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Molecular Cytogenetics as a Tool for Brassica Genome Research

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In this study we present the use of molecular cytogenetics as a tool for the structural genome research of *B. rapa*. Assigning certain probes such as BACs, YACs and repeat sequences on the chromosome level is one of the most prerequisite steps to start genome sequencing project. This work can be integrated with molecular map data to select marker BACs more accurately and efficiently. Therefore, we settled the chromosome number based on the morphology and molecular organization of heterochromatin domains in interphase nuclei, and mitotic and meiotic chromosomes of *Brassica rapa*, using DAPI staining and Fluorescence *in situ* Hybridization (FISH) of rDNA and pericentromere tandem repeats. We established the DAPI karyotyping using the 45S and 5S rDNAs and 176 bp centromere satellites repeats to clearly distinguish 10 *B. rapa* chromosomes. We characterized centromeric repeat sequences from BAC end sequences that are resulted in two classes, CentBr1 and CentBr2, occupying centromere of eight and two chromosomes, respectively. We have further carried out the identification and characterization of the major repeats in the centromeric and pericentromeric heterochromatin of *Brassica rapa*. We identified a centromere-specific retrotransposons of *Brassica* (CRB) and various pericentromere-specific retrotransposons (PCRBr). Three copies of the CRB were identified in one BAC clone, as nested insertions within a tandem array of 24 copies of 176 bp centromeric repeat, CentBr. A complex mosaic structure consisting of nine PCRBr elements and large blocks of 238-base pair degenerate tandem repeats (TR238) were found in or near a derivative of 5S-25S rDNA sequences. The chromosomal positions of selected repeats were determined using in situ hybridization. These revealed that CRB is a major component of all centromeres in three diploid *Brassica* species and their allotetraploid relatives. However, CentBr was not detected in the more distantly related of the diploid species analyzed, *B. nigra*. PCRBr and TR238 were found to be major components in the pericentromeric heterochromatin blocks of four chromosomes of *B. rapa*. These repetitive elements were not identified in *B. oleracea* and *B. nigra*, indicating that they are A genome-specific.