Karyological Investigation on Seven *Centaurea* L. (Asteraceae) Taxa from Turkey

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Summary The somatic chromosome numbers and karyotypes were examined in seven species of genus *Centaurea* L. distributed in Turkey. The chromosome numbers were determined as $2n=2x=16$ in *C. behen* L. and *C. solstitialis* L.; $2n=2x=18$ in *C. cynarocephala* Wagenitz and *C. aggregata* Fisch. & Mey. ex DC. ssp. *aggregata*; $2n=2x=20$ in *C. iberica* Trev. ex Sprengel; $2n=2x=26$ in *C. balsamita* Lam.; $2n=4x=36$ in *C. virgata* Lam. All of the chromosomes had centromeres at median point (M), median region (m), and submedian region (sm). Satellite chromosomes (sat-chromosomes) were observed only in *C. virgata*. The findings for each of the analyzed taxa are compared each other and with the results of previous studies.

Key words Asteraceae, *Centaurea*, Chromosome number, Karyotype, Turkey.

The genus *Centaurea* L. is a polymorphous genus belonging to the tribe Cardueae Cass. of the family Asteraceae, and comprises 400–700 species of annual, biennial and perennial grasses, rarely dwarf shrubs predominantly distributed in Europe and Asia (Dittrich 1977, Wagenitz and Hellwig 1996, Bancheva and Greilhuber 2006). *Centaurea* is considered a taxonomically unnatural group and recent approaches have split this taxon into four genera of *Centaurea* s.str., *Cyanus* Miller, *Psephellus* Cassini, and *Rhaponticoides* Vaillant (Greuter 2003, Hellwig 2004).

Common names for *Centaurea* species is a star-thistle, corn flower, and knapweed. Their Turkish names are “peygamber çiçeği”, “zerdali diken”, “çoban kaldiran” and “Timur diken” (Baytop 1999). Generally, *Centaurea* is grown on various habitats of stony calcareous cliffs, vineyards, roadsides, seashores, gypsum fields, open woods and shrubs, waste places, steppe, fallow fields, maquis, sandy beaches, forests, dry meadows, rocky slopes and on maritime limestone cliffs in Turkey. Particularly the aerial parts with flowers or only flowers of some *Centaurea* species are used in Turkish folk medicine to alleviate a wide range of symptoms (Yesilada *et al.* 2004, Gurbuz and Yesilada 2007).

In Turkey, the *Centaurea* is represented by 182 species including 113 endemics, distributed particularly in the southwest, central and eastern parts of the country (Davis 1975, Altintas *et al.* 2004). The ratio of endemic species to common species is quite high of 62.1%. Turkey is one of the main centers of diversity for the *Centaurea* (Wagenitz 1986). It is ca. 195 *Centaurea* species

growing in Turkey (Wagenitz 1975, Wagenitz and Hellwig 1997, Davis *et al.* 1988, Guner 2000).

A close correlation among karyology, pollen morphology and systematic in the subtribe Centaureinae have been demonstrated where basic chromosome numbers are considered a key character for sectional classification (Romaschenko *et al.* 2004, Garcia-Jacas *et al.* 2006). The basic chromosome number of *Centaurea* is known to be $x=7-15$ (Gomurgen 2006).

Centaurea is a taxonomically complicated genus because it contains many species that show a great degree of morphological variations. Therefore, analyses of karyomorphological features are important for the revision and systematics. The purpose of this study was to improve the knowledge of karyomorphological features of *Centaurea* species in Turkey.

Materials and methods

Plant materials of seven species were collected from natural habitats during the fruiting season between 2011 and 2013. The localities geographical position, altitude, and voucher number of the species are presented in Table 1. The voucher specimens were deposited at the Firat University Herbarium.

The karyological studies are conducted on the meristematic cells of root tips. The seeds were germinated on a moist filter paper in Petri dishes at 25°C. The actively growing root tips were pretreated with 0.05% colchicine for 3–3.5 h at room temperature. Afterwards, the root tips were fixed with Carnoy fixative (1:3 glacial acetic acid-absolute ethanol) for at least 24 h at 4°C, hydrolyzed in 1 M HCl at 60°C for 15 min, then rinsed in tap water for 3–5 min. Finally, they were stained in the Feulgen

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Table 1. Localities and voucher numbers of *Centaurea* taxa investigated.

