

BRIEF COMMUNICATION

Karyotype analysis of *Hypericum perforatum* L.

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*P.J. Šafárik University, Faculty of Science, Department of Experimental Botany and Genetics, Mánesova 23, 041 67 Košice, Slovak Republic***Abstract**

A karyotype study was made on *Hypericum perforatum* using plants differentiated *in vitro* with different ploidy level. The chromosomes of this species are small, morphologically similar, median and submedian. In the basic chromosome set the most distinguishable is chromosome number 1 which was subjected to detail analysis. It was found that there are two types of this chromosome which contribute differentially in diploid, triploid and tetraploid plants.

Additional key words: apomixis, cluster analysis, St. John's wort.

Naturally produced pharmaceutically important metabolites have become of renewed interest. *Hypericum perforatum* L. (St. John's wort) is an abundant source of pharmaceuticals from which the anthraquinone hypericin and its derivatives are widely studied. They show antiviral and anticancer activity (Meruelo *et al.* 1988, Hudson *et al.* 1991, Lavie *et al.* 1995). *H. perforatum* is a tetraploid hybrid ($2n=4x=32$) with the basic chromosome number $x=8$. The chromosome number $2n=4x=32$ is frequently reported, however, occasionally $2n=16$ and $2n=48$ occurs (Robson and Adams 1968). According to a hypothesis of Campbell and Delfosse (1984), this species is a product of an ancient hybridization between two diploids with subsequent chromosome doubling. It reproduces by facultative type of apomixis (Noack 1939, Brutovská *et al.* 1998). Previously, we have reported on an effective regeneration system from seedlings of *H. perforatum*, on somaclonal variation at morphological, biochemical, cytogenetical and molecular level (Čellárová *et al.* 1992, 1994, 1995, 1997, Halušková and Čellárová 1997, Brutovská *et al.* 1998). Regenerated plants obtained from tetraploid seedling showed different ploidy levels. Cytogenetic variability was observed not only in regenerated plants but also in their seed progenies (R_1 and R_2 generations) (Brutovská *et al.* 1998). Interestingly, the most stable diploid plants showed the highest hypericin content throughout several years (Čellárová *et al.* 1997).

The aim of this contribution is to present the results of karyotype study of *H. perforatum* plants with different ploidy level.

Regenerated plants of *Hypericum perforatum* L. were obtained by adventitious shoot formation as described previously (Čellárová *et al.* 1992). After adaptation to *ex vitro* conditions they were grown in soil. All plants were open-pollinated and seeds were harvested at the end of the vegetation period. The seed progenies of these regenerants (R_1 and R_2 generations) were grown firstly in jiffy pots in the cultivation chamber and later in an experimental field. Roots were pretreated with 0.002 % (m/v) 8-hydroxyquinoline for 4 h, fixed for 16 h in a mixture of ethanol:glacial acetic acid 2:1 and hydrolyzed for 6 min in 1 M HCl at 60 °C. Root tips were squashed in 45 % acetic acid using cellophane (Murín 1960). After stripping of the cellophane, the slides were stained with Giemsa for 30 min and mounted in a synthetic resin (Solacryl, Synthesia, Kolín, Czech Republic). Selected metaphase plates of diploid, triploid and tetraploid plants and haploid metaphases from mixoploid plants were drawn by using the Abbe instrument. Each chromosome was characterized by its shape, relative length (RL), centromeric index (CI) and arm ratio (AR). These characteristics were calculated using the following formulas: $RL = Lch/Sch$, $CI = (Ls/Lch) \times 100$, $AR = Ll/Ls$ where Lch = length of individual chromosome,

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Ll = long arm length, Ls = short arm length, and Sch = total sum of the length of all chromosomes of the complement $x=8$. The position of centromeric constriction was recorded as median (m: 1.0 - 1.7) and submedian (sm: 1.8 - 3.0) by the arm ratio (Levan *et al.* 1964). Normal distribution was tested by Shapiro-Wilk test and χ^2 goodness of fit-test, both at $\alpha = 0.05$. The set of AR values was analyzed by cluster analysis by the UPGMA method (unweighted pair-group method using arithmetic averages). As a measure, the Euclidean distances were used:

$$\text{Distance}(X,Y)=\sqrt{\sum (X_i - Y_i)^2}.$$

Using the best metaphase plates of root tip meristematic cells of cells with different ploidy level, we have performed karyotype analysis (Fig. 1, Table 1). The chromosomes of *H. perforatum* are small (0.78 to 1.52 μm) and morphologically very similar. We have started with the analysis of chromosomes in the cells with basic chromosome number $x=8$. These cells were present

