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Molecular Cytogenetic Analysis of the Cucumber Genome Using McFISH Combined with Karyotyping

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Lack of reliable karyotype for chromosome identification is the major obstacle for cytogenetics research in plant species that having a similar morphology of small chromosomes. To promote molecular cytogenetics research of cucumber (*Cucumis sativus* L.), we developed a McFISH (multi color fluorescence *in situ* hybridization)-based karyotype of a *C. sativus*. In present study, two highly repeated DNA fragments were identified and characterized by combined RAPD and FISH techniques. It was interesting that FISH detectable repeated DNA was obtained by PCR method using arbitrary random primer (20-mer) with high efficiencies. This is the first report molecular karyotyping of metaphase spreads of cucumber by FISH using RAPD products as a probe. The hybridization signals provide excellent cytological markers to tag individual cucumber chromosomes. In McFISH detection, CRS1 was clustered in centromeric heterochromatin regions of all chromosomes 14 and are located within the regions of constitutive heterochromatin observed by the C-banding technique. Also the CRS2 sequence was hybridized on five out of seven *C. sativus* chromosome comple-

ments. The fluorescently labeled probes, CRS1 and CRS2, were shown to be reliable cytogenetic markers in the *C. sativus* for identification of mitotic metaphase chromosomes. All chromosome could be identified unambiguously. We also demonstrated that the RAPD markers (CRS1, CRS2) can be mapped to specific positions on meiotic pachytene chromosomes. The excellent resolution of pachytene FISH can be used to construct a physical map of cucumber by applying specific molecular markers on pachytene chromosomes. Additionally, the combination of simultaneous and successive FISH with 5S rRNA, 17S rRNA genes, and a telomeric sequence allowed the assembly of a physical map based on cucumber karyotype.

Acknowledgments

This work was supported by a grant from the Korea Research Foundation Grant (KRF-2000-DP0401: PI Dr. YoonKang Hur) awarded to Dr. Jae-Wook Bang.