

Karyotype analysis of *Hypericum rumeliacum* BOISS.

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Abstract: Karyotype of the Balkan endemic, *Hypericum rumeliacum*, was studied using root tip meristems of *in vitro* propagated plants. The absolute length of the chromosomes varied between 0.97 and 1.59 μm . The karyotype formula of the basic chromosome set ($x = 7$) was $3m + 1m^{\text{sat}} + 3sm$. Karyotype data were compared with other karyotype data for the genus *Hypericum* available in literature. Only one common chromosome type – substantially bigger chromosome, is clearly distinguishable in some of the species. The other chromosomes are small, median to submedian and often difficult to distinguish due to their small size. Asymmetry index is low in *Hypericum rumeliacum* and other species with available karyotype data suggesting that they underwent no major karyotype rearrangements in their evolutionary pathway.

Keywords: *Hypericum* spp., endemic species, chromosomes, asymmetry index, *in vitro* cultured plants

Introduction

The genus *Hypericum* (*Guttiferae*) comprises more than 450 species grouped into 30 sections. It comprises trees, shrubs and herbs occurring in temperate zones of the world. (Robson, 2003). *Hypericum rumeliacum* BOISS. is a Balkan endemic species. It belongs to the section *Drosocarpium* of the *Olympia* group.

This section includes about 12 species distributed mainly in the Mediterranean region (ROBSON 1977, 1981).

The chromosome number for *H. rumeliacum* has been determined for the first time by NIELSEN (1924) as $2n = 14$. According to ROBSON (1981) the *Hypericum* species with the basic chromosome number $x = 7$ do not exist at polyploidy level. So far there are no records on karyotype study of the species *H. rumeliacum*. Karyotype data in *Hypericum* are scarce. KOGI (1984) presented detailed karyotype data on the following taxa: *H. erectum* THUNB., *H. pseudopetiolatum* KELLER, *H. tosaense* MAKINO (sect. *Hypericum*) and *H. ascyron* L. (sect. *Roscyna*). Karyotype data in *H. perforatum* L. were published by BRUTOVSKÁ et al. (2000).

The pharmacological potential of *H. rumeliacum* is high, comparable to that of some other representatives of the genus *Hypericum*. The presence of the photodynamic pigments hypericin and pseudohypericin characteristic especially for the more advanced representatives of the genus was recently reported for *H. rumeliacum* by several authors (KITANOV 2001, SMELCEROVIC et al. 2006, SMELCEROVIC & SPITELLER 2006, GALATI et al. 2008). The essential oil composition of *H. rumeliacum* was investigated by COULADIS et al. (2003), SAROGLU et al. (2007), SMELCEROVIC et al. (2007) and the polyphenol compounds by KITANOV (1979) and recently by GALATI et al. (2008). Among the pharmacological effects the antimicrobial, antiinflammatory and antioxidant have been reported so far. RADULOVIC et al. (2007) determined broad spectrum of antibiotic properties of the methanol extract of *H. rumeliacum*. It has been shown that the antimicrobial activity is connected with the presence and composition of essential oils (COULADIS et al. 2003, SAROGLU et al. 2007). The essential oil composition of some *Hypericum* species including *H. rumeliacum* was used for chemotaxonomic purposes as well. It was shown that the phylogenetic reconstruction supports the existing divisions of *Hypericum* into taxonomic sections based on terpenoids (PETRAKIS et al. 2005). SMELCEROVIC et al. (2006) found a positive correlation between the secondary metabolite content in some *Hypericum* species including *H. rumeliacum* and certain SSR and RAPD markers.

The aim of this paper was to perform karyotype analysis of endemic - *H. rumeliacum* plants grown *in vitro* and to compare these findings with the results established for other *Hypericum* species.

Material and Methods

Plant material:

Intact plants of *Hypericum rumeliacum* Boiss. were collected in the Rhodopes Mountain, Bulgaria in August 2006. Herbarium specimen was deposited at the Institute of Botany, Bulgarian Academy of Sciences, Sofia - SOM 163 524. The mononodal stem segments of the plants were used after surface sterilisation with 70% ethanol and 0.1% HgCl₂, followed by triple washing in sterile distilled water for establishment of *in vitro* culture. The explants were cultured on MURASHIGE

and SKOOG (1962) culture medium supplemented with 0.3 mg/l 6-benzyladenine. The differentiated shoots were then transferred to the same medium without 6-benzyladenine for rooting. The root tips were isolated and used for preparation of slides for karyological analysis.