in preparations of mixoploid plants originated during *in vitro* culture. Subsequently, the set of 8 chromosomes was used to set up the karyotypes of diploid, triploid and tetraploid plants. Because of small chromosome size, plants with higher ploidy level were much more difficult to analyse karyomorphologically. Nevertheless, it was possible to notice that genomes of these plants consist of the multiplied basic chromosome set. Basically, the haploid chromosome set includes one exceptionally long median centromeric chromosome - chromosome number 1 - which could be easily distinguished from the rest. We have found that there are two types of this chromosome concerning its length. The results of statistical analysis of the AR values of the chromosome number 1 are not unambiguous. Shapiro-Wilk test rejected the hypothesis about normal distribution ($W = 0.937$, corresponding $P = 0.0044 < 0.05$) but χ^2 goodness of fit-test found no significant evidence for different distributions than normal ($\chi^2 = 12.6752$, corresponding P value for 7 degrees of freedom: $P = 0.0804295 > 0.05$). It indicates

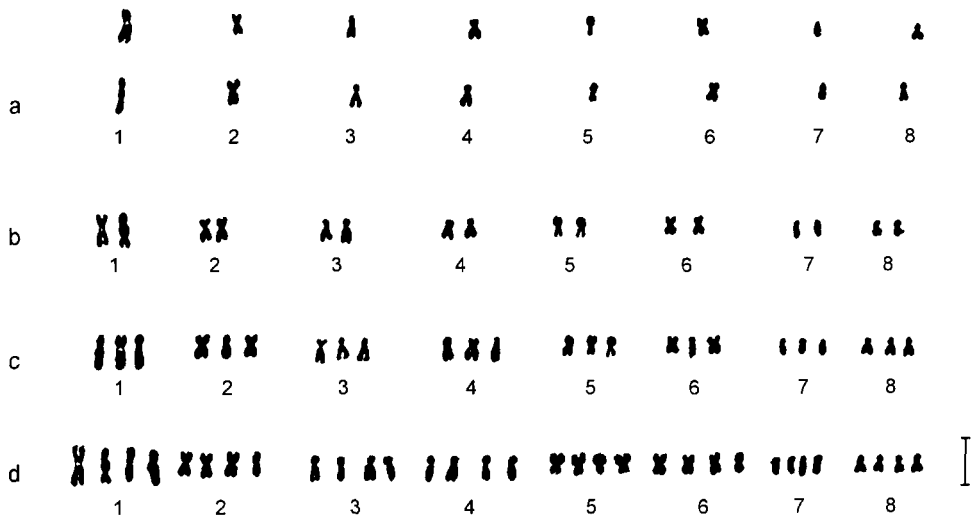


Fig. 1. Serial arrangement of the metaphase chromosomes of *H. perforatum*: a - haploid, b - diploid, c - triploid, d - tetraploid. Bar = 2 μm .

Table 1. Characteristics of *H. perforatum* chromosomes ($x=8$): L - length of a chromosome (mean \pm SD), N - number of metaphases. RL - relative length, CI - centromeric index, AR - arm ratio, m - metacentric chromosome, sm - submetacentric chromosome.

Chromosome	L	N	RL	CI	AR	Type
1a	1.278 \pm 0.377	48	0.193	45.20	1.22	m
1b	1.438 \pm 0.449	15	0.183	37.93	1.64	m
2	1.062 \pm 0.090	11	0.137	44.44	1.25	m
3	1.006 \pm 0.102	11	0.132	34.62	1.89	sm
4	0.947 \pm 0.120	11	0.127	34.00	1.94	sm
5	0.862 \pm 0.028	11	0.109	41.86	1.39	m
6	0.813 \pm 0.075	11	0.107	47.62	1.10	m
7	0.795 \pm 0.077	11	0.099	35.90	1.79	sm
8	0.782 \pm 0.078	11	0.099	41.03	1.44	m

slight deviation from normal distribution. Finally, cluster analysis showed the occurrence of two sets of chromosomes, one consisting of chromosomes with AR 1.00 to 1.44 and the other with AR between 1.49 and 1.89 (Fig. 2). Each of these two types of the chromosome number 1 was found in various contribution in plants with different ploidy level: one or two in diploids, one to tree in triploids, one to four in tetraploids which may indicate hybrid origin of this species. In the rest of haploid set there are four median (number 2,5,6,8) and three submedian (number 3,4,7) chromosomes. As stated above, the small size of chromosomes made discri-

mination between chromosomes ambiguous, e.g., between chromosomes number 3 and 4 which are submedian and between median centromeric chromosomes number 5 and 6. The differences in length between these chromosomes were less than 0.05 μm . Chromosome number 7 was morphologically the least identifiable and its length varied considerably. The occurrence of secondary constriction was difficult to identify. One type of the longest chromosome showed a constriction on the central position of the longer arm but this could be a result of artificial twisting of chromosome during squash preparation.

