Biodiversity, biotechnology and research cooperation for sustainable development

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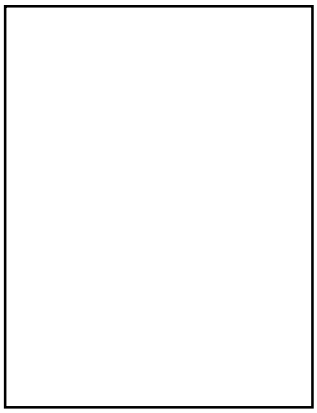
Biotechnology is the enabling technology of the future, and is likely to have far-reaching applications in health care, agriculture and environment management. Its importance in the conservation of biological diversity calls for research cooperation among developed and developing countries. This article focusses on research cooperation efforts between Japan and southeast Asian countries on the conservation and sustainable use of biological diversity through modern biotechnology.



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Introduction

Human beings have long relied on diverse biological resources for their food, clothing and housing. Many pharmaceuticals we use are derived from living organisms, and renewable industrial materials depend on biological resources. Ecosystems, made up of an enormous variety of living organisms, are a vital element of a healthy and comfortable environment.

However, as a result of the population growth in recent years and the associated industrial development, the pollution of air, water and soil, the increase of desert area and the deforestation of tropical rain forests continue, causing a loss of biological diversity.

Taking stock of the situation, the United Nations passed a resolution in 1984 calling for the establishment of a World Commission for Environment and Development. In 1987, a report, “Our Common Future”, was released, putting forward the idea of sustainable development. This idea passed on to the United Nations Conference on Environment and Development in 1992, where Agenda 21, an action programme designed to achieve sustainable development, and other agreements were adopted. The Convention on Biological Diversity (CBD), signed at the Conference, has been ratified in more than 170 countries.

Agenda 21 has chapters for conservation of biological diversity and environmentally sound management of biotechnology. The CBD defines its objectives as the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and technologies, and by appropriate funding. Here, “biological diversity” means the variability among living organisms,

including diversity within species, between species and of ecosystems.

The CBD provides articles for the *in situ* conservation, the *ex situ* conservation, and the sustainable utilization of components of biological diversity. In relation to research and training, the Convention stipulates that the Contracting Parties, taking into consideration the needs of developing countries, shall establish and maintain programmes for scientific and technical education and training, and support such activities. The Article on access to genetic resources recognizes the sovereign rights of States over their natural resources. Another Article states that access to and transfer of technology, including biotechnology, shall be provided with adequate and effective protection of intellectual property rights in the case of technology subject to patents and other intellectual property rights. Yet another Article emphasizes that technical and scientific cooperation, where necessary, through appropriate international and national institutions, should promote human resource development, institutional building, exchange of experts, and the establishment of joint research programmes and joint ventures.

An enabling technology

Biotechnology is widely recognized as an enabling technology for sustainable development.

UNIDO, designated as the Task Manager for Environmentally Sound Management of Biotechnology for Agenda 21, defines biotechnology to include any technique that uses living organisms or parts of organisms to make or modify products, to improve plants or animals, or to develop microorganisms for specific use. It ranges from traditional biotechnology to the most advanced modern biotechnology. For practical application in health care, agriculture and industry, an expanding range of interrelated techniques, procedures and processes are used in combination.

Agenda 21 lists, as areas in which biotechnology can make significant contributions, better health care, enhanced food supply, improved supply of potable water, more efficient industrial processes for transforming raw materials, support for more sustainable methods of afforestation and reforestation, and detoxification of hazardous wastes.

Research cooperation

As an example of research cooperation involving biological diversity and biotechnology, I would like to introduce "International Research Cooperation Project on Conservation and Sustainable Use of Tropical Biological Diversity" implemented by Southeast Asian countries and Japan. Japan Bioindustry Association (JBA) served as its secretariat.

The Bilateral Research Cooperation projects between Japan and Thailand, Indonesia and Malaysia respectively started in April 1993 and continued for six years until March 1999. The projects exchanged a total of 591 Japanese and Southeast Asian scientists, installed the most-needed equipment and instruments in local research facilities, and sponsored domestic research programmes. JBA carried out these projects under a contract with the New Energy and Industrial Technology Development Organization (NEDO), Japan.

Objectives of cooperation

The primary objectives of the projects were:

- To assist the Southeast Asian countries in their own efforts to conserve and use biodiversity in a sustainable manner;
- To train the Southeast Asian scientists and help develop their scientific skills through collaborative research; and
- To pave the way for future international research cooperation.

This research cooperation addressed both conservation and utilization aspects. The three participating countries, all located in Southeast Asia, have different situations of biodiversity and different sets of priority issues. The research cooperation, therefore, had to cover a broad area, from conservation to utilization, with individual projects to meet different countries' needs and priorities.

