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Identification of chloroplast DNA insertions in nuclear chromosomes of maize B73 line using the FISH procedure

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It is known that chloroplast DNA can incorporate itself into the nuclear genome of plants. However, the sites of chloroplast (ct) DNA integration into chromosomes of maize have not yet been analyzed. This project is the first attempt to find the location of the ctDNA on the maize chromosomes. Fluorescent *in situ* hybridization is a technique that has proved useful in karyotyping and chromosomal mapping in maize. The FISH procedure is being used in this study to discover the location of the ctDNA in the nuclear genome of the inbred line B37. In order to develop ctDNA "probes" for FISH analysis, we have used the polymerase chain reaction (PCR) to produce fragments of ctDNA. Primers were chosen to amplify fragments of 10 kb or larger. The amplified DNAs were purified and labeled with fluorescent dyes and these probes were subsequently hybridized to chromosomes. The probes recognize and bind to the corresponding DNA sequences within the chromosomes. Root tip cells were used to prepare the slides for hybridization. Because the cells are collected during the metaphase stage of division, the chromosomes are compact and more easily visible. Chromosomes that contain ctDNA can be detected using a compound microscope with fluorescent attachments. The location of the ctDNA on the chromosomes is made visible by the fluorescent labeling of the probe. Eight of eleven regions of the chloroplast genome of the B73 line have been specifically amplified and have been observed under the microscope for FISH analysis. This information will contribute to an understanding of the extent and mechanism of transfer of organellar genomes to the nucleus.