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Karyological analysis of two endemic *Centaurea* L. species from Turkey

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Abstract

In this paper, two endemic *Centaurea* species were investigated in terms of their chromosome numbers and karyomorphology. The chromosomal counts confirmed the results of previous reports, that the genus *Centaurea* has various basic chromosome numbers. *Centaurea urvillei* subsp. *hayekiana* and *Centaurea saligna* are diploid taxa. *C. urvillei* subsp. *hayekiana* has 2n=20 chromosomes and a different basic chromosome number of ten, and this basic number is relatively rare for *Centaurea*. *C. saligna* has 2=18 chromosomes, and it has basic chromosome number of nine, which is common in the genus *Centaurea*. Satellite was identified in the short arm of the chromosome IV. of *C. saligna*.

Key words: *Centaurea,* Chromosome Number, Karyotype.

1. Introduction

The genus *Centaurea* L. represents approximately 500–600 species distributed all around the world, particularly in Asia, Europe, North Africa and America (Wagenitz, 1975; Davis et al., 1988). *Centaurea* is one of the largest and taxonomically most difficult genera of the family Asteraceae (Dostal, 1976). According to Wagenitz (1975), Turkey is one of the main centers of diversity for the genus *Centaurea*. It is also the third largest genus in terms of the number of species in Turkey. Recent approaches have split this taxon into four genera: *Centaurea*, *Rhaponticoides* Vaill., *Psephellus* Cass. and *Cyanus* Mill. (Wagenitz and Hellwig, 2000; Greuter, 2003; Hellwig, 2004). The genus *Centaurea* was previously revised by Wagenitz (1975), for the *Flora of Turkey and the East Aegean Islands* without considering the splitting mentioned above. Even excluding the species now placed in these genera, Turkey

is among the richest countries in *Centaurea* diversity (Wagenitz 1975; Davis et al., 1988; Guner, 2000). In the Flora of Turkey, 172 plus six imperfectly known species *Centaurea* were accepted. Recently *Centaurea* was revised for *Türkiye Bitkileri Listesi* by Dural (2012), Ertugrul (2012) and Uysal (2012a; 2012b). According to the revised system, the number of known *Centaurea* species in Turkey is 166 [excluding 56 species which are now treated within *Psephellus* (33), *Cyanus* (16) and *Rhaponticoides* (7)] (Kose and Alan, 2013; Bancheva et al., 2014; Yuzbasioglu et al., 2015; Bona, 2015). High endemism ratio shows that Turkey is one of the gene centers of the genus (Atasagun, 2013).

The sections *Psephellus, Psephelloideae, Hyalinella, Aetheopappus, Amblyopagon, Heterolophus, Czerniakovskya, Odontolophoideae, Odontolophus, Xanthopsis, Uralepis,* and *Sosnovskya*, which used to belong to the genus *Centaurea*, have been transferred to the genus *Psephellus* Cass., therefore a total of 35 species have been excluded from the genus *Centaurea* (Wagenitz and Hellwig, 2000). The genus *Centaurea* has been a problematic taxon in the world, also in Turkey (Cassini, 1819; Boissier, 1873; Love and Love, 1961; Dostal, 1969; Holub, 1973; IUCN, 2001). The sectional classification of *Centaurea* relies heavily on the morphology of the appendage of the phillary and the achenes (Garcia-Jacaset al., 2001). Moreover, recent molecular approaches, karyological studies, and palynological data have been used effectively for the taxonomy of the genus *Centaurea* in recent years (Tasar et al., 2014; Potoglu Erkara et al., 2012; Atasagun et al., 2013; Uysal et al., 2015; Aksoy et al., 2016).

The family Asteraceae shows a high variety of chromosome numbers. Its variability is very helpful in studies of cytotaxonomy and karyotype evolution, particularly with regard to the ploidy level and basic chromosome number (Charanarsriet al., 1973; Weiss-Schneeweiss et al., 2003; Uysal et al., 2009; Uysal and Kose, 2009). Earlier karyomorphologic data were used to evaluate the numerical changes in chromosomes of Asteraceae and other families (Carr et al., 1999; Ayodele, 1999; Martin et al., 2009; Yıldız et al., 2009; Gunjan and Roy 2010; Tabur et al., 2012). The importance of karyology in *Centaurea* had been previously suggested by Guinochet and Foissac (1962), Tonjan (1980), Garcia-Jacas and Susanna (1992), Susanna et al. (1995), Garcia-Jacas et al. (1996), and Wagenitz and Hellwig (1996).

