4	3.55	2.21	1.33	1.66
5	3.51	2.21	1.29	1.71
6	3.46	2.17	1.28	1.68
7	2.88	1.68	1.19	1.40
8	2.75	1.86	0.88	2.10
9	2.70	1.68	1.02	1.65

aucheri and *C. indistincta* species for the first time. Also, meiotic behavior and karyomorphology parameters of *C. albonites, C.assadi* and *C. phaeopappa* are newly reported here. *C. albonitens, C. assadii, C. indistincta* , *C. farsestanica* and *C. phaeopappa* are diploid with n= 9, 2n=2x=18 and *C. aucheri* is tetraploid with 2n=4x=36+0-2B. All taxa had the basic chromosome number of x= 9.

By definition, a subspecies designation is applied to a plant that is geographically isolated from other members of its species in habitat and therefore does not interbreed for this reason. Five subspecies (*C. aucheri* subsp. *aucheri, C. aucheri* subsp. *elbursensis, C. aucheri* subsp. *farsestanica, C.aucheri* subsp. *indistincta, C. aucheri* subsp. *szowitsii*) which is introduced by Wagenitz (1980) and Mozafarian (2018) for flora of Iran, were often adjacent to each other [see Table 1 and pattern of distribution of these subspecies in the research article by Ranjbar and Negaresh (2013)]. Therefore, they cannot be considered as subspecies. Eight samples of the *C. aucheri* showed chromosome numbers of n=18 and 2n=36 in both meiosis and mitotic respectively. Meiosis in pollen mother cells in this taxon showed that the most common chromosome configurations were bivalents at diakinesis and first metaphase. Analysis of karyotype and behavior of meiosis indicated that this taxon is a natural allotetraploid species. Therefore, the results of meiotic behavior and karyomorphologycal parameters of five subspecies which are introduced by Wagenitz (1980) and Mozaffarian (2018) are not correct and revision of them by Ranjbar and Negaresh (2013) in five independence species are correct.

In this study, most of the chromosomes of the evaluated species were metacentric (m) or submetacentric(sm). Karyotype symmetry parameters showed that all the studied species were classified in the class 2A of the category of Stebbins (1971), except of *C. aucheri* which was located in class 2B (Table 8). Total form percentage (TF%) for *C. indistincta* and *C. phaeopappa* were 40.41 and 40.02 respectively, that indicated similarity between them. Also, this TF% similarity can be seen between *C. assadii* and *C. farsistanica* with 41.75 and 40.82 respectively. According to the data obtained from the five species (*C. aucheri*, *C. assadii* , *C. farsistanica*, *C. indisticta* and *C. phaeopappa*), the karyotype formulas were different between them (Table 8). Also, the inter and intrachromosomal asymmetry index (A1 and A2) for five taxa were different (Table 8). In this study, C. *farsistanica* had a higher DI value, which is associated with an enhanced order of karyotypic specialization. *C. aucheri* had the highest A_2 values therefore its karyotype was more asymmetric than the other species. To analyze the variability of the karyotypes among species, all karyotype characteristics of *Centaurea* species (Table 8) were compared by one-way design. Using principal components analysis (PCA), the first two independent components accounted for about 81.68% of total variation (Table 9). The first component indicated that TL, LA, SA, CI, TF% and A_2 were important characters for classification of species with about 61% of total variation. AR, LA%, SA%, DRL, A1 and DI were important traits in the second component (21%) (Table 9).