Taxa	Locality	Voucher number
<i>C. aggregata</i> subsp. <i>aggregata</i>	Elazığ: Palu Baltaşı köyü Baltaşı karakolu arkası, 1450m, 02.07.2013	Tasar 1092
<i>C. virgata</i>	Elazığ: Baskil Kayabeyli köyü, yol kenarı, 1100m, 18.06.2012	Tasar 1071
<i>C. balsamita</i>	Elazığ: Elazığ Işıkyolu köyü, yol kenarı, 1150m, 02.07.2012	Tasar 1083
<i>C. behen</i>	Elazığ: Keban yolu, Beşik köyü girişi, yol kenarı, 1090m, 15. 07. 2012	Tasar 1085
<i>C. cynarocephala</i>	Elazığ: Sivrice Gözeli köyü, Kuşakçı dağı, 1750m, 23.06.2012	Tasar 1075
<i>C. iberica</i>	Elazığ: Sürsürü Mahallesi, 1067m, 22.06.2011	Tasar 1051
<i>C. solstitialis</i> subsp. <i>solstitialis</i>	Elazığ: Sürsürü Mahallesi, 1067m, 22.06.2011	Tasar 1052

Table 2. Somatic chromosome number ($2n$), ploidy level, karyotype formula, chromosome length range (CLR), total length of haploid complement (THC), relative length (RL), arm ratio (AR), centromeric index (CI), the intrachromosomal and interchromosome asymmetry index of Romero Zarco (A_1 , A_2) and Stebbins' types of karyotype asymmetry (SC) the studied *Centaurea* taxa.

Taxa	$2n$	Ploidy level	Karyotype formula	CLR (μm)	THC (μm)	RL (%)	AR	CI	A_1	A_2	SC
<i>C. aggregata</i> subsp. <i>aggregata</i>	18	2x	1M+5m+3sm	3.65–7.51	42.84	8.52–17.53	1.00–2.08	32.41–50.00	0.34	0.26	2B
<i>C. virgata</i>	36	4x	3M+8m+7sm	1.63–6.00	63.47	2.56–9.45	1.00–3.60	22.37–50.00	0.34	0.28	2B
<i>C. balsamita</i>	26	2x	1M+7m+5sm	1.60–4.22	33.94	4.71–12.43	1.00–2.36	29.68–50.00	0.35	0.25	2B
<i>C. behen</i>	16	2x	3M+4m+1sm	3.07–4.03	27.81	11.03–14.49	1.00–1.76	36.22–50.00	0.14	0.10	1A
<i>C. cynarocephala</i>	18	2x	1M+6m+2sm	3.18–6.41	40.15	7.89–15.96	1.00–2.79	26.37–50.00	0.34	0.21	2B
<i>C. iberica</i>	20	2x	1M+8m+1sm	1.57–2.95	21.93	7.15–13.45	1.00–2.03	32.97–50.00	0.28	0.19	2A
<i>C. solstitialis</i> L. subsp. <i>solstitialis</i> L.	16	2x	1M+4m+3sm	1.81–3.53	19.80	9.14–17.82	1.00–2.09	32.29–50.00	0.28	0.21	2A

reagent for 1 h and mounted in 45% acetic acid (Kiran *et al.* 2012). Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope and an Olympus Camedia C-4000 digital camera.

The chromosome numbers of the species were identified and checked using the Flora of Turkey (Davis 1975) and supplements (Davis *et al.* 1988, Guner 2000). Also, relevant literature (Tamamschian 1963, Kazmi 1964) and the online chromosome number databases, Index to Plant Chromosome Numbers (<http://www.tropicos.org/Project/PCN>) and Index to Chromosome Numbers in Asteraceae (Info-Lib Index to Chromosome Numbers in Asteraceae, http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceaesresult-en) were checked.

Results

The number of somatic chromosomes, ploidy level, karyotype formula (Levan *et al.* 1964), chromosome length range, total length of the haploid complement, relative length, arm ratio, centromeric index, A_1 and A_2 values (Romero Zarco 1986), types for karyotype asymmetry (Stebbins 1971) were determined for each taxa (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1 and 2. The examined taxa and characteristics of somatic chromosomes are given below.

