Chromosome number and nuclear DNA content of *Centaurea kilaea* (*Asteraceae*), an endemic species from Turkey

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Abstract. Chromosome number and nuclear DNA amount of *Centaurea kilaea*, an endemic species for Turkey, were studied by karyological and flow cytometrical analysis techniques. The somatic chromosome number of the species was counted as $2n = 4x = 36$. Total 2n chromosome lengths were measured as 56.76 (±2.72) µm. For the flow cytometric analysis, nuclei were stained with propidium iodide and analyzed on an EPICS XL (Beckmann Coulter) model flow cytometer. The nuclear DNA content (2C-value) of *C. kilaea* was found to be 3.68 (±0.05) pg. Chromosome number and nuclear DNA content data are provided for the first time for the species.

Key words: *Asteraceae*, *Centaurea kilaea,* chromosome number, endemic, flow cytometry, nuclear DNA content

Introduction

The genus *Centaurea* L., one of the largest genera in the *Asteraceae*, comprises about 300 species widespread across the world (Bremer 1994). Most species are distributed in Turkey and the Balkan Peninsula (Siljak-Yakovlev & al. 2005). Turkey is one of the main centres of diversity for the genus *Centaurea* (Uzunhisarcıklı & al. 2007). The genus is represented in the Turkish flora by about 190 species and 60 % of these species are endemic (Wagenitz 1975; Davis & al. 1988; Güner 2000; Uzunhisarcıklı & al. 2007; Uysal 2008; Uysal & Köse 2009). *Centaurea kilaea* Boiss. is an endemic plant growing in Kırklareli: Kasatura (A2E); Istanbul: Domuzdere, Terkos, Kilyos (A2E); Istanbul: Yeşilçay (A2A); Adapazarı: Karasu (A3); Bolu (A3) in Turkey (Wagenitz 1975). According to the *Red Data Book of Turkish Plants* (Ekim & al. 2000), the conservation status of *C. kilaea* is Endangered.

The basic chromosome numbers of the genus range from $x = 7$ to $x = 12$ (Garcia-Jacas & al. 1996, 1997) and it has three ploidy levels ($2x$, $4x$ and $6x$) (Romaschenko & al. 2004; Siljak-Yakovlev & al. 2005). *Centaurea kilaea* is included into the section *Acrolophus* (Wagenitz 1975). The basic chromosome number of sect. *Acrolophus* is $x = 9$ and the section contains $2x$, $4x$ and $6x$ ploidy levels (Romaschenko & al. 2004; Trigas & al. 2008). The chromosome numbers in sect. *Acrolophus* are known only for half of the species (Garcia-Jacas & al. 1997; Güner 2000; Romaschenko & al. 2004; IPCN 2008; Uysal 2008). However, the chromosome number is an important karyological feature for plant taxonomy and there is close correlation between karyology and systematics in *Centaurea* (Romaschenko & al. 2004).

Centaurea is a taxonomically problematic genus (Romaschenko & al. 2004; Bancheva & Greilhuber 2006). In addition to the formerly carried out morphological, palynological and classical karyological studies (Garcia-Jacas & al. 1997, 1998a, 1998b, 2006; Villodre & Garcia-Jacas 2000; Romaschenko & al. 2004; Siljak-Yakovlev & al. 2005; Bancheva & Greilhu-

ber 2006), molecular biological studies have recently increased. Cytological data are insufficient for the solution of taxonomical problems in the genus, and further studies are needed, particularly karyological and molecular biological. This study aims to contribute to the present knowledge of chromosome numbers and DNA amounts of *Centaurea* for the solution of taxonomical problems of the genus.

