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Application of Laser Optics to Plant Cell and Molecular Biology

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Although laser optics has been shown to be an effective tool in the various fields of animal cell and molecular biology, its application and utility to plant cell and molecular biology have been limited. The main reason, besides the number of researchers in the plant fields, is that the plant cell has a characteristic feature of the cell wall. The cell wall interrupts a good sample preparation of chromosomes and prevents the smooth incorporation of antibodies into the cytoplasm. It is also true, in the case of the production of transgenic plants, that the cell wall is a barrier for DNA to be introduced into the cytoplasm and nucleus.

Nevertheless effective applications of laser optics in the plant cell and molecular biology are being explored and some achievements have recently been attained. Three major applications of laser optics, laser microdissection of plant chromosomes, laser poration of plant protoplasts and laser as the optical tweezers in plant cell biology, are reported.

LASER DISSECTION OF PLANT CHROMOSOMES

After establishment of an improved enzyme maceration method to digest and remove cell walls from plant cells (Fukui and Kakeda 1990, Fukui and Iijima 1991), good chromosome sample preparations without cell debris became a routine protocol. Microdissection of defined chromosomal regions of barley and rice chromosomes has been reported (Fukui et al. 1992).

Fig. 1. shows the representative steps of microdissection of barley chromosomes prepared by the improved maceration method. Barley chromosomes, air-dried on a polyester membrane, were subjected to laser microdissection. The 488 nm line from a 5 W argon ion laser through a 100x objective lens dissected the chromosomes by 0.5 μm in width. All the barley chromosomes except for the two pairs of the satellite chromosomes, chromosome 6 and 7, were ablated by using the stronger laser beam. Then the specific regions of chromosome 6 (6p1.5) and chromosome 7 (7p1.1 and 7q1.1) were dissected out from the rest of the chromosomal regions.

Direct cloning of the part of 45S and 5S rDNA from the nuclei cytologically prepared and the dissected chromosomal nucleolar organizing regions have successfully been carried out by using a PCR method. The amplified DNAs were also labeled during the PCR cycle by incorporation of biotinylated dUTP. The fluorescence *in situ* hybridization (FISH) confirmed that the amplified DNAs had been originated from the nucleolar organizing region dissected (Nakamura and Fukui in press). Direct cloning and direct labeling of certain DNA sequences from dissected chromosomes and nuclei becomes a standard chromosome technique. Fig. 2. shows a multi-color FISH of 5S and 45S rDNAs in rice using the probes prepared by the laser dissection method (Ohmido and Fukui in press).

LASER-PORATION OF PLANT PROTOPLASTS

After the establishment of protoplast preparation and culture methods (Takebe et al. 1968), a living plant cell without the cell wall became available in the various experiments. The protoplast system has been used in electroporation for producing transgenic cells and subsequently transgenic plants. Laser-poration has been tested to explore the possibility of developing an alternative

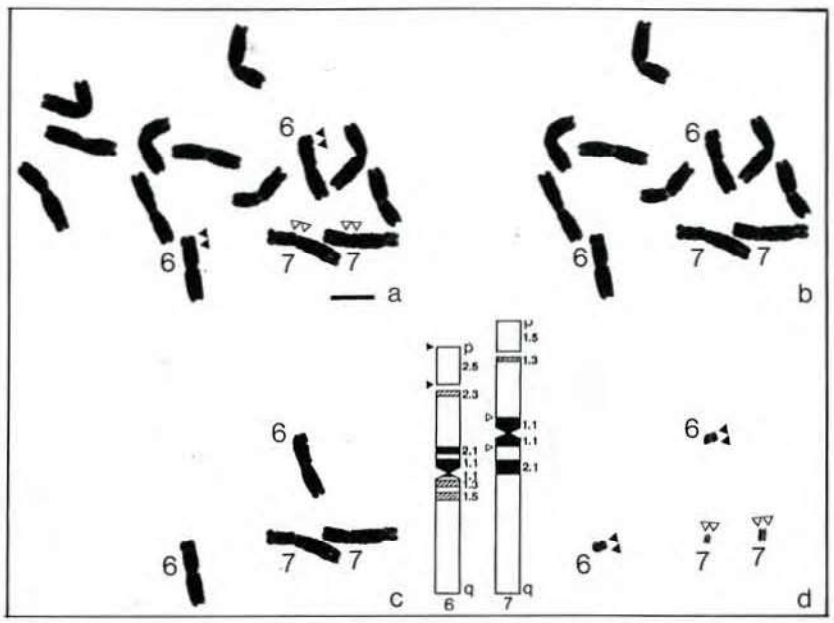

Fig. 1. Representative steps of laser dissection for the defined regions of barley chromosomes.

