



## Nuclear DNA content of an endemic species for Turkey: *Silene sangaria*

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### Abstract

The nuclear DNA content of *Silene sangaria* Coode & Cullen, an endemic species for Turkey, was determined by flow cytometrical analyses techniques. The plant samples were collected from their natural habitats. They transferred into pots and placed in the growth chamber. For flow cytometry analysis, young leaves were chopped in an MgSO<sub>4</sub> buffer with propidium iodide on ice in a petri dish. Nuclei, which were stained with propidium iodide, were analyzed on an EPICS XL model flow cytometer. The nuclear DNA content (2C-value) of *S. sangaria* was found to be  $4.76 \pm 0.20$  pg. The somatic chromosome number of the species was determined using conventional karyological methods and counted as  $2n = 4x = 48$ . This study contributes to the data on the nuclear DNA content of angiosperm taxa.

**Key words:** Caryophyllaceae, *Silene sangaria*, Endemic species, Nuclear DNA content, Flow cytometry

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### Türkiye için endemik bir türün çekirdek DNA miktarı: *Silene sangaria*

### Özet

Türkiye florası için endemik bir tür olan *Silene sangaria* Coode & Cullen 'nın çekirdek DNA miktarı flow sitometri yöntemiyle analiz edildi. Bitkiler doğal ortamlarından toplandı ve saksılara ekilerek büyüme kabineye yerleştirildi. Flow sitometri analizi için, genç yapraklar buz üzerinde, bir petri içinde propidium iodid ilaveli MgSO<sub>4</sub> tamponu içinde parçalandı. Propidium iodid ile boyanan çekirdekler EPICS XL (Beckmann Coulter) model flow sitometri ile analiz edildi. *Silene sangaria*'nın çekirdek DNA miktarı (2C-değeri)  $4.76 \pm 0.20$  pg olarak hesaplandı. Türün somatik kromozom sayısı klasik karyolojik metodları kullanılarak belirlendi ve  $2n = 4x = 48$  olarak sayıldı. Bu çalışmanın angiosperm taksonlarının çekirdek DNA miktarı bilgilerine katkı sağladığı düşünülmektedir.

**Anahtar kelimeler:** Caryophyllaceae, *Silene sangaria*, Endemik tür, Çekirdek DNA içeriği, Flow sitometri

### 1. Introduction

Genus *Silene* L. (Caryophyllaceae) comprises about 700 species divided into 44 sections widely distributed throughout the Northern hemisphere (Siroky et al., 2001). *Silene* is represented in 136 species in the flora of Turkey and 40% of these species are endemic (Coode and Cullen, 1967; Davis et al., 1988; Yildiz and Cırpıcı, 1996; Tan and Vural, 2000; Duran and Menemen, 2003; Deniz and Dusen, 2004). Genus *Silene* is one of the largest genera of flowering plants in the world and the most species-rich taxa in Turkey.

*S. sangaria* is an endemic plant in the flora of Turkey and grows only in Karasu, Sakarya; Kilyos, Istanbul; and Igneada, Kırklareli. According to "Red Data Book of Turkish Plants", the conservation status of *S. sangaria* is vulnerable (Ekim et al., 2000). Moreover, this plant is listed in Appendix 1 (strictly protected flora species), which covers the "convention on the conservation of European wildlife and natural habitats" (Council of Europe, 1979).

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*Silene* species have  $2n = 20$ ,  $2n = 24$ , and  $2n = 48$  ( $x = 10$  and  $x = 12$ ) chromosome numbers (Petrova, 1995; Yildiz and Cirpici, 1996). The chromosome number of *S. sangaria* has been reported earlier as  $2n = 48$  by Yildiz and Cirpici (1996). The DNA 1C-values for twelve species of the *Silene* genus are known to be between 1.00 and 3.30 pg (Suda et al., 2003; Bennett and Leitch, 2004). However, the nuclear DNA content of *S. sangaria* is unknown. Nuclear DNA C-values are an important genomic character used in many diverse disciplines including taxonomy, systematics, genome evolution, phylogeny, ecology, plant breeding, conservation, and cell and molecular biology (Bennett et al., 2000). The DNA amount in plant nuclei has been estimated since 1950, leading to the discovery of the constancy in organisms and the key role in biology of DNA (Swift, 1950). Unfortunately, the DNA amount is known only for about 1.4% of the angiosperm taxa and approximately 50% of angiosperm families (Bennett and Leitch, 2005; Dolezel and Bartos, 2005). The DNA amount of angiosperm taxa in global flora is expected to be easily determined using new technologies (e.g., flow cytometry) in the future.