In recent year, flow cytometry has been widely used for estimating the nuclear DNA content, since it is easy, quick and reliable to apply. Analysis of the nuclear DNA amount is the most widespread application of flow cytometry in modern plant biosystematics. DNA C-values have been estimated in plant species with flow cytometer for twenty five years now (Galbraith & al. 1983). C-values are known for only 1.4 % of angiosperms (Doležel & Bartoš 2005), although the data of C-values is helpful for understanding the plant molecular biology, population biology, genome evolution, taxonomy, ecology, physiology, and development (Bennett & al. 2000). There are various problems in the estimation of DNA C-values, such as geographical distribution, plant life form and requirement of costly equipment. However, the quick increase of recent studies on the angiosperm DNA amount seems rather hopeful. This study is expected to contribute to the Angiosperm C-value database (Bennett & Leitch 2004) and to the solution of taxonomical problems of genus *Centaurea*.

Material and methods

The plants and their seeds were collected from a natural population in Kırklareli, İğneada (European Turkey) (Fig. 1). The locations of plants were determined with a GPS. Their coordinates were 41°51'55" N and 27°58'43" E. Some plants were prepared as herbarium materials and voucher specimens were deposited in the EDTU Herbarium (Trakya University, Edirne, Turkey). Other plants were placed in a growth chamber at 27 °C and with a 16/8 h photoperiod.

The chromosome preparations were made by a standard root-tip squash technique (Johansen 1940). Seeds were germinated in darkness at 25 °C on moist filter paper in petri dishes. Actively growing root tips, 4 mm long were excised from the germinating seeds. Root tips for chromosome counts were pretreated with 0.5 % colchicine for 3 hours at room temperature (RT) and then fixed in Carnoy (3 ethyl alcohol:1 acetic acid) for 15 min. the root tips were subsequently hydrolyzed with 5N HCl for 1 h at RT. They were stained with Schiff's reagent (Sigma) for 2 hours in darkness at RT. Dissected meristems were squashed and mounted in 45 % acetic acid. The slides were examined with an Olympus BH2 light microscope (Tokyo, Japan) and images were taken with a ProgRes C12 Plus digital camera (Jenoptik, Germany). The measurements of chromosome lengths were performed using Image-Pro Plus, version 5.1 (Media Cybernetics, Silver Spring, MD). For analysis, chromosomes from ten cells with well spread metaphase plates were measured. Average and standard deviation of data were calculated.

Healthy young leaves were prepared for a flow cytometric analysis, according to Tuna & al. (2001). Diploid *Hordeum vulgare* ($2n = 2x = 14$ and 2C-value 10.68 pg) was used as internal standard (Tuna & al. 2001). Fresh leaf fragments of *C. kilaea* and *H. vulgare* were chopped with a razor blade on ice in a petri dish containing 1 ml of $MgSO_4$ buffer (ice-cold) with 1 $mgml^{-1}$ dithiothreitol (Sigma), 100 μgml^{-1} propidium iodide (Sigma) and 2.5 μgml^{-1}

Fig. 1. Location of *C. kilaea.*

triton X-100 (Sigma). Then the suspension was filtered through a 40 µm nylon mesh (BD Falcon) and centrifuged at 13 000 rpm for 2 min. The supernatant was removed; and the pellet was homogenized in 600 µl of the above-mentioned $MgSO_4$ buffer after adding 2.5 µlml^{-1} RNase (DNase free, Roche). The homogenate was incubated for 20 min at 37 °C in oven before the flow cytometric analysis. The prepared material was analysed in Trakya University, Faculty of Medicine on EPICS LX model flow cytometer (Beckmann Coulter). Analyses were repeated three times for ten different plants. The mean DNA content per plant was based on 10 000 scanned nuclei. The formula used for converting fluorescence values to DNA content was: Sample nuclear 2C DNA content = [(sample G_1 peak mean) / (standard G_1 peak mean)] x standard 2C DNA content (pg DNA) (Doležel & Bartoš 2005).

Results and discussion

The chromosome number and nuclear DNA content of *C. kilaea,* an endemic species for Turkey, are given for the first time in this study. *Centaurea kilaea* has $2n = 4x = 36$ chromosome number and is a tetraploid with $x = 9$ (Fig. 2). The species has small median or submedian chromosomes, with few morphological distinctions between them. The $2n$ total chromosome lengths are 56.76 (±2.72) µm and the average chromosome length is individual for 1.58 µm (total chromosome length / chromosome number).