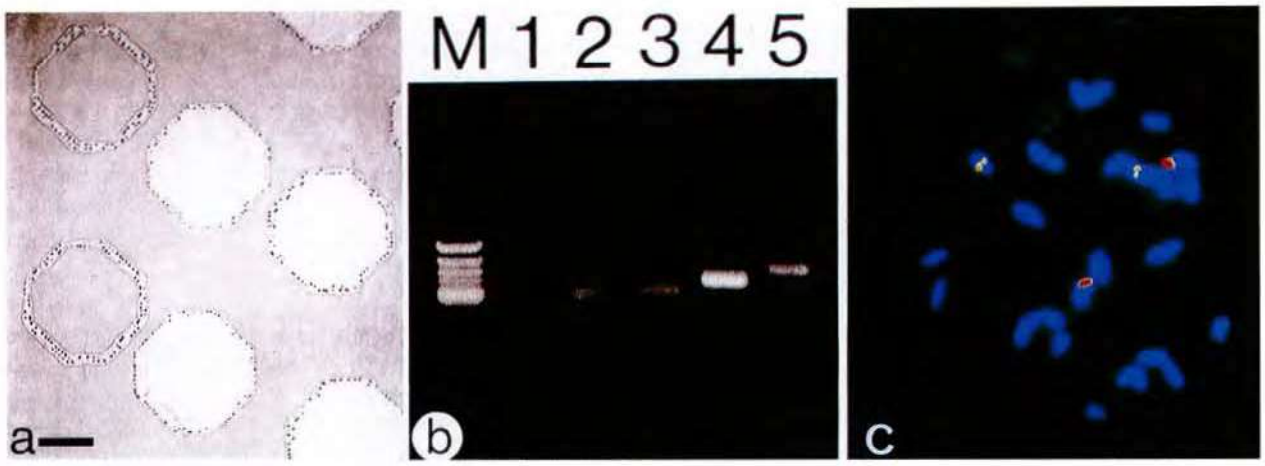

Fig. 2. Multi-color FISH of african rice 5S and 45S rRNA genes using the probes directly cloned and labelled. a) Laser dissection of the membrane with nuclei on the surface. b) Direct cloning and direct labeling of the 5S and 45S rDNAs. c) Multi-color FISH using the probes. Yellow: 5S rDNA. Red: 45S rDNA.

transformation system using protoplasts.

Laser-poration was tested by the incorporation of a dye, Evans Blue, which is not incorporated into living protoplasts. Fig. 3. shows a protoplast before and after optical poration. The living protoplast with green chloroplasts were stained dark blue after laser beam irradiation. It is shown that laser-poration induced the successful incorporation of the dye into the cytoplasms of the protoplasts. Further investigation is needed to clarify the relationship between the strength of the laser beam and the pore sizes for minimizing protoplast damage. The incorporation of DNA/RNA into protoplasts is underway.

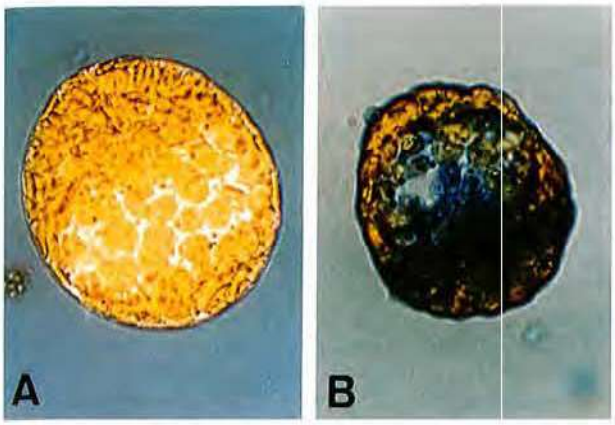

Fig. 3. A protoplast before and after optical poration.

Table 1. Optical tweezers for pollen grains[1)]

Laser power in mW	Laser scan strength (%)[2)]				
	100	80	60	40	20
100	+	+	+	-	-
80	+	+	+-		
60	+-	+-	-		
40	+-	-			
20	-				

1) Glycerol concentration: 20%
2) +: Good movement, +-: Poor movement, -: Not moved

OPTICAL TWEEZERS FOR CHROMOSOME MEDIATED GENE TRANSFER

Chromosome mediated genetic transfer is an alternative method for development of transgenic plants, although the number of reports have been quite limited. The method of random incorporation of the chromosomes and subsequent selection is not an effective way to produce transgenic plants as there is no selection markers for the protoplasts with specific chromosomes.

Thus specific chromosome should be introduced into the plant protoplasts. To meet the objective, flow cytometry is a possible way , although the method needs much more conditions to be refined.

Optical tweezers combined with laser-poration provide an alternative method for chromosome mediated genetic transfer by using a single targeted chromosome and a single cell. To develop the method, the ability of optical tweezers in a viscous solution like cytosol has been examined using cells of *Sacchromyces cereviciae*, pollen grains of Erigeron *annuus*, and nuclei separated from suspension cultured tobacco cells. The optical power of grasping objects was measured by using the combinations of the different laser scan strength and different glycerol concentrations.

Table 1 shows the results of optical tweezing on the pollen grains. In the case of 20% glycerol, pollen grains were easily grasped by the laser beam with 60% or more of the scan strength at 100mW laser power. They could readily be moved around by the movement of the laser beam under the above conditions. As a result the optical tweezers have been showed to have the capacity to pick up and transfer any plant chromosome in viscous solutions like cytosol. Thus it is anticipated that the combination of laser tweezers and laser poration will provide an alternative transgenic system.

As the three types of applications of laser optics described here are all in progress now, there are several areas for refinement. It is, however, clearly demonstrated that the applications of laser optics particularly effective also in the field of plant cell and molecular biology.

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