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

2. Material and Methods

Plant materials were collected from natural habitats during fruiting season between 2011 and 2012. The voucher specimens were deposited at the Fırat University Herbarium. The localities geographical position, altitude, and voucher number of the species are presented in Table 1.

The karyological studies are conducted on the root tips meristematic cells. The seeds were germinated on moist filter paper in Petri dishes at 25 °C. The actively growing root tips were pretreated with aqueous colchicine (0.05%) for 3–3.5 h at room temperature. Afterwards, the root tips were fixed with Carnoy (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4 °C, hydrolyzed in 1 N HCl at 60 °C for 15 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least 5 well-spread metaphase plates were taken using an Olympus BX51 microscope, and were recorded with an Olympus Camedia C-4000 digital camera. The short arm (s), long arm (l) and total lengths (tl) of each chromosome were measured, and the relative lengths, arm ratios, and centromeric indices were determined from images of selected cells. Chromosomes were classified according to the nomenclature of Levanet al. (1964). The intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2) followed those of Romero-Zarco (1986). The karyotype symmetry nomenclature followed Stebbins (1971). Coefficient of variation of chromosome length (CV_{CL}), coefficient of variation of the centromeric index (CV_{CI}), asymmetry index (AI) of Paszko (2006). Also, relevant literature the online chromosome number databases, Index to Plant Chromosome Numbers (IPCN).

Table 1. Localities of the studied *Centaurea* species.

Taxon	**Locality**	**Collection Number**
C. urvillei subsp. *hayekiana*	B7/Elazığ; Baskil Haroğlu Mountain, South-East slopes, rocky area, 1520 m. 13.06.2011	Tasar, 1010
C. saligna	B7/Elazığ; Palu Baltaşı Village, Hilly, 1450 m. 17.07.2012	Tasar, 1016

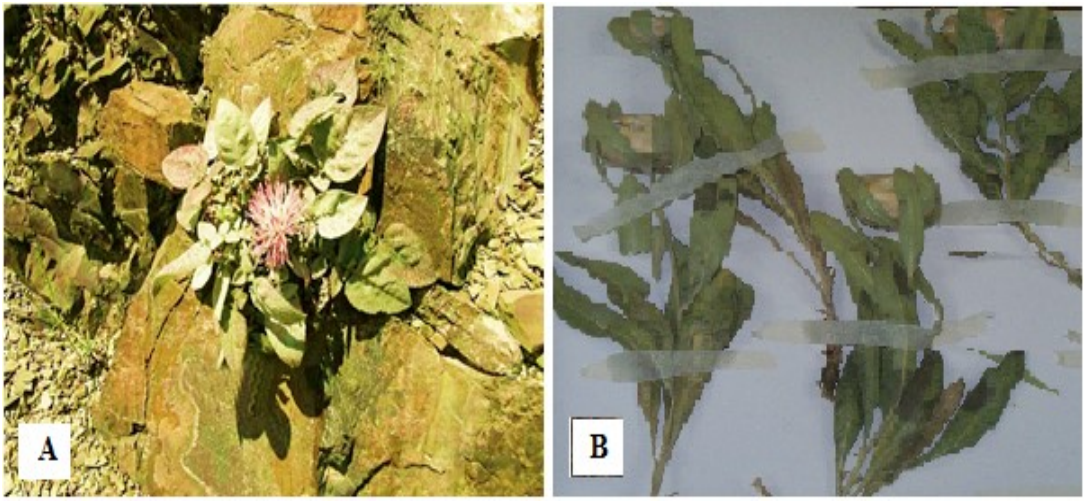

Figure 1. Habitus of *Centaurea* taxa **A:** *C. urvillei* subsp. *hayekiana* **B:** *C. saligna.*

3. Results and Discussion

Chromosome numbers and detailed chromosome morphology of two endemic *Centaurea* species were reported in this study. The chromosome number of *C. urvillei* subsp. *hayekiana* was 2n=20 and that of *C. saligna* was 2n=18. Karyological features of studied *Centaurea* taxa are represented in Tables 2, 3 and 4.

