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SHORT COMMUNICATION

Flow cytometric determination of genome size and ploidy level of some frost-resistant cultivars and species of *Rhododendron* L. native to Asian Russia

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ABSTRACT

Rhododendrons of Asian Russia represent a valuable genetic resource for development of new ornamental cultivars for cold environment. Assessment of their genome size and ploidy level is critical for creation of new rhododendron hybrids and polyploids as well as for solving taxonomic problems. In spite of genome sizes of some European and American rhododendron genotypes have been already determined with the use of flow cytometry, this approach is not used for analysis of species native to Asian Russia. Here, we present flow cytometric data on genome size, DNA content and ploidy level of frost-resistant cultivars of rhododendrons (*R. catawbiense* 'Grandiflorum', 'Helsinki University', 'Haaga') and species (*R. adamsii*, *R. brachycarpum*, *R. dauricum*, *R. ledebourii*, *R. mucronulatum*, *R. parvifolium*, *R. schlippenbachii* and *R. sichotense*).

Keywords: genome size, DNA content, flow cytometry, *Rhododendron*

РЕЗЮМЕ

Зайцева Ю.Г., Амброс Е.В., Каракулов А.В., Новикова Т.И. Цитометрическое определение размеров генома и уровня плоидности некоторых морозоустойчивых сортов и видов *Rhododendron* L., произрастающих в Азиатской России. Рододендроны азиатской России являются ценным генетическим ресурсом для создания новых сортов для холодных климатических зон. Оценка их размера генома и уровня плоидности имеет решающее значение для создания новых гибридных и полиплоидных рододендронов, а также для решения таксономических проблем. Несмотря на то, что размеры геномов для некоторых европейских и американских генотипов рододендронов уже установлены с использованием проточной цитометрии, этот подход не используется для анализа видов азиатской России. Здесь мы впервые приводим данные, полученные с использованием проточной цитометрии, о размере генома, содержании ДНК и уровне плоидности морозоустойчивых сортов (*R. catawbiense* 'Grandiflorum', 'Helsinki University', 'Haaga') and species (*R. adamsii*, *R. brachycarpum*, *R. dauricum*, *R. ledebourii*, *R. mucronulatum*, *R. parvifolium*, *R. schlippenbachii* and *R. sichotense*).

Ключевые слова: размер генома, содержание ДНК, проточная цитометрия, *Rhododendron*

The genus *Rhododendron* L. (Ericaceae) comprises more than 1,000 species (Kondratovich 1981, Chamberlain 1996) and over 10,000 varieties wordwide. In the Russian Federation there are 16 rhododendron species, 13 of them occur only in Siberia and the Russian Far East (Budantsev 2009). Rhododendrons native to Asian Russia are highly ornamental, frost resistant and able to grow on slightly acidic soils (Babro et al. 2007, Vrishch 2010, Tikhonova et al. 2012) that allows to use these species as a valuable genetic resource for breeding new ornamental cultivars for Siberia and the Russian Far East.

To obtain improved rhododendron cultivars plant breeders use natural polyploids and/or induce polyploidy since it often results in the extension of flower longevity, increase of flower size and deep flower colors (Jones et al. 2007). Furthermore, the important consequences of polyploidy for plant breeding are increased heterozygosity and heterosis as well as enhanced tolerance to both biotic and abiotic stresses (Sattler et al. 2016). There are a large number of induced polyploid rhododendron cultivars of commercial value; however, wild species of Siberia and the Russian Far East have not been involved in the breeding program yet. The first step in this direction is the assessment of relative genome size and ploidy level in the genotypes studied using flow cytometry.

Cytometric studies were conducted in *Rhododendron* species and cultivars (De Schepper et al. 2001, Jones et al. 2007, Lattier et al. 2013). Availability of these data are important not only for plant breeding but also for various fields of basic research, including rhododendron taxonomy and evolutionary changes (Chahal & Gosal 2002, Contreras et al. 2007, Kron et al.). The objective of this study was to de-

termine genome size and ploidy level of frost-resistant cultivars (*R. catawbiense* 'Grandiflorum', hybrids 'Helsinki University', 'Haaga', created on the base of *R. brachycarpum* var. *tigerstedtii*) and species native to Asian Russia (*R. dauricum*, *R. mucronulatum*, *R. ledebourii*, *R. sichotense*, *R. schlippenbachii*, *R. brachycarpum*, *R. parvifolium* and *R. adamsii*).