Karyological analysis:

Roots isolated from *in vitro* grown plants were washed in distilled water for 1 hour at 4 °C. For pre-treatment, the root tips were placed in 0.002 M aqueous solution of 8-hydroxyquinoline at 4 °C for 4 hours. Root tips were then fixed in acetic ethanol (glacial acetic acid and 96% ethanol at a ratio 1:3) and then hydrolyzed for 6 minutes in 1N HCl at 60 °C. The root tips were squashed using cellophane technique (MURIN 1960) in a drop of 45 % acetic acid and stained in 10% Giemsa stain solution in Sörensen phosphate buffer, pH 7. The slides were then washed in distilled water, dried and observed in a drop of immersion oil. The best metaphase plates were selected for calculation of karyotype characteristics. Microphotographs of these metaphase plates were taken and chromosomes were measured. For the chromosome identification and comparison, the following characteristics were used: absolute chromosome length, relative chromosome length – RL value (the ratio of the length of particular chromosome to the sum of length of all chromosomes in the metaphase plate studied), arm index (the ratio of the length of longer to shorter arms). Since RL is dependent on chromosome number (2n) of the species studied, for the sake of comparisons among different species (with different chromosome numbers and even ploidy), RLC value was calculated $RLC = RL \cdot 2n$. The classification of chromosomes was made according to LEVAN *et al.* (1964). Karyotype asymmetry index AI was calculated and interpreted according to PASZKO (2006)

Results and Discussion

Chromosome counts of metaphase cells of *in vitro* cultured plants confirm diploid chromosome number $2n = 2x = 14$ in all samples studied. The chromosomes of *H. rumeliacum* are small ranging in size between 0.97 and 1.59 μm . Further reports of chromosome size in the genus give similar values: 0.5 to 2.2 μm according to ROBSON & ADAMS (1968), 0,9 to 2,5 in KOGI (1984) and between 0.78 and 1.52 μm for *H. perforatum* according to BRUTOVSKÁ *et al.* 2000. In *H. rumeliacum* the position of centromere is median or submedian. Chromosome characteristics for individual homologous pairs are given in Tab. 1. The karyotype formula for *H. rumeliacum* is $3m + 1m^{\text{sat}} + 3sm$. Photograph of c-metaphase is shown in Fig. 1 and idiogram of the chromosomes is given in Fig. 2. For the sake of visualization of karyotype data, combined BoxPlot graph was drawn for karyotype of *H. rumeliacum* (Fig. 3). It can be seen there that the chromosome pair I (the pair of the biggest chromosomes) is a very well distinguishable one. The pair two is another detectable chromosome pair. The smaller the chromosomes, the worse the detection is, especially the chromosome pairs III and IV, as well as V and VI are difficult to distinguish from each other.

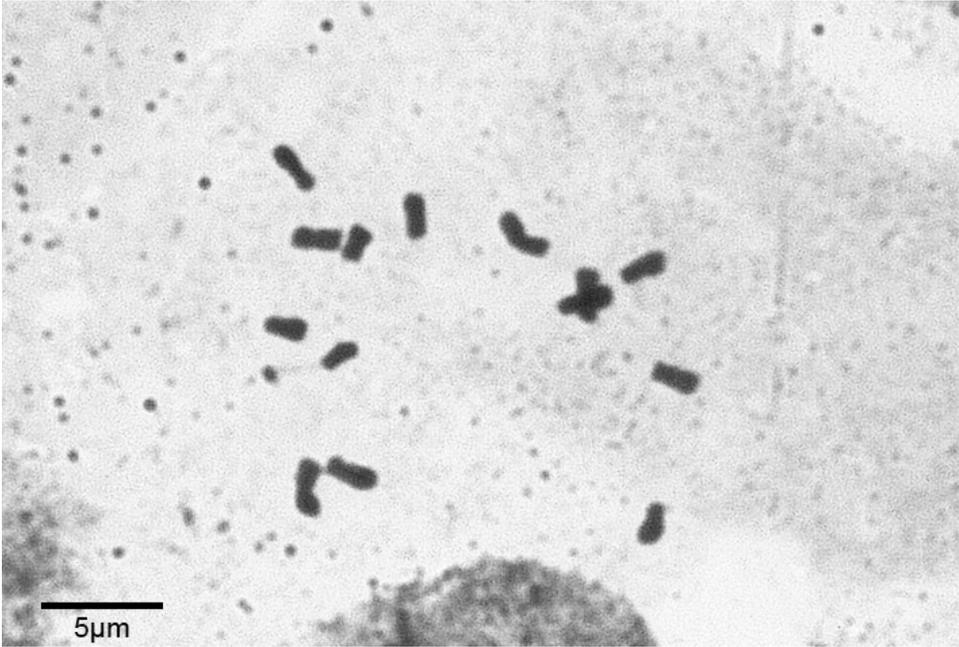


Fig. 1. Metaphase plate of *H. rumeliacum* Boiss. grown *in vitro*.

