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The estimation of relative DNA content in the genus *Spiraea* L., section *Calospira* C. Koch.

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ABSTRACT

The estimation of relative DNA content in cell nuclei in the genus *Spiraea* L. section *Calospira* C. Koch. from the Russian Far East is given for the first time. The cell nuclei were isolated from a leaf tissue. The relative intensity of fluorescence was measured by the nuclei stained with propidium iodide using flow cytometry. The values of relative DNA amounts in *Spiraea* differ significantly within a high statistical significance ($p<0.001$). It is revealed that the relative DNA content of *S. betulifolia* Pall. (2C = 0.91 and 1.01 pg) is twice higher than those values in other species. The *S. beauverdiana* Schneid. (2 C = 0.55 and 0.57 pg) and the *S. aemiliana* Schneid. (2C = 0.45 and 0.48 pg) are close to the amount of DNA they contain.

Keywords: *Spiraea betulifolia*, *Spiraea beauverdiana*, *Spiraea aemiliana*, nuclear DNA amount, flow cytometry, DNA C-value

РЕЗЮМЕ

Костикова В.А., Воронкова М.С., Банаев Е.В., Полякова Т.А. Оценка относительного содержания ДНК у растений рода *Spiraea* L. секции *Calospira* C. Koch. методом проточной цитометрии. Впервые исследовано относительное содержание ДНК у растений рода *Spiraea* L. секции *Calospira* C. Koch., произрастающих в российской части ареала. Ядра клеток выделены из тканей листьев. Относительная интенсивность флюоресценции измерена на ядрах, окрашенных йодидом пропидия, с помощью проточной цитометрии. Значения относительного содержания ДНК у *Spiraea* достоверно отличаются ($p<0.001$). У *S. betulifolia* Pall. (2C = 0.91 и 1.01 пикограмм) относительное содержание ДНК в два раза превышает значения у остальных видов. *S. beauverdiana* Schneid. (2C = 0.55 и 0.57 пг) и *S. aemiliana* Schneid. (2C = 0.45 и 0.48 пг) более близкие таксоны по содержанию ДНК

Ключевые слова: *Spiraea betulifolia*, *Spiraea beauverdiana*, *Spiraea aemiliana*, относительное содержание ДНК, проточная цитометрия

The studies on changes in the genome size of one species or closely related species of plants from different areas allow us to judge the evolutionary processes and their types as well as predict next steps of evolution (Marda at al. 2008). The genome size can be an informative marker in plant taxonomy at the species and subspecies level along as with chromosome numbers, morphological or anatomical features (Talluri & Murray 2009).

The species in the section *Calospira* C. Koch. differ from all other species in the genus *Spiraea* L. by their inflorescences. The inflorescences make a broad complex corymbose or oval panicles. Some authors consider that this polymorphic group from the *Calospira* section is a single species – *S. betulifolia* Pall. (Nedoluzhko 1995). Other authors distinguish between 2 and 4 species in this section (Vorobyev 1968, Yakubov 1996, Koropachinsky & Vstovskaya 2002). Within this section, Tzvelev (2008) identifies 3 species and 2 notospecies of hybridogenic origin. There are still many questions to understand the taxonomic status of closely related species *S. betulifolia*, *S. beauverdiana* Schneid. and *S. aemiliana* Schneid. This happens due to a different understanding of the volume of the species by different taxonomists and a choice of discriminant features on which to distinguish these species. The species *S. betulifolia* and *S. beauverdiana* growing on the territory of the Russian Far East are characterized by a high flexibility of morphological parameters (Kostikova & Polyakova 2014). These species have intermediate forms in places of joint growth in Khabarovsk Krai, Sakhalin and the Republic of Sakha (Yakutia). The *S. betulifolia* also grows in China and Japan, and the *S. beauverdiana* – in China, Korea and North America (Koropachinsky & Vstovskaya 2002). In the study of the variability of morphological characters it was confirmed that the species *S. beauverdiana* and *S. betulifolia* differ from each other by the following characteristics: a dense pubescence of pedicel and follicle, a curved position of follicle rostrum. The qualitative composition of phenolic compounds in water-ethanol extracts of leaves of the *Spiraea* were also defined as species-specific. The *S. beauverdiana* and *S. betulifolia* slightly differ from each other in morphometric morphological characteristics (Kostikova & Polyakova 2014). The *S. aemiliana* has a low height of the shrub to 30 cm and has rounded leaves. In terms of its habitus the

S. aemiliana is closer to the *S. beauverdiana*. The *S. aemiliana* also grows in Japan (Vorobyev 1968).

The purpose of this study is to compare the relative DNA amounts in cell nuclei of plants in the genus *Spiraea* section of *Calospira*.

The materials for the study were fresh leaves of three species of the genus *Spiraea* section of *Calospira*, collected in 2017 from plants introduced at the experimental plot of land of the Laboratory of Phytochemistry of the Central Siberian Botanical Garden SB RAS (CSBG) and from plants growing in native populations (Table 1). Almost all studied plants are brought from the native populations; some of them are grown from seeds collected also in the native populations. This study included specimens which morphological description corresponds to the species identifying specific differences in the relative content of DNA. For each species, 10 plants from each of two populations were examined. All tests were carried out in 3 replicates.