The basic chromosome numbers of genus *Centaurea* range from $x = 7$ to $x = 12$ (Garcia-Jacas & al. 1996, 1997). Romaschenko & al. (2004) suggested that the chromosome number is a good character in the *Jacea* group. Similarly, the chromosome number is also characteristic in sect. *Acrolophus,* including *C. kilaea*. Sect. *Acrolophus* has $x = 9$ basic chromosome number and contains $2x$, $4x$ and $6x$ ploidy levels (Romaschenko & al. 2004; Trigas & al. 2008). Our result supports the sectional classification of *C. kilaea* that sect. *Acrolophus* has $x = 9$ basic chromosome number. *Centaurea kilaea* has small chromosome sizes (the mean is 1.58 µm).

The 2C nuclear DNA content of *C. kilaea* is found as 3.68 (±0.05) pg (Fig. 3). Each chromosome has individually about 0.10 pg DNA amount (DNA amount/chromosome). The results of chromosome counts and 2C DNA contents are given in Table 1. The data are presented by combining the results of this study with those available in literature.

Nuclear DNA contents of *Centaurea* are known for 55 taxa (Bennett & Leitch 2004; Siljak-Yakovlev & al.

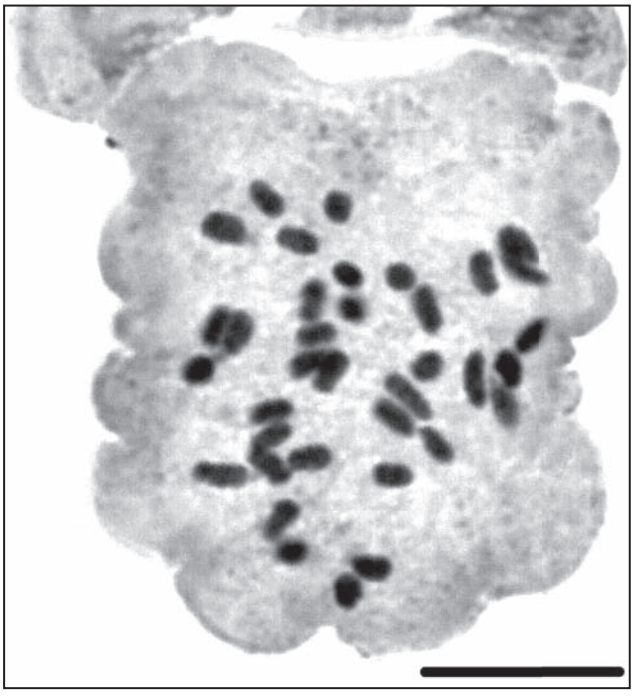

Fig. 2. Mitotic metaphase chromosomes of *C. kilaea* ($2n = 4x = 36$). Scale bar: 10 µm.

