

Nuclear DNA content of *Dianthus ingoldbyi* (Caryophyllaceae)

Çiler Meriç & Necmettin Güler

¹ Department of Biology, Faculty of Arts and Sciences, Trakya University, 22030 Edirne, Turkey. e-mail:cilermeric@trakya.edu.tr; e-mail:nguler@trakya.edu.tr

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Abstract. The nuclear DNA content of *Dianthus ingoldbyi*, an endemic species to the Balkan Peninsula, was analyzed by flow cytometry. The plant samples were collected from their natural habitats. Propidium iodide-stained nuclei were analyzed on an EPICS XL (Beckmann Coulter) model flow cytometer. The nuclear DNA content (2C-value) of *D. ingoldbyi* was found to be 2.48 ± 0.03 pg.

Key words: *Dianthus ingoldbyi*, endemic species, flow cytometry, nuclear DNA content, propidium iodide

Introduction

Caryophyllaceae is a large family containing 86 genera and 2200 species of annual or perennial herbs, and seldom shrubs distributed worldwide in temperate and warm-temperate regions (Fior & al. 2006). The genus *Dianthus* L. comprises about 300 species that are widespread in the northern temperate regions, but concentrate in the Mediterranean region (Jürgens & al. 2003). *Dianthus* is represented by 70 species in the flora of Turkey, and 45 % of these species are endemics (Reeve 1967; Davis & al. 1988; Uysal & al. 1992; Güner 2000). *Dianthus ingoldbyi* Turrill is an endemic plant growing in rather limited regions of the Balkan Peninsula, such as Çanakkale (Bozcaada-Sulubahçe and Eceabat-Arıburun, Turkey), Edirne (Keşan-Mecidiye, Turkey), and Greece (NE) (Reeve 1967; Uysal & al. 1992; Basak & Güler 2000; Trigas & al. 2007). According to the *Red Data Book of Turkish Plants*, the conservation status of *D. ingoldbyi* is Critically Endangered (Ekim & al. 2000).

Genus *Dianthus* contains diploid, tetraploid and hexaploid species. *Dianthus* species have $2n = 2x = 30$, $2n = 4x = 60$ and $2n = 6x = 90$ ($x = 15$) chromosome numbers (see, IPCN 2008). Furthermore, the genus includes diploid, tetraploid and hexaploid cytotypes

within the species (Weiss & al. 2002). DNA 2C-values for two species of *Dianthus* are known as 1.48 and 2.57 pg for *D. caryophyllus* L. cv. “98sp1651” and *D. japonicus* Thunb., respectively (Nimura & al. 2003). Also, the 2C DNA amount of *D. caryophyllus* L. cv. “White Sun” was reported to be 1.25 pg by Figueira & al. (1992). However, the nuclear DNA content of *D. ingoldbyi* is unknown.

The nuclear DNA amount is a specific karyological feature that determines the evolutionary considerations for a taxon. According to Soltis & al. (2003), the small genome size is ancestral for angiosperms. Within the extant seed plants, the small genome size is characteristic of the angiosperms, while extant gymnosperms have larger C-values than angiosperms (Soltis & al. 2003). Unfortunately, it is known only for the 1.4 % of the angiosperm taxa; and the DNA C-value data of global angiosperm flora are still found to have major gaps (Doležel & Bartoš 2005). There are various problems in the estimation of DNA C-values due to the geographical distribution of the taxa and the need in expensive equipment. The estimation of nuclear DNA content of endemic taxa growing in limited geographical regions is found to be a major problem. This study contributes to Angiosperm DNA C-values database (Bennett & Leitch 2004).

Material and method

Plants were collected from a rather limited region in Mecidiye (Edirne), on coastal maritime rocks and limestone (Fig. 1). The location of plants was determined with a GPS. Their location coordinates were 40°36'N and 26°32'E. Some plants were prepared as herbarium materials and voucher specimens were deposited in the Herbarium of Trakya University, Edirne (EDTU). Others were transferred into pots and placed in a growth chamber at 27°C, with a 16/8 h photoperiod. Young leaves of growing plants were prepared for flow cytometry according to Tuna & al. (2001). Diploid *Hordeum vulgare* L. cv. Hitchcock ($2n = 2x = 14$ and 2C-value 10.68 pg) was used as internal standard (Tuna & al. 2001).

Fresh leaf fragments of *D. ingoldbyi* and *H. vulgare* were chopped with a razor blade on ice in a Petri dish containing 1 ml of $MgSO_4$ buffer, with 1 $mg\ ml^{-1}$ dithiothreitol, 100 $\mu g\ ml^{-1}$ propidium iodide and 2.5 $\mu g\ ml^{-1}$ triton X-100. The suspension was then filtered through a 40 μm nylon mesh and centrifuged at $16060 \times g$ 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 $\mu l\ ml^{-1}$ RNase (DNase free). The suspension was incubated at 37°C for 15 min in an oven prior to the flow cytometric analysis (Galbraith & al. 1983; Tuna & al 2001).

The prepared materials were analyzed at the Trakya University, Faculty of Medicine on an EP-

ICS XL model flow cytometer (Beckman Coulter). Analyses were performed on twenty different plants and averages and standard deviations of the measures were taken. The mean DNA content per plant was based on 10 000 scanned particles. 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)] \times standard 2C DNA content (pg DNA) (Doležel & Bartoš 2005).