The flow-cytometric measurements were run in the Laboratory of Dendrology in the CSBG. The content of plant DNA was determined by a flow cytometry with staining isolated nuclei by propidium iodide (PI). The analysis was carried out on a CyFlowSpace device (Germany, Sysmex Partec) with a laser radiation source of 532 nm. The fresh leaves of *Solanum lycopersicum* cv. 'Stupice' (2C DNA content = 1.96 pg) were used as an internal standard which seeds were obtained from the Centre of the Plant Structural and Functional Genomics of the Institute of Experimental Botany AS CR, Olomouc, Czech Republic (Doležel et al. 1992).

A part of the fresh leaf of a 0.5 cm^2 size was ground using an acute blade along with an appropriate amount of internal standard in 500 µl of a cooled commercial buffer (Nuclei Extraction Buffer) (Germany, Sysmex Partec) according to the manufacturer's protocol in plastic Petri dishes adding polyvinylpyrrolidone (MB ~ 29,000) (USA, Sigma-Aldrich). The samples incubation was carried out at room temperature for 2 minutes. The samples were filtered through the Cellecrics Partec 50 micron (Germany, Sysmex Partec) and then 2 ml of a staining solution were added containing another commercial buffer (Staining Buffer) (Germany, Sysmex Partec), PI (50 µg/ml) and RNAse A (50 µg/ml). The incubation (staining) was performed at room temperature in a dark place for 40 minutes. The prepared samples were stored in a refrigerator for no more than 4 hours. 15,000 events were collected for each sample. The DNA 2C value content was calculated on a linear relationship between fluorescent signals from the stained nuclei of the internal standard and studied specimens.

The received data is carried out with the help of the software Statistica 6.0. (StatSoft, Inc. 1984-2001). The taxa differences by the relative DNA amounts were studied by a nonparametric variance analysis (ANOVA) using the Kraskel-Wallis criterion.

The nuclear DNA content of plants of the genus *Spiraea* section *Calospira* growing in the Russian Far East is determined for the first time by the flow cytometry method according to the fluorescence relative intensity (Table 1). The nuclear DNA content varies from 0.45 pg in the *S. aemiliana* to 1.01 pg in the *S. betulifolia*.

The colored nuclei generate histograms of the relative DNA content of the sample and the comparative standard (Fig. 1). The histograms of the flow cytometry represent two dominant peaks corresponding to the sample G0/G1 nuclei and the reference standard as well as a peak corresponding to the standard G2 nuclei.

The relative DNA (2C) content in angiosperms changes from 0.22 to 254.8 pg (Leitch et al. 2005). The variability of the relative DNA content in the Rosaceae family is low. The subfamily Spiraeoideae plant genome size is referred to the smallest among angiosperms (Dickson et al. 1992). The nuclear DNA content values of the genus *Spiraea* species are comparatively small and according to the gradation of Leitch et al. (2005) fall into the category of "very small": *S. chamaedryfolia* 2C = 0.90 pg; *S. chinensis* 0.40; *S. crenata* 0.45; *S. pubescens* 0.95; *S. wilsonii* 1.60; *S. nipponica* 1.75 and *S. sargentiana* 1.85 (Dickson et al. 1992, Siljak-Yakovlev et al. 2010, Bennett & Leitch 2012). The range of relative DNA content in plants of the genus *Spiraea*, section *Calospira* is within the values previously recorded for *Spiraea*. A comparative analysis of the relative DNA content of the studied *Spiraea* revealed the facts that the values differ at a high statistical significance ($p<0.001$) (Fig. 2). The *S. betulifolia* occupies plain and low mountain forest lands in middle and southern regions of the Asian Russia. The *S. beauaverdiana* acquired new adaptive features (dense pubescence of organs, a tendency to a general reduction in plant size) that allow it to settle in upland alpine tundra areas of the range northern part (Koropachinsky & Vstovskaya 2002, Kostikova & Polyakova

Table 1. The nuclear DNA content and the site of material collection of close species of the genus *Spiraea*, section *Calospira* (standard *Solanum lycopersicum* cv. 'Stupice' (2C DNA content = 1.96 pg) (Doležel et al. 1992)

Sample №	Sites of material collection	2C DNA content, pg, ±SD***	1C**** (genome value), (Mbp)
S. betulifolia			
1	Kunashir Island, Golovnino village environs	1.01±0.01	493.89
2	Primorsky Krai, Anisimovka village environs, Livadia Range, Mount Litovka *	0.92±0.01	449.88
S. beauverdiana			
3	Khabarovsky Krai, Kur-Urmia Range	0.55±0	268.95
4	Republic of Sakha (Yakutia), Maly Nimnyr settlement environs**	0.57±0.007	278.73
S. aemiliana			
5	Kunashir Island, Golovnino volcano caldera	0.48±0.005	234.72
6	Sakhalin Island, Chekhov peak	0.45±0.01	220.05

