3D PLANT NUCLEUS STRUCTURE STUDIED WITH IMAGE CYTOMETRY

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Introduction: In plant cells during differentiation DNA can be replicated without a subsequent cell division, which leads to an increase of ploidy level. Owing to this endoreduplication nuclei coming from one organ but differing with respect to their DNA content may exhibit different chromatin organisation. The nuclear DNA content of plant nuclei is routinely measured using flow cytometry (FC). This technique permits rapid measurement of ploidy in large population of nuclei. However, it does not provide spatial (ex. specific for a region in tissue or organ) information. Moreover, further measurements on already processed nuclei are difficult without additional equipment. Alternatively, one may use image cytometry (IC) to estimate ploidy from microscope images of nuclei. This technique permits easy application of fluorescent in situ hybridization (FISH) to obtain information about spatial organisation of chromatin. Here we combine of IC with 3D FISH to study relationship between ploidy and organisation of plant nuclei.

Materials and methods: Nuclei of Arabidopsis thaliana Col-0 were isolated from stem leaves and fixed in 4% paraformaldehyde. Arabidopsis 180 bp centromere tandem repeat sequence, pA11 was used as for FISH procedure. The probe was labelled with dig-dUTP and detected using anti-dig antibody. Nuclei were imaged using SCAN^R imaging cytometer equipped with OlympusXI inverted microscope and CCD camera (Orca AG, Hamamatsu). The slides were scanned in 2D to construct the DNA content (ploidy) histogram of the population. The nuclei corresponding to three ploidy levels (2C-8C) were then imaged in 3D using deconvolution microscopy.

Results: Using image cytometry we evaluated DNA content of single nuclei labelled with FISH with probes for specific chromosome regions. Thus the information on chromatin content was correlated with that on 3D chromatin architecture. The proposed method provided a fast and precise alternative to combination of flow cytometry and confocal microscopy.