Results and discussion

The applied cytometric procedure was appropriate for the material analyzed in this study. No technical problems were encountered during the analysis of the samples. The average coefficients of variation (CV) of the 2C nuclei population were always less than 3% (2.42 ± 0.19). The 2C-value of *D. ingoldbyi* was found to be 2.48 ± 0.03 pg (Fig. 2). Nuclear DNA amounts are known only for two species in genus *Dianthus*. *Dianthus caryophyllus* cv. "98sp1651" and *D. japonicus* have 1.48 pg and 2.57 pg 2C-values, respectively (Nimura & al. 2003). Also, Figueira & al. (1992) have reported that 2C DNA amount of *D. caryophyllus* cv. "White Sun" is 1.25 pg. The 2C-values and chromosome numbers of *Dianthus* species are shown in Table 1. The data are presented by combining the results of this study with those available in literature.



Fig. 1. Locations of *D. ingoldbyi*.

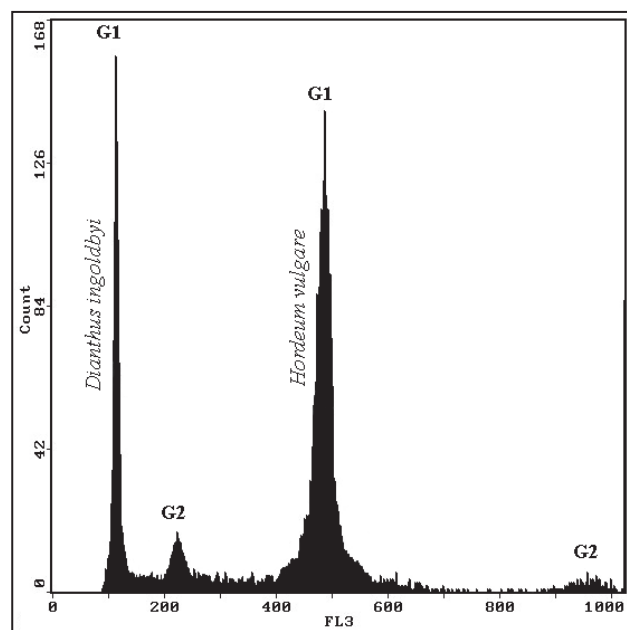


Fig. 2. Flow cytometric histogram of *D. ingoldbyi*.

Table 1. Nuclear DNA contents and chromosome numbers of the *Dianthus* species

Species	Chromosome number (2n)	DNA amount 2C (pg)
<i>D. caryophyllus</i> cv. "98sp1651"	30 (Murín 1993)	1.48 (Nimura & al. 2003)
<i>D. caryophyllus</i> cv. "White sun"	30 (Murín 1993)	1.25 (Figueira & al. 1992)
<i>D. ingoldbyi</i>	30 (Basak & Güler 2000)	2.48 (in present study)
<i>D. japonicus</i>	30 (Nimura & al. 2006)	2.57 (Nimura & al. 2003)

Dianthus ingoldbyi is closely related to *D. japonicus* with respect to nuclear DNA content. These three species have the same chromosome numbers ($2n = 30$) (Murín 1993; Basak & Güler 2000; Nimura & al. 2006), but different nuclear DNA contents (Nimura & al. 2003). Some related species with identical number of chromosomes may be determined by the nuclear DNA content. Furthermore, the related taxa with identical karyotype (such as subspecies and variety) may differ from one another with respect to the nuclear DNA amount. Dimitrova & al. (1999) determined that the Bulgarian populations of *Crepis foetida* subsp. *commutata* have about 10 % smaller genome size than the subsp. *foetida* and *rheadifolia*, despite the karyotype constancy.

Although *Caryophyllaceae* contains about 2200 species, nuclear DNA amounts of only 30 species are known (Bennett & Leitch 2004; Fior & al. 2006). *Herniaria glabra* ($2n = 18$) has the smallest 2C DNA amount, with 1.05 pg, while *Silene chalcedonica* ($2n = 24$) has the largest 2C DNA content, with 6.59 pg in the family. The mean 2C-value of the *Caryophyllaceae* species is 3.40 pg (Bennett & Leitch 2004). For C-value distribution of angiosperm taxa, Leitch & al. (1998) has suggested four modalities; 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, the species with 1C-values between 3.51 pg and 13.99 pg are called "intermediate" by Soltis & al. 2003. According to these data, *D. ingoldbyi* has a very small genome with 1.24 pg 1C-values (≤ 1.4 pg). The small genome size in plants correlates with several characters for successful competition, such as rapid seedling establishment, short minimum generation times, and increased reproductive rate. Therefore, the small genome size ensures great evolutionary flexibility (Soltis & al. 2003).

Unfortunately, nuclear DNA amounts are known for only a fraction of all plant species in the global flo-

ra (1.4 %) (Doležel & Bartoš 2005). Likewise, C-values are known for only approximately 45 % of the angiosperm families on Earth (Hanson & al. 2001). Estimation of nuclear DNA contents for endemic taxa growing in limited geographical regions is the specific problem. Therefore, genome size data of endemic and rare plants should be broadened. This study will contribute to the molecular systematic studies of angiosperm taxa in the future.

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