Plant Research Group

Developments of Afforestation and Metabolic Engineering in Plants for Supply of Alternative Energy

Plant Research Group aims at sequestration of CO_2, the main cause of global warming, and develops the techniques for both afforestation and Metabolic Engineering in Plants for Supply of Alternative Energy. In the techniques for afforestation, the group tries to enhance the growth of plants/trees by both strengthening photosynthesis and tolerance against environmental stress under semi-arid lands with their genetic modification. Furthermore, on the contrary to these GM-strategies, the group tries to make molecular markers which give trees tolerance to environmental stress, using elite trees of Eucalyptus sp. obtained in semi-arid land of western Australia. The usage of the markers could increase the efficiency of selection of elite trees in the field and contribute to the sequestration of CO_2 through the expansion of afforestation area. On the other hand, Plant Research Group tries to modify metabolic profiles in plant cells for the supply of alternative bioenergy to petroleum source one through GM-technologies, which could contribute to the suppression of CO_2 emission in industrial activities. Followings are the introduction of projects in Plant Research Group.

Afforestation project

The greenhouse effect on the Earth by increased atomospheric CO_2 is the most important environmental problem. This problem is taken as a political issue in many countries. However, it is true that practical solutions for the problem are not actually executed because of a bad influence to their economy. In this project, we try to develop technologies for improving photosynthetic activity of plants and afforestation in unstable area for vegetation, such as a semi-arid area to reduce atmospheric CO_2. For this purpose, we planned to reveal the function of photosynthesis related genes by physiological and molecular biological methods. It is known that a plant photosynthetic rate is mainly determined by three factors; carboxylase reaction for CO_2 fixation, electron transport in photosystems, and recovery of phosphate into chloroplasts. It is necessary to release these rate limiting steps of photosynthesis for improving CO_2 fixation activity of plants. We obtained some Arabidopsis photosynthetic mutants by chlorophyll fluorescence analysis. Now we have detected three mutated genes in these mutants. We are going to throw light on the mechanism of rate limiting factors in photosynthesis and produce plants that have higher CO_2 fixation activity in the future.

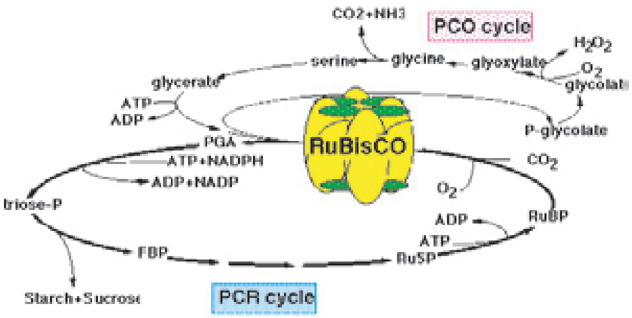

Fig. 1 Photosynthesis in higher plants

Developing a new procedure selecting stress tolerant *Eucalyptus* elites

Elite trees, which survive in infertile areas such as dry and salty lands, are potentially useful materials to increase terrestrial areas absorbing CO_2. Nippon Paper Group, Inc. has *Eucalyptus* afforestation of about 40,000 ha in Western Australia, and so far has selected about 70 *Eucalyptus* (*E. globulus*) individuals that can be candidates for the elite trees. They are maintained as clones by cutting and grafting.

However, it takes more than 10 years to access whether those elite candidates are effective with certainty. RITE has started evaluating drought/salt stress tolerance of the elite candidates and also clearing the physiological mechanisms in collaboration with Nippon Paper and Tokyo University. Our final goal is to develop a new procedure selecting the elite trees with certainty and rapidity, which would bring about effective afforestation in the infertile areas.

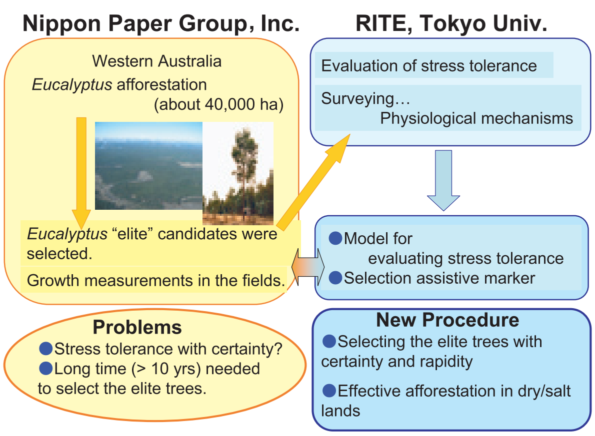

Fig. 2 Collaboration with Nippon Paper and Osaka Univ.