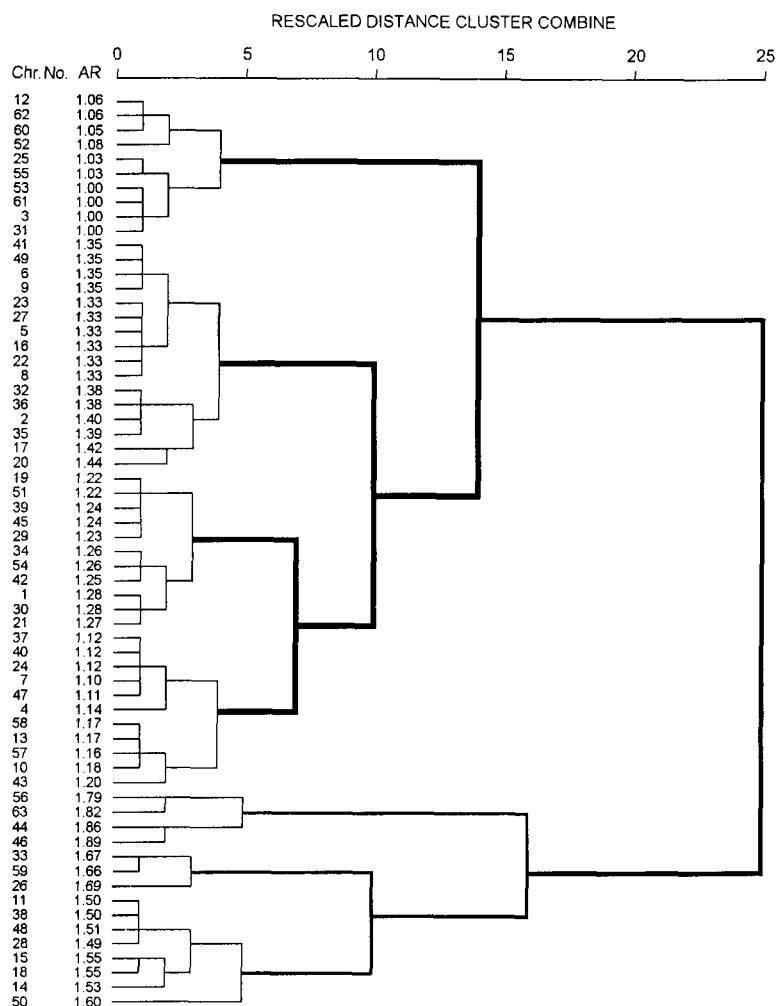


Fig. 2. Hierarchical cluster analysis of the AR values of chromosome 1 of *H. perforatum*.

Karyotype of *H. perforatum* has not been composed yet. It is the small size of the chromosomes why the karyotype studies of *Hypericum* genus have been so scarcely made (Robson 1981). The only available data come from analyses of the related *H. erectum* ($2n=16$), *H. tosaense* ($2n=16$), *H. ascyron* ($2n=18$) and *H. pseudopetiolatum* ($2n=32$) distributed in Japan (Kogi 1984). The lengths of their chromosomes are 1.5 to 3.3

μm , 0.8 to 1.9 μm , 1.0 to 1.7 μm and 1.0 to 2.5 μm , respectively. According to Reynaud (1986) the length of *H. perforatum* chromosomes is 1.0 to 1.7 μm . Our results are slightly different: 0.78 to 1.52 μm , and show that *H. perforatum* has the smallest chromosomes in a group of species of the *Hypericum* genus with known chromosomes length. Robson and Adams (1968) gave a summary of the cytological information known about

genus *Hypericum* and mentioned that this genus is cytologically relatively unspecialized. They stated that studies of chromosome morphology are not likely to yield much information about the evolutionary history of the genus. Chromosomes of the genus have median or submedian centromeres (Robson and Adams 1968, Kogi 1984). Our karyo-morphological observation is

comparable. Five of 8 chromosomes in the basic set are median centromeric and three are submedian. Chromosomes of *H. perforatum* ($2n=32$), *H. erectum* ($2n=16$) and *H. pseudopetiolatum* ($2n=32$) are very similar. The diploid chromosome set of all these three species contain one pair of long median centromeric chromosomes.

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