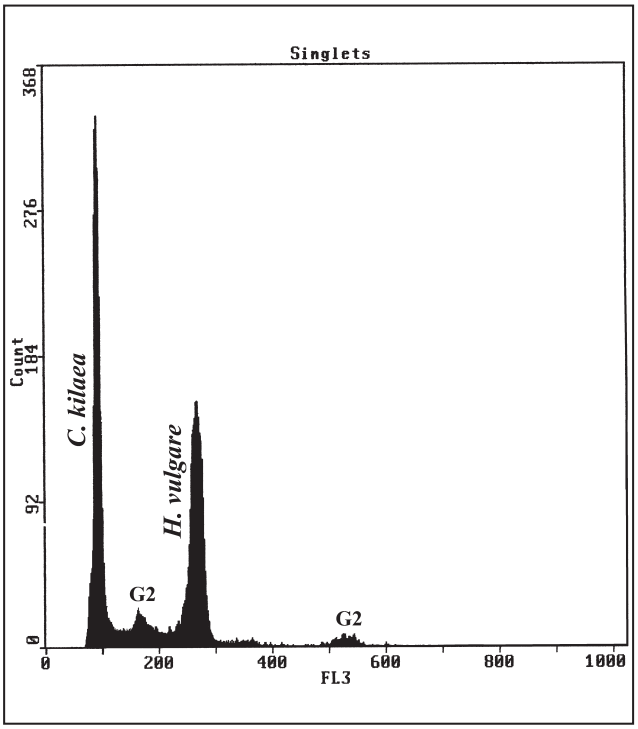

Fig. 3. Flow cytometric histogram of *C. kilaea.*

Table 1. Chromosome numbers, ploidy levels and nuclear DNA contents of *Centaurea* species.

Taxon	Chromosome number (2*n*)	Ploidy level	DNA amount 2C (pg)	DNA amount 1Cx (pg)
Centaurea achtarovii	22[3]	2x[3]	3.12[3]	1.56
C. alba	36[3]	4x[3]	3.58[3]	0.90
C. apiculata	20[3]	2x[3]	3.54[3]	1.77
C. arenaria	32[3]	4x[3]	3.28[3]	0.82
C. badensis	20[3]	2x[3]	3.46[3]	1.73
C. chrysolepis	20[3]	2x[3]	3.40[3]	1.70
C. cuneifolia	18, 36[3]	2x, 4x[3]	1.77, 3.24[3]	0.89, 0.81
C. cuspidata	18[2]	–	2.10, 2.17[2]	1.05, 1.09
C. cyanus	24[3]	2x[3]	1.47[3]	0.74
C. davidovii	44[3]	4x[3]	4.13[3]	1.03
C. debeauxii	44[3]	4x[3]	4.30[3]	1.08
C. deusta	18[2]	–	1.67[2]	0.84
C. diffusa	16[3]	2x[3]	1.79[3]	0.90
C. edith-mariae	36[2]	–	3.63[2]	0.91
C. elegantissima	36[2]	–	3.72[2]	0.93
C. glaberrima	36[2]	–	3.19–3.36[2]	0.80–0.84
C. gloriosa var. *gloriosa*	18[2]	–	2.18[2]	1.09
C. gloriosa var. *multifolia*	18[2]	–	2.17[2]	1.09
C. immanuelis-loewii	20[3]	2x[3]	3.30[3]	1.65
C. indurata	44[3]	4x[3]	3.82[3]	0.96
C. jacea	44[3]	4x[3]	4.00[3]	1.00
C. kernerana	22[3]	2x[3]	2.03[3]	1.02
C. kilaea	**36**	**4x**	**3.68**	**0.92**
C. kotschyana	20[3]	2x[3]	3.48[3]	1.74
C. kusanii	36[2]	–	3.66[2]	0.92
C. mannagettae	20[3]	2x[3]	3.32[3]	1.66
C. marschalliana	30[3]	2x[3]	3.58[3]	1.79
C. mayeri	36[2]	–	3.54[2]	0.89
C. moesiaca	44[3]	4x[3]	4.07[3]	1.02
C. napulifera	20[3]	2x[3]	2.48[3]	1.24
C. nervosa subsp. *nervosa*	22[3]	2x[3]	2.16[3]	1.08
C. nervosa subsp. *gheorghieffii*	44[3]	4x[3]	4.16[3]	1.04
C. nigra	44[4]	–	3.60[1]	0.90
C. nigrescens	44[3]	4x[3]	3.80[3]	0.95
C. orientalis	20, 40[3]	2x, 4x[3]	3.30, 6.92[3]	1.65, 1.73
C. orientalis x chrysolepis	20[3]	2x[3]	3.36[3]	1.68
C. ovina subsp. *besserana*	18[3]	2x[3]	1.76[3]	0.88
C. parilica	22[3]	2x[3]	2.18[3]	1.09
C. pichleri	44[3]	4x[3]	3.94[3]	0.99
C. ragusina subsp. *ragusina*	20[2]	–	3.43[2]	1.72
C. ragusina subsp. *lungensis*	20[2]	–	3.31[2]	1.66
C. rupestris	20[2]	–	2.33[2]	1.17
C. salonitana var. *salonitana*	20[3]	2x[3]	3.86[3]	1.93
C. salonitana var. *macracantha*	20[3]	2x[3]	3.68[3]	1.84
C. scabiosa	– 20[3]	– 2x[3]	3.55[1] 3.54[3]	1.78 1.77
C. solstitialis	16[3]	2x[3]	1.74[3]	0.87
C. stenolepis	22, 44[3]	2x, 4x[3]	2.16, 4.12[3]	1.08, 1.03
C. stereophylla	20[3]	2x[3]	3.36[3]	1.68
C. stereophylla x orientalis	20[3]	2x[3]	3.32[3]	1.66
C. stoebe	18, 36[3]	2x, 4x[3]	1.77, 3.14[3]	0.88, 0.79
C. thirkei	20[3]	2x[3]	2.18[3]	1.09
C. triumfettii subsp. *triumfetti*	22[3]	2x[3]	3.02[3]	1.51
C. triumfettii subsp. *adscendens*	22[3]	2x[3]	2.80[3]	1.40
C. tuberosa	22[2] 20[3]	– 2x[3]	2.69[2] 2.52[3]	1.35 1.26
C. visiani subsp. *visiani*	18[2]	–	2.19[2]	1.10
C. visiani subsp. *pumilla*	18[2]	–	2.15[2]	1.08

