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Review

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Core Plant Biotechnologies and Bio-venture Businesses in Japan

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Recently, waves of Biotechnology developed in the field of microbiology and mammalian science surge over plant biotechnology field. Molecular biology established in such kingdoms has been applied to plants, and genetically modified plants (GM plants) are getting popular not only in crop production but also in agricultural products industry.

However, several regulations on safety of GM plants have been set and this has suppressed the spread of GM plants and improvement of agricultural production by GM plants rather slows down in Japan. Simultaniously, the public acceptance to the GM plant cultivation must be found.

In Japan, we are facing several problems for development of GM plants. We are importing a large amount of agricultural products from U.S.A., and some foods contain the products derived from GM plants. However, Japanese farmers are not officially encouraged to grow GM plants. Japanese government rather tends to retreat from the promotion of GM plants because of the lack of public acceptance to GM plants. What is the negative reason for the promotion of GM plants? The main reason is that the cultivation of GM plants would not be accepted by citizens at present. For the GM plant cultivation the seeds should be purchased from the monopolized seed-supplying companies which have succeeded in the creation of the GM plant. Furthermore, this would be costly for farmers and GM plant cultivation has an advantage only in a large scale plantation which are often seen in U.S.A. In Japan some food production companies have declared that they will not use any GM grains until the safety in the GMP products is totally proved. Actually, the core techniques to create GM plants have been patented by the foreign companies. Such situation discourages food manufacturers to create GM plants for the improvement of agricultural productions. The tight official permission for the cultivation of GM plants in the field would rather receive a warm welcome by food manufacturers until the core patents to GM plants expire. Such a self-righteous policy has suppressed the promotion of useful GM plants.

In the near future, the population on the earth surely exceeds 7 billion and the essential requirements for life will be threatened because of the short supply of food, energy resources and the environmental deterioration. To overcome this looming threat, we must develop powerful techniques applicable to plant biotechnology. In this respect Japanese plant scientists must start the researches in various ways and improve the potentials of plants.

Laser-assisted cell wall-surgery techniques applicable for single-cell metabolome and proteome

The structure of plant cell is quite different from that of animal cell because plant cell has hard wall and fragile cell membrane. Although micro-injection techniques targeting at a single cell have been established in mammal cells, the cell wall is a tough barrier for injection of foreign materials into plant cells. To overcome these difficulties, the reliable technique for microscopic surgery should be developed to process a focused region of the surface of the targeted cell.

Recently laser-assisted micro-etching techniques have been developed in semi conductor industry. We have applied this basic technology to micro-surgery of the surface of the cell, and we have also developed own laser-assisted micro-surgery techniques to shave off the limited area of the targeted cell surface.

In a preliminary experiment we tested both YAG and excimer laser to process the cell surface of onion bulb cell. ArF excimer (193 nm) laser seemed to be more promissing since no significant damage was seen after its irradiation. After the adjustment of the basic irradiation parameter, such as pulse number, beam intensity of the excimer laser, both the area and the depth of the hollow made by the laser irradiation are good enough not only to inject foreign materials but also to extract cell components through the hollow (window) (Figure 1).

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We first succeeded in introduction of the GFP gene into onion cells and the gene expression in the targeted cell (Figure 2). The laser-assisted micro-surgery technique is confirmed as one of the most reliable methods for introduction of foreign materials into the targeted cells. Recently, the transgenic torenia plant (*Torenia fournieri*) was created by this method.

We first succeeded in introduction of the GFP gene into onion cells and the gene was determined to be expressing in the targeted cell (Figure 2), and this laser-assisted.

This micro-surgery technique has many advantages in new plant biotechnology. In the near future, chloroplast, plastid and other organelle engineering would develop dramatically with the laser-assisted micro-surgery technique we have developed.

A single cell micro-surgery technique is applicable to a single-cell proteome and metabolome

The micro-surgery technique enables us to investigate gene products as well as primary and secondary metabolites on a single cell basis.