## 2. Materials and methods

Plants and their seeds were collected from a natural population in Igneada, Kırklareli (European Turkey) (Figure 1). Some plants were prepared as herbaria materials and voucher specimens were deposited in EDTU Herbarium (EDTU 13277) (Trakya University, Edirne, Turkey). Others were transferred into pots and placed in a growth chamber at 27°C with a 16/8 h photoperiod.

The technique of standard root-tip squash was used for the chromosome preparations. Seeds were germinated in darkness at 25°C on moist filter paper in petri dishes. Actively growing 4 mm root tips were excised from the germinating seeds. The root tips for chromosome counts were pretreated with 0.5% Colchicine (Sigma) for 3 h at room temperature (RT), and fixed Carnoy (3 ethyl alcohol; 1 acetic acid) for 15 min. Then, they were hydrolyzed with 5 N HCl for 1 h at RT and were stained with Schiff's reagent (Sigma) for 2 h in darkness at RT. Dissected meristems were squashed and mounted in 45% acetic acid. For permanence, the preparations were frozen at -20°C in a deep freeze. Then they were passed through an alcohol-xylene dehydration series and mounted in Entellan (Merck). The slides were examined using an Olympus BH2 light microscope (Tokyo, Japan) and the images were taken with a ProgRes C12 Plus digital camera (Jenoptik, Germany).

For flow cytometric analysis, the young leaves of growing plants were prepared according to Tuna et al. (2001). As an internal standard diploid *Hordeum vulgare* L. cv. Hitchcock ( $2n = 2x = 14$  and 2C-value 10.68 pg) were used (Tuna et al., 2001). Fresh leaf fragments of *S. sangaria* and *H. vulgare* were chopped with a razor blade on ice in a plastic petri dish containing 1 ml of MgSO<sub>4</sub> buffer (ice-cold) with 1 mgml<sup>-1</sup> dithiothreitol (Sigma), 100 mlml<sup>-1</sup> propidium iodide (Sigma), and 2.5 mlml<sup>-1</sup> triton X-100 (Sigma). Then the suspension was filtered through a 40 µm nylon mesh (BD Falcon) and centrifuged at 13000 rpm for 2 min. The supernatant was removed and the pellet was homogenized in 600 ml of the above-mentioned MgSO<sub>4</sub> buffer after adding 2.5 mlml<sup>-1</sup> RNase (DNase free, Roche). The suspension was incubated at 37°C for 15 min in an oven before the flow cytometric analysis (Tuna et al., 2001).

The prepared materials were analyzed at Trakya University, Faculty of Medicine on an EPICS XL model flow cytometer (Beckman Coulter). Analyses were performed on 10 different plants three times, and averages and standard deviations of measures were taken. The mean DNA content per plant was based on 10000 scanned nuclei. The formula used for converting fluorescence values to DNA content was as follows: sample nuclear 2C DNA content = [(sample G<sub>1</sub> peak mean) / (standard G<sub>1</sub> peak mean)] x standard 2C DNA content (pg DNA) (Dolezel and Bartos, 2005).

## 3. Results

*S. sangaria* was collected on coastal maritime sand in a rather limited region in Igneada. The locations of the plants were determined using GPS. Their location coordinates were 40° 50' N, 27° 58' E; 41° 49' N, 27° 57' E; and 41° 51' N, 27° 56' E.

The chromosome number of *S. sangaria* was found to be  $2n = 4x = 48$  and, it was a tetraploid with  $x = 12$  (Figure 2). This result is similar to that of Yildiz's (1994). Yildiz (1994) reported that the species has 18 pairs of metacentric (M, m) chromosomes and 6 pairs of submetacentric (sm) chromosomes. Its total chromosome length is 39.81 µm (for  $n = 24$ ), and its average chromosome length is 1.658 µm (Yildiz, 1994). In the above mentioned study, the investigated specimens were collected from the Karasu coast of Sakarya, Turkey (Yildiz, 1994).