[1]Bennett & Leitch (2004); [2]Siljak-Yakovlev & al. (2005); [3]Bancheva & Greilhuber (2006); [4]IPCN (2008).

2005; Bancheva and Greilhuber 2006). The 2C-values of this species range from 4.30 to 1.47 pg (2*x* and 4*x*) (Table 1). Diploid species have 2.63-fold different values in 2C DNA (from 3.86 pg for *C. salonitana* var. *salonitana* to 1.47 pg for *C. cyanus*). The difference of 2C DNA content is 1.37-fold for tetraploid species (from 4.30 pg for *C. debeauxii* to 3.14 pg for *C. stobe*) (Bancheva & Greilhuber 2006).

According to the new genome terminology proposed by Greilhuber & al. (2005), "holoploid genome size" is the DNA content of the complete chromosome complement of an organism and is abbreviated 1C, 2C … etc. "monoploid genome size" defines the DNA content of the monoploid genome set and is abbreviated 1Cx, 2Cx … etc (the "symbol x" refers to the basic chromosome number). According to this terminology, 1Cx DNA value of *C. kilaea* is 0.92 pg, while 1C DNA value is 1.84 pg. When 1Cx value of the species is accounted for in the evaluation, it becomes significantly smaller. For C-value distribution of the angiosperm taxa, Leitch & al. (1998) have suggested four modals: the species with 1C-values of ≤ 1.4 pg and ≤ 3.5 pg are defined as having "very small" and "small" genomes, respectively. Likewise, the species with 1C-values of ≥ 14.0 pg and ≥ 35.0 pg are defined as having "large" and "very large" genomes (Leitch & al. 1998). In addition to this terminology, species with 1C-values between 3.51 pg and 13.99 pg are called "intermediate" by Soltis & al. (2003). According to these data, *C. kilaea* has a very small genome for 1Cx-value (0.92 pg), and a small genome for 1C-values (1.84 pg). Flow cytometric procedure can be quite useful for the very problematic and complex *Centaurea* genus, because of the presence of small chromosome sizes and different ploidy levels. This study determines the chromosome number, genome size and ploidy level of *C. kilaea* growing in a limited geographical region, and also contributes data on the genome sizes of *Asteraceae*.

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