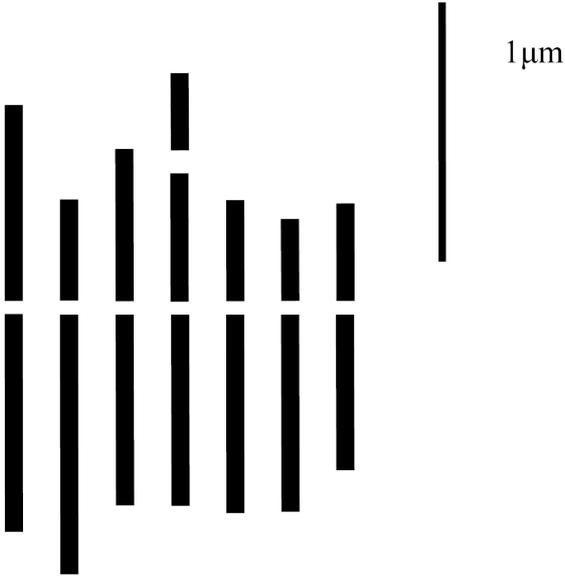


Fig. 2. Idiogram of *H. rumeliacum* Boiss.

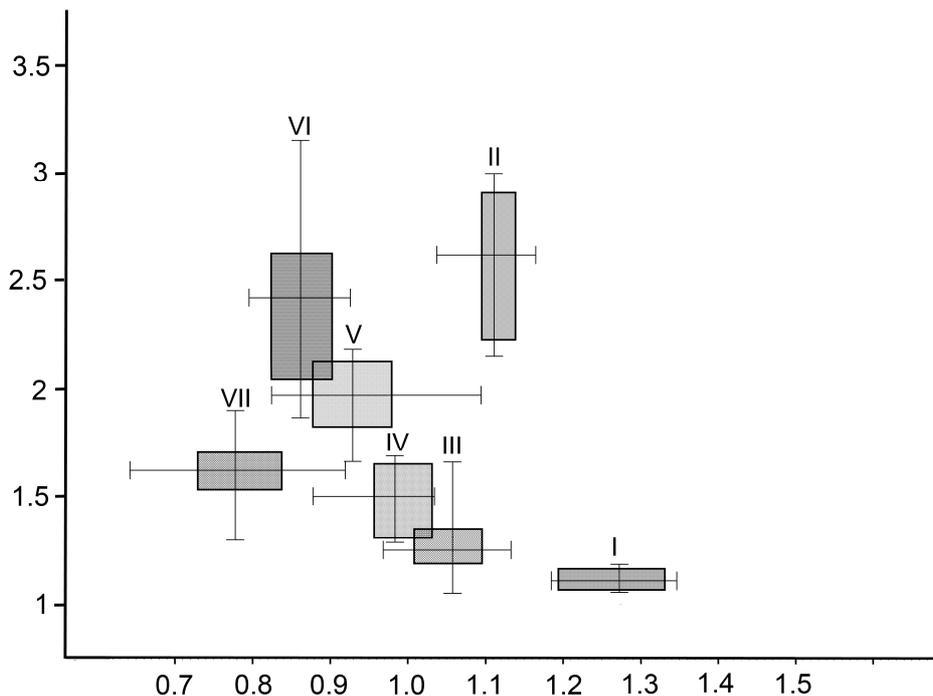


Fig. 3. Combined BoxPlot graph for the karyotype of *H. rumeliacum*: x-axis represents RLC values, y-axis AR values, the boxes with abscissas represent particular chromosome pairs (I – VII). Inside the boxes values between first and third quartiles are included, the abscissas mark whole range of the values from minimum to maximum: the intersection of the abscissas represents average values.

The results of karyotype studies in species with small chromosomes should be interpreted with caution. The measurements of chromosome characteristics are less accurate in small-sized chromosomes and discrimination of pairs of homologous chromosomes is often difficult, or even impossible.