The 2C-value of *S. sangaria* was found to be  $4.76 \pm 0.20$  pg (1C-value 2.38 pg) (Figure 3). The nuclear DNA amounts of the *Silene* genus are known only for twelve species although the genus contains about 700 species (Siroky et al., 2001; Suda et al., 2003; Bennett and Leitch, 2004). The 1C-values of *Silene* range from 1.00 (*S. coeli-rosa*) to 3.30 (*S. chalcadonica*) pg (Suda et al., 2003; Bennett and Leitch, 2004). The difference between 1C-values of these species is approximately three-fold. The average of 1C-values of *Silene* species were 2.37 pg and the standard deviation was estimated at 0.73. The 1C-value of *S. sangaria* (2.38 pg) was almost identical to the mean value of known species (2.37 pg). This species has a  $2n = 48$  chromosome number although the others have  $2n = 24$  chromosome counts (Löve and Löve, 1982; Halkka, 1985; Siroky et al., 1999; Siroky et al., 2001; Suda et al., 2003; IPCN 2012). It is surprising that the DNA amount of *S. sangaria* with  $2n = 48$  chromosomes is smaller than those of other species with  $2n = 24$

chromosomes such as *S. chalcedonica* and *S. nutans*. Yildiz (1994) reported that the chromosomes of polyploid species are generally smaller than those in the diploid species. Similarly, Bennett et al. (2000) reported that the genome sizes of polyploids are smaller than their diploid relatives. The reason for the decrease in genome size is usually the deletion and elimination of the repeating parts on chromosomes (Siljak-Yakovlev et al., 2005). More data are needed for the evaluation of nuclear DNA amounts of *Silene* concerning 1C-values of this genus. The 1C-values and chromosome numbers of the *Silene* species whose genomes are known are shown in Table 1. The data are presented by combining available data from current literature and the results of this study. *S. sangaria* is the only polyploid species among the ones whose genome size is known.



Figure 1. The location of *Silene sangaria* (◀)

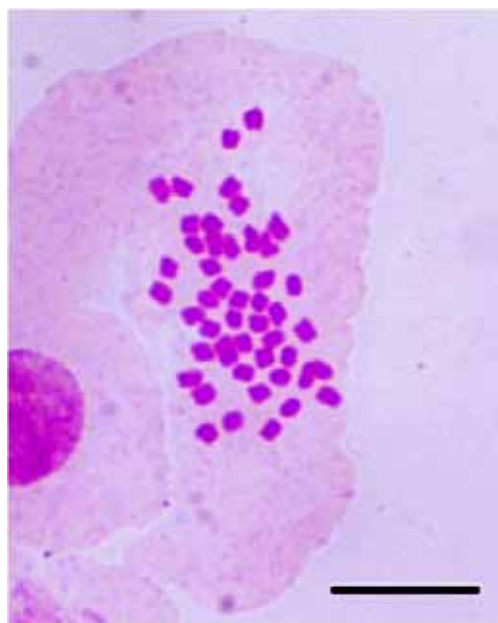


Figure 2. Mitotic metaphase chromosomes of *Silene sangaria* ( $2n = 4x = 48$ ) Scale bar: 10  $\mu$ m.

