Abstract

The gene-engineered agricultural plants have been mainly cultivated in Canada and U.S.A. It is important to apply the biotechnology to agriculture in future. It is thought that the two fields are influencable in plant biotechnology in particular. One is the analysis of plant stress responces and another one is the new gene transfer method. Thus, these two points are noted in this report.

For the analysis of plant stress responces, two methods were applied to plant. The methods were the elechtrochemical detection of the reduction activity and the analysis of phloem sap.

In elechtrochemical analysis, *Nicotiana tabacum* cultured BY2 cell was used. BY2 cell exhibited the electrochemical reduction of 1,4-benzoquinone (BQ), 2,6-dichloroindophenol (DCIP), hexacyanoferrate (ferricyanide). The reduction of electron acceptors were detected by an amperometry with fixed potential (400 mV vs Ag/AgCl) using a film-coated ϕ 1.6 mm platinum disk electrode as a working electrode. The electrochemical activities of BQ, DCIP, and ferricyanide mixed with BY2 cell were changed after the addition of various stress compounds such as elicitors, plant hormones, metabolic and electron transport inhibitors. Different stress compounds gave different electrochemical patterns probably due to the different positions of electron acceptors affected by these bio-materials.

The microdialysis method was applied to collect phloem sap. Tobacco mosaic virus (TMV) was used as stress against the plant (TMV-resistant tobacco). phloem sap was collected through the dialysis membrane of microdialysis probe. Collected sap was analized by HPLC and mass spctroscopy. The results suggested that there were some fluorescence compounds in phloem sap and this material increased with TMV treatment. The mass spectra of these compounds showed that the compounds may be new stress-relative compounds.

Two gene transfer methods were developed for plant. One is a electroporation with comb-type electrode and another one is the gene transfer using infrared radiation with free electron laser (FEL).

Electroporation is well studied for gene transfer. We also previously reported that the electroporation using comb-type microelectrode tranferred gene into Medaka egg cell. In this research, we fabricated three thin film interdigitated electrodes of which gap width are 50, 100, 400 μm, respectively and attempted DNA transfer into rice (*Ozyza sativa* L.) cultured Oc cells by electroporation. The highest expression of transferred gene was observed when a 400 V pulse was applied three times using electrodes of 400 μm gaps.

The Free Electron Laser (FEL) has potential for various biotechnological application due to its advantages such as flexible wavelength tunability, short pulse and high peak power. We could successfully introduced the Green Fluoresecnt Protein (GFP) gene into tobacco BY2 cell by FEL irradiation of 5.5-6.1 μm with 2000-2,5000J/cm^2. FEL irradiation at wavelength of 5.75 and 6.1 μm, which were mainly absorbed by the ester bond of lipid and the amide I bond of protein respectively, was able to cause Introduction of fluorescent dye into cell. Transient expression of GFP fluorescence was observed after irradiation of 5.75 μm.