Note: * plant grown of seeds; ** natural material; *** SD – standard deviation (Std. Dev.); **** 1 pg of DNA = 978 Mbp (Doležel et al. 2003)

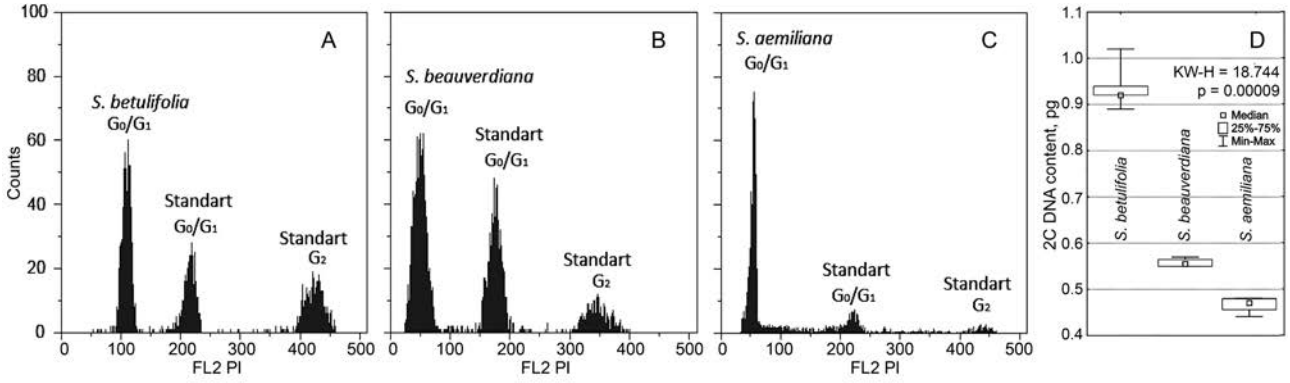

Figure 1 Histogram of PI fluorescence intensity (A–C): *S. betulifolia* (A); *S. beauverdiana* (B); *S. aemiliana* (C). A standard is *Solanum lycopersicum* cv. 'Stupice' (2C DNA content = 1.96 pg) (Doležel et al. 1992) and the relative nuclear DNA content (D)

2014). In the places of joint growth these species are not clearly separated from each other, perhaps these taxa represent different ecotypes of the same species (Svyazeva 1967). The relative DNA content in the *S. betulifolia* is the higher – 0.91 and 1.01 pg (Table 1, Fig. 2). The values of the relative DNA content of the *S. beauverdiana* are almost twice lower than in the *S. betulifolia* – 0.55 and 0.57 pg. The southeast Asia is the center of *Spiraea* distribution. The species were dispersed northward, from more to less favorable climatic conditions, so it is possible to trace the tendency of decreasing the relative DNA content of plants because of habitat conditions deterioration. That means that the relative DNA content in plants of the genus *Spiraea*, section *Calospira* decreases northward.

The *S. aemiliana* occurs on the Russia's island part – the Kurils and Sakhalin Islands. The nuclear DNA content of this species is the lowest – 0.45 and 0.48 pg. The *S. aemiliana* differs from *S. betulifolia* even more than twice and it is closer to the *S. beauverdiana* by the relative DNA content. As the population No. 1 of *S. betulifolia* and the populations No. 5 of *S. aemiliana* are located relatively close each other on the Kunashir Island, they grow under the same climatic conditions, it is likely that differences in DNA content are related to the species taxonomic position.

Within the same chromosomal composition, the nuclear DNA content can differ significantly between various taxa (species) within the genus (Talluri & Murray 2009). There are diploids and polyploids: tetraploids, octoploids among the genus *Spiraea* plants (Zhukova 1980, Oginuma et al. 2004, Polyakova & Muratova 2015, http://www.tropicos.org/ Project / IPCN). The plants of the whole subfamily Spiraeoideae including meadowsweets are characterized by the main number of chromosomes n = 9. The karyotypes of the *S. betulifolia* and the *S. beauverdiana* from the Russian Far East contain 18 chromosomes and are diploids (2n = 18) with a base number equal to 9 (Dickson et al. 1992, Zhukova 1980, Polyakova & Muratova 2015). Probably, the studied *Spiraea* species are diploids with different nuclear DNA contents. The obtained values of the nuclear DNA content should not be used to establish *Spiraea* ploidy due to the lack of reliable information on chromosome numbers in the studied specimens. It is planned to count the chromosome number of the plant samples within a study and to identify their relation to the relative DNA content.

Thus, the revealed values of the relative DNA content in *Spiraea* differ from each other greatly at the high statistical significance ($p<0.001$). The relative DNA content in the *S. betulifolia* (2C = 0.91 and 1.01 pg) is twice than in the other species. The *S. beauverdiana* (2C = 0.55 and 0.57 pg) and the *S. aemiliana* (2C = 0.45 and 0.48 pg) are closer in DNA content.

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