To determine relative DNA content and genome size in some frost-resistant genotypes, flow cytometry (FCM) analysis with CyFlow® Space (Partec, Germany) was performed. *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg) (Doležel et al. 1998) and P*etroselinum crispum* L. (2C = 4.5 pg) (Obermayer et al. 2002) were used as reference standards with a known DNA content for calculating the relative DNA content of the test samples. Young leaves of 2 to 6 individuals of the same species or varieties grown in the collection of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (CSBG) were used to prepare samples for the analysis. For nuclei extraction, approximately 1 cm^2 of leaf blade of a sample and reference standard were simultaneously chopped with razor blade in a Petri dish with 0.5 ml of commercial nuclei extraction buffer (from reagent kit CyStain® PI Absolute, Partec, Germany). According to manufacturer protocol, 1 % polyvinylpyrrolidone (mol wt 29000) (Sigma-Aldrich, USA) was added to the buffer. After 90 seconds of extraction, the suspension was filtrated using Partec CellTrics filters (Sysmex Partec GmbH, Germany) with 30 μM of pore size. Then the nuclei were stained for an hour in the dark at room temperature with commercial staining buffer (from reagent kit CyStain® PI Absolute, Partec, Germany) containing propidium iodide (PI) and RNAse. The samples were analyzed by FCM using a green laser (532 nm) at 30–50 events per minute to determine relative DNA fluorescence. The fluorescence data were obtained from the detection of 10,000 events. DNA content was determined by comparing a mean fluorescence of 2C-peak of each sample and reference standard. The relative DNA content (2C) was calculated using the formula (Doležel et al. 2007): 2C DNA content of sample = 2C DNA content of standard – mean fluorescence value of 2C sample / mean fluorescence value of 2C standard.

The genome size of samples was calculated on the basis of that 1 pg DNA = 978 Mbp (Doležel et al. 2003). The histograms of DNA content were evaluated with FloMax 2.9 software package (Partec, Germany).The obtained results were processed using the Statistica 8.0 software (StatSoft Inc., USA). The data were analyzed by clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA).

The genus *Rhododendron* is divided into eight subgenera, four most important subgenera being *Tsutsusi*, *Pentanthera*, *Rhododendron* and *Hymenanthes* (Chamberlain 1996). In this study we analyzed by FCM method the genotypes belonging to the last three subgenera according to the classification of Chamberlain (1996) or to the subgenera *Rhodorastrum*, *Rhododendron*, *Sciadorhodion* and *Leiorhodium* according to the classification of Aleksandrova (1975) (Table1).

Previous studies showed that the basal chromosome number of the genus *Rhododendron* is 13, and most of the species and hybrids are diploids, with 2n = 26, although tetraploids, hexaploids, and also dodecaploids (2n = 12x = 156), are found in different species (Ammal et al. 1950, Jones et al. 2007). In early studies, the light microscopy was used for counting the chromosome number in rhododendron roots and for determination of ploidy levels (Sax 1930). However, due to problems of root isolation and small size of rhododendron chromosomes, it was a laborious, time consuming and difficult task. Modern FCM approach provides a fast and accurate determination of genome size and nuclear DNA content which is related directly to ploidy level of taxa (Doležel et al. 1998). Polyploidy was found to be common in the genus *Rhododendron* and considerably more prevalent in the subgenus Pentanthera (Eeckhaut et al. 2004). However, all samples studied including *R. schlippenbachii* (subgenius *Pentanthera*) were found to be diploid. The FCM profiles indicated stability of the ploidy level in leaf cells of the genotypes studied (Fig. 1).