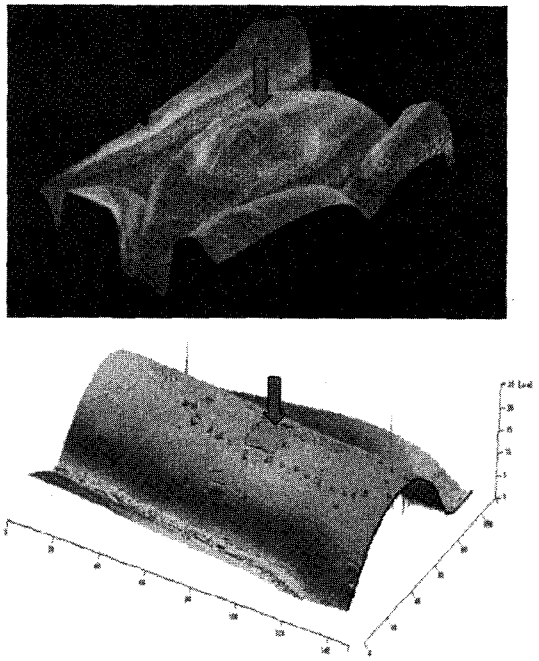

Figure 1. 3D microscopic view of laser-processed cell (epidermal onion cell) Red arrows indicate the laser-irradiated region of the cell wall

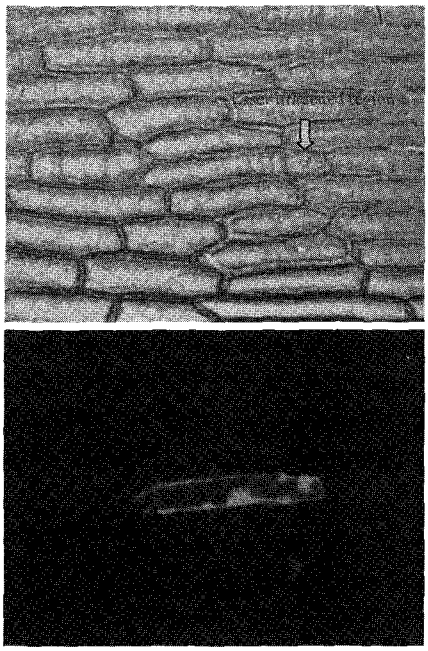

Figure 2. Transient expression of GFP gene introduced by laser-assisted microinjection

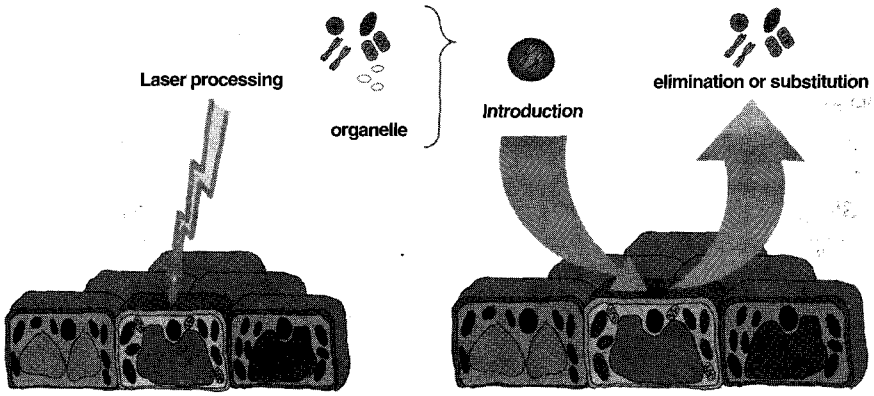

Figure 3. Concept scheme of laser-assisted organelle substitution

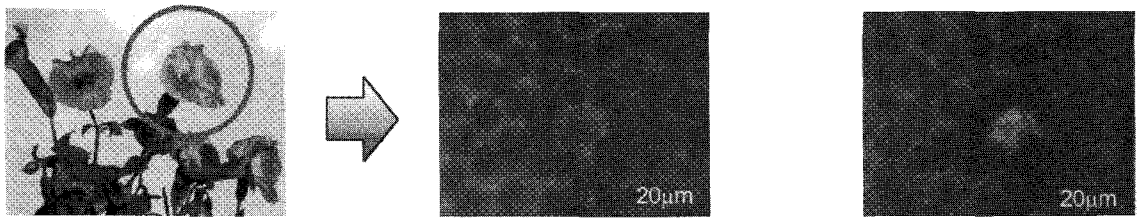

Figure 4. Laser assisted single cell sampling (Torenia petal cell)