ROS -regulation project-Enhancement of HDP-and APX -systems by chloroplast engineering-

Extension of vegetation area to the unused lands is thought to be an actual and effective method to reduce the level of atmospheric carbon dioxide. In many cases, plants suffer severe growth inhibition due to abiotic stress, such as drought, salinity, cold and high temperature, in the unused areas. Under such abiotic stress, plants close their stomata to avoid the loss of water by transpiration. However, the close of stomata leads to decrease CO_2 influx to chloroplasts, and decline in the rate of photosynthesis. As the result, absorbed photon energy, which is usually consumed by photosynthesis, becomes excess, and the excess photon energy stimulates the production of reactive oxygen species (ROS) by reduction of dioxygen in chloroplasts. Furthermore, ROS induce oxidative damage, i.e. "sunburn", to cellular components of plants, and finally lead plants to death.

We have studied intensively the mechanisms of protection of cellular components from ROS attack. We clarified that two mechanisms, those are called "HDP system" and "APX system", play significant roles in the protection of chloroplasts against ROS attack. In HDP system, excess absorbed photon energy is converted to heat, and the production of ROS in chloroplasts is repressed. In APX system, H_2O_2 produced in chloroplasts is immediately converted to water by an enzyme, ascorbate peroxidase (APX).

In this project, we are trying to develop the fundamental technology to produce stress-tolerant transgenic arabidopsis and tobacco plants by chloroplast transformation as the model systems. Now, three research subjects aimed to enhance the abilities of HDP-and APX systems in chloroplasts are carried out as described below:

(1) Clarification of the molecular mechanism to drive HDP system and identification of a gene encoding master protein in the regulation of HDP system
(2) Localization of H_2O_2-insensitive ascorbate peroxidase from red alga Galdieria partita to thylakoid membranes in chloroplasts to scavenge H_2O_2 produced immediately.
(3) Production of oxidative-stress tolerant transgenic plants by introducing genes for master protein in HDP system and Galdieria APX into chloroplasts

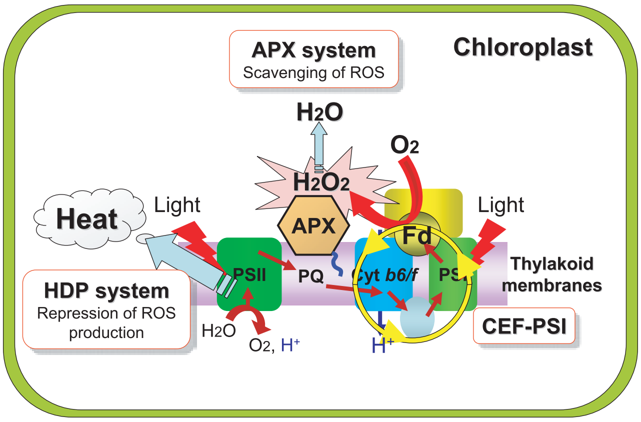

Fig. 3 Fundamental Technology for Controlling the Material Production Process of Plant Chloroplasts

Industrial utilization of material production functions of higher plants is one of the promising technologies that can alternate energy-intensive petrochemical process. The purpose of this project is to identify substance production process and to improve the capacity of useful material production by using gene-recombination technology in plants.

A chloroplast is the center of photosynthesis, which converts CO_2 to various substances by using light energy. We have already established the systems of both proteome analysis that covers chloroplastically-located proteins and metabolism analysis that enables to exhaustively profile metabolic compounds produced in plants by collaboration with Osaka University. In 2007, we also succeeded in the establishment of new metabolic analysis system which makes it possible to observe production rate of metabolic intermediates.

Chloroplast transformation, one of the key technologies in this project, is developing, which has potential to exceed the ability of conventional gene-recombination technique. In fact, we succeeded in producing high-value added compound, astaxanthin, in tobacco plants by using chloroplast transformation. Now we are developing the technology for controlling gene expression in chloroplast to produce high levels of useful materials in a tissue-specific manner by collaboration with Kyoto Prefectural University and Nagoya City University.