C. aggregata Fisch. & Mey. ex DC. ssp. *aggregata*: This taxon growing on dry rocky slopes and forests between altitudes of 700 and 1800 m (Davis 1975). The

somatic chromosome number of this species was found to be $2n=18$. The chromosome length ranges 3.65 to 7.51 μm and the total length of the haploid complement was 42.84 μm . It consists of 1M, 5m, and 3sm chromosomes. Chromosome arm ratios are 1.00–2.08. The centromeric index is 32.41–50.00, and relative lengths are 8.52–17.53% (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1a and 2a.

C. virgata Lam.: This taxon perennial with the woody base and several stems. It's growing on dry hills, steppe, dry waste ground, between 100 and 2000m (Davis 1975). The somatic chromosome number of this species was found to be $2n=36$. The chromosome length ranges 1.63 to 6.00 μm , and the total length of the haploid complement was 63.47 μm . It consists of 3M, 8m, and 7sm chromosomes. Chromosome arm ratios are 1.00–3.60. Centromeric index is 22.37–50.00, and relative lengths are 2.56–9.45%. Two of the sm chromosomes have satellites (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1b and 2b.

C. balsamita Lam.: This taxon is annual, Iran–Turan Element. It's growing on the steppe and fallow fields, between altitudes of 650 and 1900m (Davis 1975). The somatic chromosome number of this species was found to be $2n=26$. The chromosome length ranges from 1.60 to 4.22 μm , and the total length of the haploid complement was 33.94 μm . It consists of 1M, 7m, and 5sm chromosomes. Chromosome arm ratios are 1.00–2.36. Centromeric index is 29.68–50.00, and relative lengths are 4.71–12.43% (Table 2). Somatic metaphase chromo-

Fig. 1. Somatic metaphase chromosomes in *Centaurea* taxa. a. *C. aggregata* ssp. *aggregata* ($2n=18$), b. *C. virgata* ($2n=36$), c. *C. balsamita* ($2n=26$), d. *C. behen* ($2n=16$), e. *C. cynarocephala* ($2n=18$), f. *C. iberica* ($2n=20$), g. *C. solstitialis* ssp. *solstitialis* ($2n=16$). Scale bars= $10\mu\text{m}$.

Fig. 2. Haploid idiograms in *Centaurea* taxa. a. *C. aggregata* ssp. *aggregata*, b. *C. virgata*, c. *C. balsamita*, d. *C. behen*, e. *C. cynarocephala*, f. *C. iberica*, g. *C. solstitialis* ssp. *solstitialis*.

somes and idiograms are given in Figs. 1c and 2c.

C. behen L.: This taxon is perennial, Iran-Turan Element. It's growing on rocky slopes, fallow fields, between 340 and 1730m (Davis 1975). The somatic chromosome number of this species was found to be $2n=16$. The chromosome length ranges from 3.07 to $4.03\mu\text{m}$

and the total length of the haploid complement was $27.81\mu\text{m}$. It consists of 3M, 4m, and 1sm chromosomes. Chromosome arm ratios are 1.00 – 1.76 . Centromeric index is 36.22 – 50.00 , and relative lengths are 11.03 – 14.49% (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1d and 2d.

C. cynarocephala Wagenitz: This taxon is biennial, Iran-Turan Element. Its distributed on limestone cliffs, dry hills, between 1150 and 1200 m (Davis 1975). The somatic chromosome number of this species was found to be $2n=18$. The chromosome length ranges from 3.18 to $6.41\ \mu\text{m}$ and the total length of the haploid complement was $40.15\ \mu\text{m}$. It consists of 1M, 6m, and 2sm chromosomes. Chromosome arm ratios are 1.00–2.79. The centromeric index is 26.37–50.00, and relative lengths are 7.89–15.96% (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1e and 2e.

C. iberica Trev. ex Sprengel: This taxon growing on fields, roadsides and waste land, c. 2300 m (Davis 1975). The somatic chromosome number of this species was found to be $2n=20$. The chromosome length ranges from 1.57 to $2.95\ \mu\text{m}$ and the total length of the haploid complement was $21.93\ \mu\text{m}$. It consists of 1M, 8m, and 1sm chromosomes. Chromosome arm ratios are 1.00–2.03. The centromeric index is 32.97–50.00, and relative lengths are 7.15–13.45% (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1f and 2f.