There are several papers dealing with chromosome characteristics of some *Hypericum* species which concern *H. perforatum* (BRUTOVSKÁ *et al.* 2000), *H. erectum*, *H. tosaense*, *H. ascyron*, *H. pseudopetiolatum*, *H. samaniense* MIYABE ET KIMURA, *H. hakonense* FRANCH. ET SAV., *H. sikokumontanum* MAKINO, *H. kamtschaticum* LEDEB. and *H. yojiroanum* TATEW. ET KOJI ITO (KOGI 1984). Among the nine studied species belonging to the sections *Hypericum* and *Roscyna* KOGI (1984) identified three different karyotypes. He gave karyotype details for *H. erectum*, *H. pseudopetiolatum* (first karyotype), *H. tosaense* (second karyotype) and *H. ascyron* (third karyotype). We compared the published karyotype data in the genus *Hypericum* with the karyotype data for *H. rumeliacum* and found only one well distinguishable chromosome type – big

median chromosome, which is common for *H. perforatum*, *H. erectum* and *H. pseudopetiolatum*. The other chromosomes are small and mostly median (AR from 1 to 1.7). This fact is also expressed in low karyotype asymmetry index for the species concerned (Tab. 2). This means that neither *H. rumeliacum*, nor other species studied by KOGI (1984) and BRUTOVSKÁ et. al. (2000) underwent substantial karyotype reorganization (chromosome translocations, deletions, etc.) on their evolutionary pathway from ancestral species. However, the chromosomes in the genus *Hypericum* are small, therefore the smaller changes in chromosome morphology are not detectable by means of light microscopy.

Tab. 1. Chromosome characteristics of *Hypericum rumeliacum* (m – metacentric chromosome, sm – submetacentric chromosome, sat - satellite).

Chromosome pair no.	Relative chrom. length	Absolute chrom. length (µm)	Arm index	Chrom. type
I	0.090938	1.598558	1.112682	m
II	0.079389	1.394231	2.566446	sm
III	0.07571	1.329327	1.249722	m
IV (sat)	0.070315 (0.01694)	1.233173 (0.295673)	1.502915	m ^{sat}
V	0.066438	1.158654	1.968561	sm
VI	0.061603	1.079327	2.418781	sm
VII	0.055607	0.978365	1.621398	m

Tab. 2. Karyotype asymmetry indices (AI) for different *Hypericum* taxa

Taxon name	AI
<i>H. rumeliacum</i>	2.80
<i>H. perforatum</i>	2.15
<i>H. erectum</i>	3.13
<i>H. pseudopetiolatum</i>	1.61
<i>H. tosaense</i>	1.68
<i>H. ascyron</i>	1.65

Due to peculiarities in reproduction mechanisms of the species, the chromosomal and karyotype findings for *Hypericum* ssp. should be considered in a wider context of the genus. ROBSON & ADAMS (1968) concluded that the basic chromosome numbers in the genus *Hypericum* form a descending series from 12 to 7, with a possible extension to 6 if the count of $2n = 24$ for *H. gentianoides* BRITTON proved to indicate tetraploidy. Tetraploidy has been recorded on the basic numbers $x = 8, 9$ and 10 but not on $x = 7$ or definitely on $x = 12$; higher degrees of polyploidy appeared to be confined in nature to section IX *Hypericum* and are associated with the largely apomictic *H. perforatum* L. ($2n = 32, 48$) and its hybrid with *H. maculatum* Crantz ($2n = 32, 40, 48$) (ROBSON, 1981). The flow

cytometric seed screen analysis of 67 species and 3 subspecies of the genus *Hypericum* belonging to 21 sections including six representatives of the section *Drosocarpium*, namely *H. barbatum* Jacq., *H. montbretii* Spach, *H. perforatum* L., *H. richeri* Vill., *H. rumeliacum* and *H. spruneri* Boiss. revealed that all the studied species are characterised by obligate sexual mode of reproduction (MATZK *et al.* 2003). The authors characterised all the studied species of the section as diploid but, surprisingly, for *H. barbatum* Jacq. and *H. rumeliacum* they mentioned $2n = 2x = 16$ instead of $2n = 2x = 14$.

The results presented here provide the first karyotype data and basic chromosome characteristics of the endemic *H. rumeliacum*. They also reveal feasibility of the use of *in vitro* propagated plantlets as a material for karyological studies.

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