3.1. C. urvillei DC. subsp. *hayekiana* Wagenitz

The chromosome number of *C. urvillei* subsp. *hayekiana* is 2n=20 and therefore it has a basic chromosome number of x=10. The shortest chromosome length is 2.29 µm, the longest is 6.07 µm, and haploid chromosome length is 36.34 µm. Chromosome arm ratios are between 1.00 and 1.70. Relative lengths vary from 6.27 to 16.73 (Table 2). The karyotype formula of this taxon 3M+6m+1sm. Somatic metaphase chromosomes and ideograms are given in Figures 2 and 3. In this study, chromosome number and detailed chromosome morphology are given for the first time in *C. urvillei* subsp. *hayekiana.*

Table 2. Parameters of mitotic metaphase chromosomes of *Centaurea urvillei* subsp. *hayekiana.*

Chromosome Number	Total Length (µm)	Long Arm (µm)	Short Arm (µm)	Arm Ratio	Relative Length	Centromereic Index	Centromer Position
1	6.08	3.04	3.04	1.00	16.73	0.50	M
2	4.54	2.54	2.00	1.27	12.49	0.44	m
3	3.86	2.43	1.43	1.69	10.62	0.37	m
4	3.75	1.93	1.82	1.06	10.31	0.48	m
5	3.68	2.18	1.50	1.45	10.12	0.40	m
6	3.68	2.32	1.36	1.70	10.12	0.36	sm
7	3.00	1.64	1.36	1.20	8.25	0.45	m
8	2.89	1.64	1.25	1.31	7.95	0.43	m
9	2.58	1.29	1.29	1.00	7.09	0.50	M
10	2.28	1.14	1.14	1.00	6.27	0.50	M

3.2. C. saligna (C. Koch.) Wagenitz

The chromosome number of *C. saligna* is 2n=18, and therefore it has a basic chromosome number of x=9. The shortest chromosome length is 3.18 µm, the longest is 6.09 µm, and haploid chromosome length is 40.20 µm. Chromosome arm ratios are between 1.00 and 1.85. Relative lengths vary from 7.91 to 15.14 (Table 3). The karyotype formula of this taxon 1M+6m+2sm. Satellite was identified in the short arm of the chromosome IV. somatic metaphase chromosomes and ideograms are given in Figures 2 and 3.

Table 3. Parameters of mitotic metaphase chromosomes of *Centaurea saligna*.

Chromosome Number	Total Length (µm)	Long Arm (µm)	Short Arm (µm)	Arm Ratio	Relative Length	Centromereic Index	Centromer Position
1	6.09	3.44	2.65	1.29	15.14	0.43	m
2	5.26	2.95	2.31	1.27	13.08	0.43	m
3	5.10	2.55	2.55	1.00	12.68	0.50	M
4	4.66	3.03	1.63	1.85	11.59	0.34	sm
5	4.52	2.52	2.00	1.26	11.24	0.44	m
6	4.02	2.18	1.84	1.18	10.00	0.45	m
7	3.90	2.52	1.38	1.82	9.70	0.35	sm
8	3.47	2.02	1.45	1.39	8.63	0.41	m

9	3.18	1.98	1.20	1.65	7.91	0.37	m

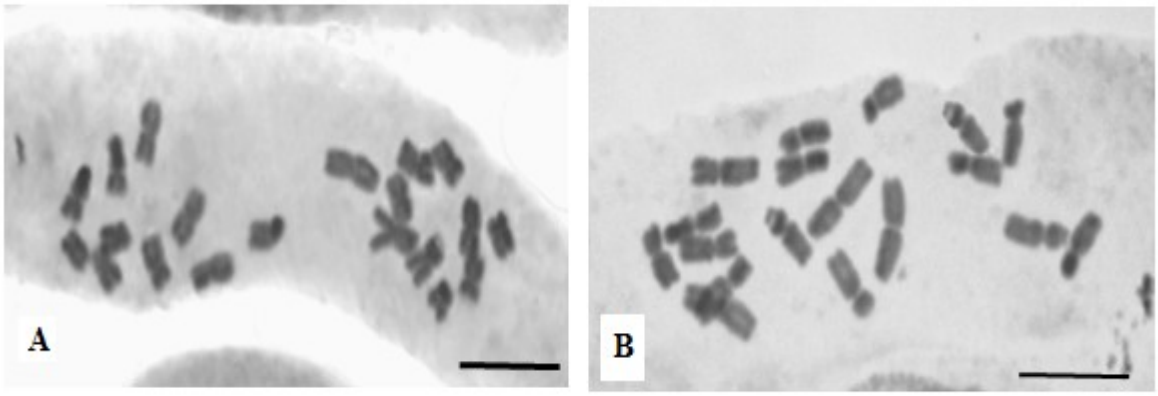

Figure 2. Somatic chromosomes of *Centaurea* taxa **A:** *C.urvillei* subsp. *hayekiana* **B:** *C. saligna.*