C. solstitialis L. ssp. *solstitialis*: This taxon distributed on *Pinus* forests, dry slopes, fallow fields and waste places, c. 1900 m (Davis 1975). The somatic chromosome number of this species was found to be $2n=16$. The chromosome length ranges from 1.81 to $3.53\ \mu\text{m}$ and the total length of the haploid complement was $19.80\ \mu\text{m}$. It consists of 1M, 4m, and 3sm chromosomes. Chromosome arm ratios are 1.00–2.09. The centromeric index is 32.29–50.00, and relative lengths are 9.14–17.82% (Table 2). Somatic metaphase chromosomes and idiograms are given in Figs. 1g and 2g.

Discussion

The genus *Centaurea* has been a problematic taxon in the world, also in Turkey. High endemism ratio shows that Turkey is one of the gene centers of the genus. Therefore, in this study, we focused on this genus. Karyotype analysis of the examined *Centaurea* taxa showed that the taxa have $x=8, 9, 10$ and 13 basic chromosome number and all of them are diploid except for *C. virgata*. Analysis of the karyotype formulae indicated that chromosomes of seven studied *Centaurea* taxa generally have m and sm karyotypes. An M karyotype is also observed. The mean chromosome length is 1.57– $6.00\ \mu\text{m}$. The smallest chromosome length is in diploid specimens of *C. iberica* while the longest chromosome length was seen in tetraploid specimens of *C. virgata*. The centromeric index ranges from 22.37 to 50.00, and relative lengths from 2.56 to 17.82%. The intrachromosomal asymmetry index (A1) ranges from 0.14 to $0.35\ \mu\text{m}$. Interchromosomal asymmetry index (A2) varies between 0.10 and 0.28.

Chromosome number of *C. aggregata* ssp. *aggregata* was first reported by Bakhshi Khaniki, and its $2n=18$

(Bakhshi Khaniki 1996). Our investigations confirm these dates, the determined chromosome number being of $2n=18$. A tetraploid chromosome numbers were observed $2n=4x=36$ in the specimens of *C. virgata*. Two of the submedian chromosomes have satellites (sat-chromosome) was observed in *C. virgata* (Figs. 1b and 2b). Ghaffari was reported that chromosome number of *C. virgata* $2n=18$ (Ghaffari and Chariat-Panahi 1985, Ghaffari 1989). By Bakhshi Khaniki and Shahraki were reported chromosome number of *C. balsamita* $2n=26$ (Bakhshi Khaniki 1996, Ghaffari and Shahraki 2001). Our investigations confirm these dates, the determined chromosome number being of $2n=26$. Somatic chromosome number is $2n=26, 32, 34$ of *C. behen* were reported in the literature (Chouksanova *et al.* 1968, Ghaffari and Shahraki 2001, Romaschenko *et al.* 2004). Our investigations confirm these dates, the determined chromosome number being of $2n=16$. The somatic chromosome numbers were determined as $2n=20$ in the specimens of *C. iberica*. $2n=20$ chromosomes of this species were reported in the literature (Kuzmanov *et al.* 1986, Ghaffari 1989, Hellwig 1994, Garcia-Jacas *et al.* 1997). The somatic chromosome numbers was determined as $2n=2x=16$ of *C. solstitialis* ssp. *solstitialis*. $2n=2x=16$ chromosomes of this species were reported in the literature (Van Loon 1980, Georgiadis and Christodoulakis 1984, Ozturk *et al.* 2009).

In this study, chromosome numbers of seven Turkish *Centaurea* taxa were established. Numerous counts were reported for *C. aggregata* ssp. *aggregata*, *C. virgata*, *C. balsamita*, *C. behen*, *C. iberica* and *C. solstitialis* in previous studies. But, as far as we know, the chromosome number and morphology of the taxa *C. cynarocephala* are newly reported here. This study will contribute to further studies on the karyotype properties and implications on systematic of genus *Centaurea* from Turkey.

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