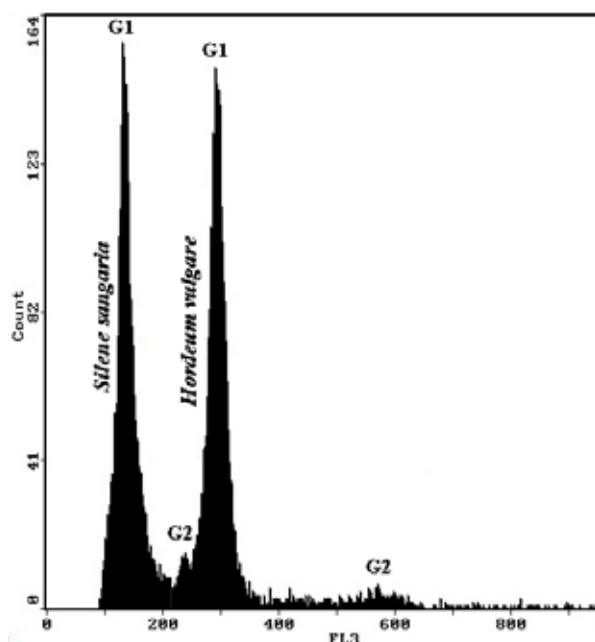


Figure 3. The flow cytometric histogram of *S. sangaria*

Leitch et al. (1998) suggested that the species with 1C-values less than 1.4 pg are defined as having “very small” genomes whereas species with 1C-values less than 3.5 pg are defined as having “small” genomes. Likewise, species with 1C-values greater than 14.0 pg are defined as having “large” genomes, and species with 1C-values greater than 35.0 pg are defined as having “very large” genomes (Leitch et al., 1998). In addition to this terminology, species with 1C-values between 3.51 pg and 13.99 pg are called “intermediate” by Soltis et al. (2003). According to these data, *S. sangaria* has small genome since it has 2.38 pg 1C-value (£ 3.5 pg). However, Greilhuber et al. (2005) proposed the new genome terminology as “holoploid genome size” (the DNA content of the complete chromosome complement of an organism, abbreviated as 1C, 2C, etc.) and “monoploid genome size” (the DNA content of the monoploid genome set, abbreviated as 1Cx, 2Cx, etc). The “symbol x” refers to the basic chromosome number (Greilhuber et al. 2005). According to this terminology, the 1Cx DNA value is 1.19 pg while the 1C DNA value is 2.38 pg of *S. sangaria*.

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

Species	Chromosome number (2n)	1C (pg)
<i>Silene bertelotiana</i>	24 <sup>1</sup>	2.55 <sup>1</sup>
<i>Silene chalcedonica</i>	24 <sup>6</sup>	3.30 <sup>1</sup>
<i>Silene coeli-rosa</i>	24 <sup>3</sup>	1.00 <sup>1</sup>
<i>Silene dioica</i>	24 <sup>2</sup>	2.70 <sup>1</sup>
<i>Silene lagumensis</i>	24 <sup>1</sup>	2.59 <sup>1</sup>
<i>Silene latifolia</i>	24 <sup>5</sup>	2.70 <sup>1</sup>
<i>Silene nocteolens</i>	24 <sup>7</sup>	2.58 <sup>1</sup>
<i>Silene nutans</i>	24 <sup>4</sup>	3.20 <sup>1</sup>
<i>Silene pendula</i>	24 <sup>6</sup>	1.18 <sup>1</sup>
<i>Silene pogonocalyx</i>	24 <sup>1</sup>	2.61 <sup>1</sup>
<i>Silene rubra</i>	---	2.85 <sup>1</sup>
<b><i>Silene sangaria</i></b>	<b>48</b>	<b>2.38</b>
<i>Silene vulgaris</i>	24 <sup>6</sup>	1.13 <sup>1</sup>

<sup>1</sup>Bennett and Leitch, 2004; <sup>2</sup>Halkka, 1985; <sup>3</sup>IPCN, 2012; <sup>4</sup>Löve and Löve, 1982; <sup>5</sup>Siroky et al., 1999; <sup>6</sup>Siroky et al., 2001; <sup>7</sup>Suda et al., 2003

Genome sizes correlate with many factors such as ecological environment, live form, cell sizes, reproductive features, minimum generation times, and ancestral aspects. Small genome size is ancestral for Caryophyllales, and Caryophyllaceae also have a small ancestral genome size. In Caryophyllales clade, the mean of 1C-value is 1.7 pg (Soltis et al., 2003). To explain the genome size variation, the biological factors that depend on the organism are not adequate. This variation is also related to ecological factors such as temperature (Bennett et al., 2000). Humanity faces the mass extinction of biodiversity because of global warming and pollution. Although the relationships between genome sizes and plant species loss are unclear, a relationship likely exists (Bennett et al., 2000). Therefore, the data on DNA amounts helps to improve conservation strategies.

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