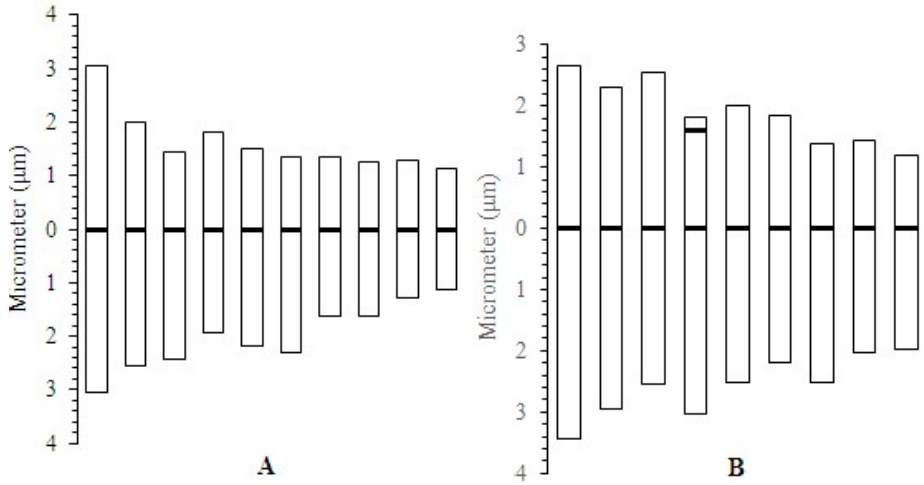

Figure 3. Idiogram of *Centaurea* taxa **A:** *C.urvillei* subsp. *hayekiana* **B:** *C. saligna.*

Table 4. Karyological features of two *Centaurea* taxa.

Taxon	**2n**	**Ploidy Level**	**Karyotype Formule**	**TKL (µm)**	**A_1**	**A_2**	**SC**	**CV_{CL}**	**CV_{CI}**	**AI**
C. urvillei ssp. *hayekiana*	20	2x	3M+6m+1sm	36.34	0.18	0.30	1B	30.10	11.47	3.45
C. saligna	18	2x	1M+6m+2sm	40.20	0.26	0.20	1A	21.00	11.94	2.47

TKL: total karyotype length; A_1: intrachromosomal asymmetry index; A_2: interchromosomal asymmetry index; SC: karyotype symmetry nomenclature followed Stebbins; CV_{CL}: coefficient of variation of chromosome length; CV_{CI}: coefficient of variation of centromeric index; AI: karyotype asymmetry index.

In the genus *Centaurea* in Turkey, the somatic chromosome number varies from 2*n* =16 to 66 (Wagenitz, 1975; Davis et al.,1988; Guner, 2000; Romaschenko et al., 2004; Martin et al., 2006; Inceer et al., 2007). There are some cytological and karyological studies of the genus *Centaurea*; the basic chromosome number of *Centaurea* species varies from x=7 to 16 and four ploidy levels (2x, 3x, 4x, 6x) have been determined (Khaniki, 1995;1996; Garcia-Jacas et al.,1997; Routsi and Georgiadis, 1999; Garnatje et al., 2001; Peruzzi and Cesca, 2002; Romaschenko et al., 2004; Martin et al., 2009; Uysal et al., 2009; Gomurgen et al., 2010; Meric et al., 2010; Bona, 2013; Kocyigit and Bona, 2013; Silvestre, 2013). Uysal et al. (2015), reported that the number of chromosomes of *C. saligna* was 2n=4x=36. *C. saligna* is tetraploid species having a basic chromosome number of nine. Polyploidy (rarely 3x and generally 4x, and 6x) is common in the genus *Centaurea* (Garcia-Jacas et al., 1998; Romaschenko et al., 2004; Uysal et al., 2009; Uysal and Kose, 2009; Gomurgen et al., 2010).

4. Conclusion

The results increase our karyological knowledge of these species. Moreover, this paper gives short taxonomic and karyomorphologic notes to complement the information provided by Flora of Turkey with regards to the studied species.

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