Suppression and enhancement of the expression of the targeted gene provide us a variety of new information of the metabolism even on a single cell basis. Recently, RNAi technique was found to be a very useful tool for down regulation of the desired genes and it is very useful to understand its function by analyzing the loss of function.

RNAi technique will be a powerful tool for the focused metabolome and proteome.

We have focused on the regulation of the metabolic pathway of the pigments in the purple torenia flower. RNAi technique was found to be very effective to suppress the biosynthetic pathway of the purple-color pigment. We have so far succeeded in preparation of transgenic torenia with different petal color. Some pico-liter cell component was successfully extracted and subjected to mass spectrometric analysis.

The cell component was found to contain purple pigments, and their structures were elucidated to be peonidin-3,5-O-diglucoside and malvidin-3-glucoside-5-(p-coumaroyl)-glucoside by no flow LC-ESI MA/MS and MALDI TOF MS. This combined method would be one of the most powerful tools for studying metabolic profiling of individual plant cells.

New business development in Korea-Japan joint plant biotechnology venture

I have started a joint venture, GreenGold BioSystem Co., Ltd. (GGBS) with Korean scientists since 2001.

The peg of the start of GGBS was that I was very much interested in the core technology developed by a bio-venture company, MicroPlants Co. Ltd. in Korea which has developed own micro-propagation technique for medicinal plants. The company also has accumulated a plenty of experiences for establishing the regeneration systems in various plant species. Regeneration techniques should be essential for creation of transgenic plants from transformed cells, and those are also applicable to preparing artificial seeds. One joint project with Microplants Co. Ltd. has been conducted on a platform provided by GGBS. GGBS is very unique in many respects. GGBS is said to be a company which incubates a pre-venture under its own unique incubation system. GGBS offers a platform on which a group of scientists, business managers and technical assistants play their own roles in their individual task. GGBS helps to set up a laboratory where the research project is operated. Before a new project is launched the research proposal is disclosed to a client (investor) under the confidential agreement. GGBS calls another investors and Angel funds to support the project. Microplants Co. Ltd. is developing a research & development project on the GGBS platform with a good-standing company, Nihonshokusei Co. Ltd., locating at Okayama Prefecture.

The laser-assisted microsurgery technique will be used for such platform projects. The core plant biotechnology that has been developed so far or will be developed by scientists, should be tested on the GGBS platform and evaluated whether or not the business plan deserves to succeed as a pre-venture. After a certain incubation period, inventors are ready to judge the future success of business developing on the platform.

Okayama Prefecture recently constructed an incubation building where 63 incubation rooms are offered to entrepreneurs and looks for foreign business partners to start joint ventures with Japanese partners. Korean companies are invited to the incubation center, and the prefecture government is preparing some financial supports for new tenants. Okayama city is very near from Seoul, and it takes about an hour from Seoul by airplane. BBGS is now occupying 6 rooms in the incubation building and seeks business partners from foreign countries.

In the plant bio-venture business, the needs and seeds shift quickly as time slides. Contrarily, it takes many years to grow up from the infant stage to the steady stage (a good-standing company). Especially, in plant biotechnology field a proper supporting system is required to overcome these difficulties, and federal financial supports are definitely needed for young entrepreneurs to raise bio-ventures. Plants should play many important roles in the agricultural production and the environmental remediation.

We have to make an effort to optimize the proper infrastructure for bio-venture incubation under the international business partnership.

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