Table 8. Karyotype parameters of *Centaurea* taxa; Total Length of chromosome(TL), Long Arm (LA), Short Arm (SA), Arm Ratio(AR), Centromeric Index(CI), Long Arm percent(LA %), Short Arm percent(SA %); Total Form percent (TF %), Difference of Relative Length (DRL), intrachromosome asymmetry index(A_1), interchromosome asymmetry index(A_2), Dispersion Index(DI), symmetry classes of Stebbins(SC), Haploid Karyotype Formula(H.K.F.) (m: metacentric , sm: submetacentric).

species	TL	LA	SA	AR	CI	%LA	%SA	%TF	DRL	A_1	A_2	DI	SC	H.K.F
C. assadi	3.68	2.14	1.54	1.47	0.41	6.47	4.64	41.75	5.89	0.28	0.19	8.11	2A	7m+2sm
C. aucheri	4.11	2.43	1.68	1.48	0.41	3.29	2.27	40.83	4.41	0.31	0.22	8.41	2B	15m+3sm
C. farsistanica	2.79	1.65	1.14	1.52	0.41	6.58	4.54	40.82	6.87	0.30	0.21	9.12	2A	6m+3sm
C. indistincta	3.11	1.85	1.26	1.52	0.40	6.62	4.49	40.41	5.55	0.32	0.17	7.65	2A	5m+4sm
C. phaeopappa	3.38	2.03	1.35	1.56	0.40	6.66	4.45	40.02	4.96	0.33	0.15	5.47	2A	6m+3sm

Table 9. Eigenvectors from the first two principal components for 12 karyotype parameters to classify *Centaurea* species.

Parameters	First component	Second component
TL	0.328	-0.054
LA	0.336	-0.050
SA	0.316	-0.061
AR	0.294	0.308
CI	-0.320	-0.206
LA%	-0.275	0.381
SA%	-0.304	0.316
TF%	-0.326	-0.169
DRL	-0.204	0.528
A_1	0.133	-0.176
A_2	0.332	0.218
DI	0.215	0.471
Eigen value	7.3257	2.4761
Percentage of Variance	61.0479	20.6346
Cum percentage of variance	61.0479	81.6824

The tree phylogeny (Figure 4) of the five species indicated two major clades. The first major clade consists of two species (*C. indistincta* and *C. phaeopappa*) showed a degree of affinity and were placed close to each other, while, *C. aucheri* joined the other species at a great distance. The second clade contained *C. assadii* and *C. farsestanica*. Thus, these studies could greatly help us in the classification and taxonomic studies. The diagram of species' dispersion, based on two first components, showed that the species separated in three groups, which completely fits with results obtained through the grouping analysis by Ward's method (Figure 5).

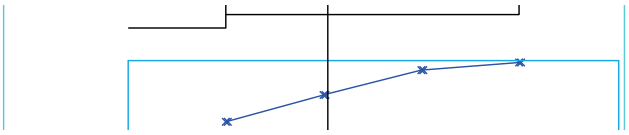

Figure 4. Dendrogram of cluster analysis (Ward) of *Centaurea* species based on karyotype characteristics. Cophenetic correlation r=0.94.

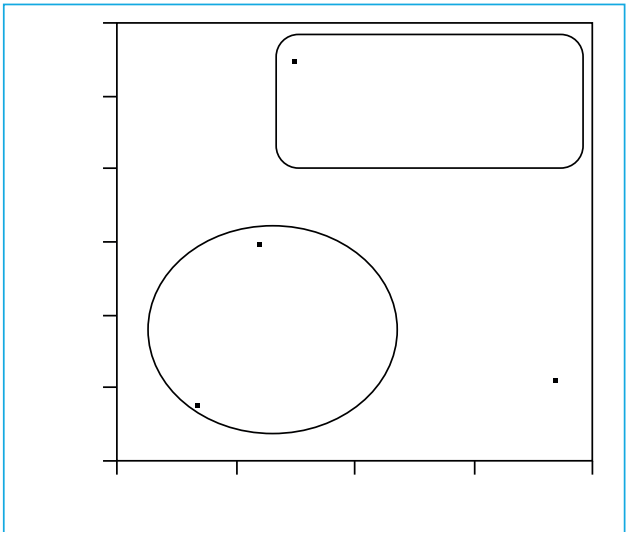

Figure 5. Scatter plot of *Centaurea* species for the first two principal components.

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