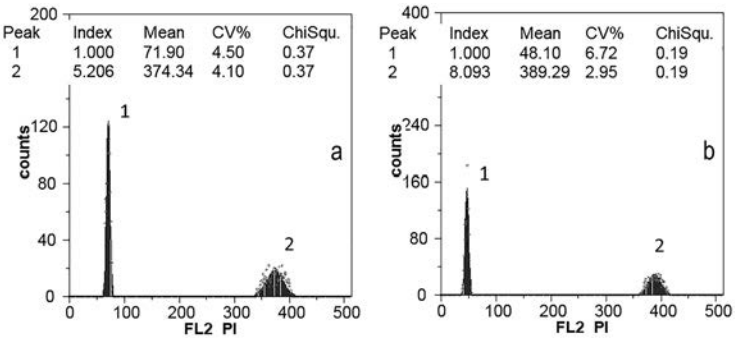

Figure 1 FCM histogram of relative fluorescence intensity of propidium iodine-stained nuclei harvested from young leaves: a – *R. parvifolium*, 1 – G1 pic of *R. parvifolium*, 2 – G1-pic of *Pisum sativum* L. 'Ctirad' (reference standard); b – *R. schlippenbachii*, 1 – G1-pic of *R. schlippenbachii*, 2 – G1-pic of *Pisum sativum* L. 'Ctirad' (reference standard)

Table 1. Classification of the tested genotypes of genus *Rhododendron* L.

Species or variety	Classification by Aleksandrova (1975)		Classification by Chamberlain (1996)	
	Subgenus	Row	Subgenus	Section
R. sichotense Pojark., *R. mucronulatum* Pojark., *R. dauricum* L., *R. ledebourii* Pojark.	*Rhodorastrum*	*Daurica* Pojark.	*Rhododendron*	*Rhododendron*
R. parvifolium Adams = *R. lapponicum* (L.) Wahlenb.	*Rhododendron*	*Parvifolia* E. Busch		
R. adamsii Rehd. = *R. fragrans* (Adams) Maxim.		*Fragrantia* E. Busch		*Pogonanthum*
R. schlippenbachii Maxim.	*Sciadorhodion*	–	*Pentanthera*	*Sciadorhodion*
R. catawbiense 'Grandiflorum'	*Leiorhodium*	–	*Hymenanthes*	*Pontica*
R. brachycarpum D. Don ex G. Don = *R. fauriei* Franch. 'Helsinki University', 'Haaga'		*Caucasia* (Pojark.) Alex.		

Relative genome sizes (2C) of the genotypes studied ranged between 1.15 pg and 1.74 pg whereas 1C varied from 564.0 to 850.94 Mbp (Table 2). It was revealed that *R. schlippenbachii* from the subgenus *Pentanthera* possessed the smallest genome size (1.15 pg) among the taxa studied of the genus *Rhododendron*. The result obtained showed similarity with *R. canadense*, another representative of this subgenus with the same genome size (1.15 pg) (Lattier et al. 2013). However, this data contrasted with other reports on genome sizes of deciduous diploid azaleas which ranged between 1.51 pg and 1.74 pg (Jones et al. 2007, Zhou et al. 2008). At the same time the genome size of *R. parvifolium* (subgenus *Rhododendron*) was the biggest one among the genotypes studied (1.74 pg).

Due to the large variability of *Rhododendron* species, it is difficult to establish phylogenetic relationships within it (Chamberlain 1996, Aleksandrova 1975). Until now, there is no generally accepted classification of the genus. Handling the data on DNA content and genome size by clustering can provide a useful tool for taxonomy. In this research, UPGMA analysis of the DNA content of the samples distinguished four groups (Fig. 2): A–D. *R. sichotense*, *R. mucronulatum*, *R. lebeburii* and *R. dauricum* belong to group A; *R. schlippenbachii* a species with the smallest genome size belongs to group B; *R. adamsii*, *R. brachycarpum* and *R. catawbiense* 'Grandiflorum', 'Helsinki University' and 'Haaga' belong to group C; and *R. parvifolium* a species with the largest genome size belongs to the group D. This division overlaps with the classification of Chamberlain (1996). In addition, the grouping of *R. sichotense*, *R. mucronulatum*, *R. lebebourii* and *R. dauricum* is in agreement with previous studies based on modern biochemical and molecular genetic data (Goetsch et al. 2005, Kusev & Karakulov 2010, Baranova et al. 2014).

To summarize, we report the data on nuclear DNA content and genome size of frost-resistant cultivars 'Helsinki University', 'Haaga' and *R. catawbiense* 'Grandiflorum' and *R. dauricum*, *R. lebeburii*, *R. mucronulatum*, *R. sichotense*, *R. schlippenbachii*, *R. brachycarpum*, *R. parvifolium* and *R. adamsii* native to Asian Russia, which could serve as a base for further breeding research programs including hybridization and polyploidy induction among the frost-resistant *Rhododendron* species and at the same time useful for determining the phylogenetic evolution of the rhododendrons. Moreover, the obtained data on nuclear genome sizes are also important for the mapping of rhododendrons genomes and the development of strategies for isolation of plant genes.

