APPLICATION OF TEXTURE PARAMETERS TO MEASURE CHANGES IN 3D ARCHITECTURE OF NUCLEI INDUCED BY THE PRESENCE OF EXTRA (B) CHROMOSOMES

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Introduction: B-chromosomes constitute optional complement to basic (A) chromosome set. These extra chromosomes are usually small and highly heterochromatic. The presence of B-chromosomes may reduce plant vigor and fertility. These adverse effects increase with chromosome number and are higher when the number is odd. Although they may organize nucleoli B-chromosomes are, in general, genetically silent. This notion indicates that effects of B-chromosomes presence may be caused by epigenetic mechanisms. However, relevant studies have been limited to 2D light microscopy of isolated metaphase chromosomes. Therefore, effects of presence of B-chromosomes on 3D chromatin architecture of interphase nuclei remain yet to be elucidated.

Methods and Results: Meristem parts of roots of Crepis Capilaris containing varied number of B-chromosomes (from 0 to 3) were obtained from in vitro culture of transformed tissue. The nuclei were fixed in physiological ionic strength and pH and stained with DAPI. Series of optical sections were registered with confocal fluorescence microscope in photon-counting mode. Image registration was optimized so as to eliminate photodynamic effects while preserving good light penetration depth

The nuclei were segmented using newly developed algorithm based on iterative local thresholding followed by growing and merging of regions belonging to different nuclei. The result of segmentation was validated by a human observer. Sets of resulting binary masks (belonging to correctly isolated interphase nuclei) were used to calculate several morphological parameters with original (uncorrected) image data as second input. The parameters included shape parameters (volume, linear dimensions, circularity), global intensity distribution measures (mean, variance) and local intensity distribution parameters (haraclick features, wavelet energy, runlength features). The sets of parameters corresponding to populations of nuclei with different number of B-chromosomes were subjected to discriminant analysis using classification and regression trees (CR&T) method. The parameters constituting optimum classifier for nuclei with different numbers of B-chromosomes were isolated. These parameters were then correlated with depth in tissue at which a given nucleus was positioned.

Conclusions: The proposed algorithm provides accurate and efficient 3D segmentation of nuclei in plant tissue. The presence of B-chromosomes correlates with change of local chromatin distribution measured at and above Rayleigh resolution distance. The effect is manifested by increase of the size of chromatin blocks, increase of regularity of spatial chromatin distribution and decrease of differences in distribution pattern between interphase and prophase nuclei. Therefore, one may hypothesize that the presence of B-chromosoemes influences large-scale architecture of A-chromosome set.