

CYTOGENETIC ANALYSIS OF RRNA GENES IN *CREPIS CAPILLARIS* GENOME USING CONFOCAL LASER SCANNING MICROSCOPY AND HIGH CONTENT SCREENING STATION

Hanna Sas Nowosielska, Marta Dydak, Tytus Bernaś, Jolanta Maluszynska
Department of Plant Anatomy and Cytology
University of Silesia
Jagiellonska 28, 40-032 Katowice, Poland
E-mail : h.sasnowosielska@gmail.com

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Laser scanning confocal microscopy and high content screening station were used to study rRNA gene condensation in *Crepis capillaris* interphase nuclei and metaphase chromosomes. rRNA genes are conservative, housekeeping genes present on chromosome in thousands of copies. Transcription of these genes is key for nucleolus formation but they can be also used as chromosome marker in cytogenetic studies.

C. capillaris is good object for plant cytogenetic analysis because of small number of easily distinguishable chromosomes ($2n = 6$) and possibility of occurrence in genome different numbers of B chromosomes. Only one pair of chromosomes with nuclear organizing region (NOR) 25S and 5S rDNA *loci* is present. Thanks to this feature, using fluorescence in situ hybridization (FISH) with appropriate rDNA probes, NOR chromosomes can be distinguished from other chromosomes in interphase nuclei. 25S rDNA is also present on *C. capillaris* B chromosomes. It is still unclear if rDNA loci on B chromosomes are active and participate in forming of the nucleolus. Presence of B chromosomes increases genome size but it is unknown if it also influences interphase genome structure[1].

The goal of presented work was to answer the question if the presence of B chromosomes can change the state of rDNA fiber. Experiments were conducted on hairy roots lines with different numbers of B chromosomes (0-3 B). We used laser scanning confocal microscopy (Olympus FV1000) to study 3D architecture of nucleolus in nuclei with reference to rRNA gene condensation and high content screening station (Olympus Scan^R) for cytosine methylation analysis [2]. Our results show that presence of B chromosomes can influence rDNA fiber condensation. In all analyzed nuclei two bright signals of rDNA from standard chromosomes were seen adjusted to the nucleus. Additionally in nuclei containing B chromosomes small, bright signals usually distant from nucleolus appeared. The number of signals was correlated with the number of Bs. Interestingly in these nuclei also bright signals connected with DNA fiber could be seen. The brightness and size of signals seemed to be proportional to the number of B chromosomes present in *C. capillaris* genome, with the brightest and largest signals in 3B nuclei. We confirm on metaphase chromosomes *C. capillaris* that inactive rRNA genes are correlated with cytosine methylation.

[1] R.N. Jones, W. Viegas and A. Houben "A century of B chromosomes in plants: so what?", *Ann Bot* 1-9 (2007)

[2] P.J. Shaw, A.F. Beven, B. Wells, M.I. Highett and E.G. Jordan "The organization of nucleolar activity in plants" *J Microsc* **181**, 178-185 (1996)