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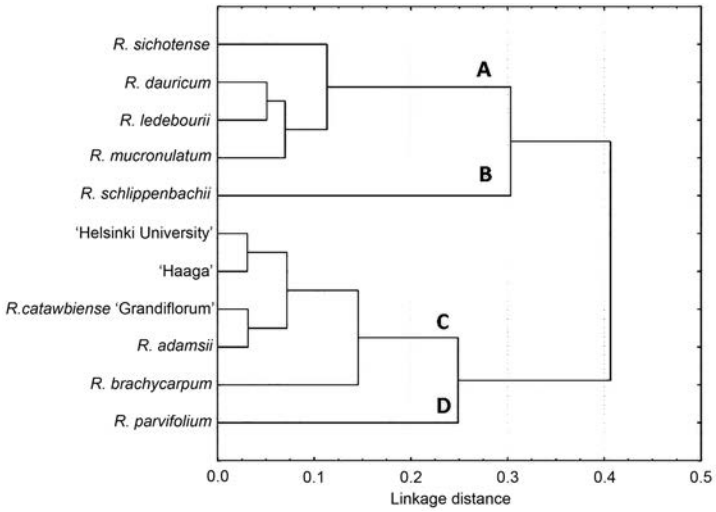

Figure 2 Tree diagram based on DNA-content of some *Rhododendron* genotypes

Table 2. Relative DNA content and genome size of some genotypes of genus *Rhododednron* L.

Species or variety	N*	index **	2C (pg)	1C (pg)	Genome size (Mbp)	Coefficient of variation (%)	Reference standard
R. sichotense	6	3.27 ± 0.07	1.38 ± 0.03	0.69 ± 0.02	674.23 ± 14.88	5.59 ± 1.09	*Petroselinum crispum* L.
R. mucronulatum	5	6.91 ± 0.13	1.32 ± 0.03	0.66 ± 0.01	643.44 ± 12.39	3.26 ± 0.55	*Pisum sativum* L. 'Ctirad'
R. dauricum	3	6.70 ± 0.10	1.36 ± 0.02	0.68 ± 0.01	663.37 ± 10.01	3.62 ± 0.24	*Pisum sativum* L. 'Ctirad'
R. ledebourii	3	6.89 ± 0.21	1.32 ± 0.04	0.66 ± 0.02	645.06 ± 19.97	6,47 ± 0.51	*Pisum sativum* L. 'Ctirad'
R. adamsii	3	5.60 ± 0.08	1.62 ± 0.02	0.81 ± 0.01	793.35 ± 11.39	4.13 ± 0.67	*Pisum sativum* L. 'Ctirad'
R. parvifolium	2	5.22 ± 0.05	1.74 ± 0.02	0.87 ± 0.01	850.94 ± 8.46	4.04 ± 0.18	*Pisum sativum* L. 'Ctirad'
R. shlippenbachii	3	7.88 ± 0.19	1.15 ± 0.03	0.57 ± 0.01	564.01 ± 13.42	3.61 ± 0.25	*Pisum sativum* L. 'Ctirad'
R. catawbiense 'Grandiflorum'	6	2.78 ± 0.03	1.62 ± 0.02	0.81 ± 0.01	791.07 ± 9.09	4.87 ± 0.54	*Petroselinum crispum* L.
R. brachycarpum	2	6.12 ± 0.15	1.49 ± 0.06	0.74 ± 0.02	726.69 ± 17.38	6.68 ± 0.47	*Pisum sativum* L. 'Ctirad'
'Helsinki University'	7	2.92 ± 0.04	1.54 ± 0.02	0.77 ± 0.01	754.83 ± 10.24	4.34 ± 0.39	*Petroselinum crispum* L.
'Haaga'	5	2.92 ± 0.05	1.54 ± 0.03	0.77 ± 0.01	754.57 ± 13.97	4.77 ± 0.39	*Petroselinum crispum* L.

Data is presented as means ± standard deviation (SD)
* Number of samples analyzed
** The index is the ratio of mean fluorescences of G1 peaks (2C) of the standard and sample
2C – diploid DNA content, pg
1C – haploid DNA content, pg

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