After a series of consultations, including the exchanges of scientists, the research cooperation projects were fixed as follows:

Japan-Thailand project

A Memorandum of Understanding (MOU) was drawn up between NEDO, Japan and The National Science and Technology Development Agency (NSTDA), Thailand. Scientists and researchers from many national research institutes and universities of Thailand participated in the joint research activities. The subjects of the cooperation projects were:

- *Taxonomic analysis, ecosystem evaluation and monitoring*
- Feeding strategies of primates:
  1. Evaluation of the ecosystems of tropical plant, animal and microbial habitats, and their time-course changes; and
  2. Screening of primates' food plants for new bioactive substances.
- Improvement of microbial culture collection systems:
  1. Use of classification/identification methods based on DNA; and
  2. Establishing a network of individual culture collections.
- *Conservation of biodiversity through man-made ecosystems*
- Interactions among different organisms within a man-made ecosystem: Evaluation of the effectiveness of man-made ecosystems by the analysis of the interactions among different organisms and by physiological and biochemical analysis.
- Genetic diversity analysis of artificial ecosystems:
  1. Genetic diversity analysis of artificial ecosystems, and evaluation of inbreeding effects;
  2. Construction of gene library; and
  3. Examination of the models for maintaining and improving genetic diversity.
- Socio-economic and ethnological analysis of an artificial ecosystem: Analysis of the effects of the most important constituent of an ecosystem, i.e. human society, to develop the best approaches to ecosystem conservation and sustainable use.
- *Use of bioresources*
- Screening of new bioactive substances found in plants and their applications.
- Study of traditional use of plant resources.

Japan-Indonesia project

The MOU was concluded between NEDO, Japan and The Agency for the Assessment and Application of Technol

ogy (BPPT), Indonesia. The subjects of the joint research were:

- *Taxonomic analysis, ecosystem evaluation and monitoring*
- Microbial culture collection systems:
  1. Taxonomic analysis of lactic and acetic acid bacteria found in fermented foods; and
  2. Improving culture collection network.
- Plant conservation techniques:
  1. Conservation of plant diversity: Basic research on plant inventory and diversity, and analysis of genetic make-up and phylogenic relationships of species;
  2. Tissue and cell cultures of tropical plant species: Research and development of tissue and cell culture and micropropagation techniques for tropical plant species; and
  3. Development of DNA techniques for the evaluation of biological diversity.
- *Utilization of tropical bioresources*
- Utilization of microbial resources: Screening microorganisms for their capabilities of producing antibiotics, polysaccharide-degrading enzymes and other useful substances; and
- *Searching for microorganisms that are capable of producing new oils and degrading cyanides.*
- Utilization of plant resources: Feeding strategies of primates - conservation of tropical rainforest, examination of primates' food plants for bioactive substances, and investigation of techniques to produce these useful substances.
- Study of symbiosis between plant and microorganism and its utilization.
- *Promoting the establishment of a Tropical Bioresources Industrial Development Centre in Indonesia*
- Feasibility study.

Japan-Malaysia project

An MOU was concluded between NEDO, Japan and The Standards and Industrial Research Institute of Malaysia (SIRIM), but the actual administration of the cooperation project on the Malaysian side was conducted by National Biotechnology Directorate (NBD) of The Ministry of Science, Technology and the Environment (MOSTE) Malaysia. The subjects of the research cooperation were:

- *Ecosystems and monitoring*
- Biodiversity databases and gene banks:
  1. Gene diversity evaluation techniques using Malaysian plants; and
  2. Techniques involved in the separation and identification of microorganisms.
- Evaluation and monitoring of marine ecosystems:
  1. Techniques for separating and storing marine life, and field survey in Sabah; and
  2. Joint research on Porifera and symbiotic microorganisms.
- Ecosystem evaluation and inventory development based on advanced technologies:
  1. Development of biodiversity inventory and database system with online data search and exchange capabilities; and
  2. Advanced technologies and automatic sequencing to analyze genetic variations in tropical trees.
- *Utilization of tropical bioresources*
- Screening and separation of bioactive compounds produced by microorganisms and plants;
- Evaluation of therapeutic and toxic potentials of natural products:
  1. Techniques to evaluate therapeutic and toxic potentials such as anti-tumour, neuro-protective and anti-allergic activities; and
  2. Cell culture techniques for medicinal plants.

Achievements of the projects

Most of the research activities were conducted by scientists from universities and public research institutes. A total of 389 Japanese scientists were despatched to the three tropical countries for on-site collaborative research, whereas a total of 202 scientists from the three countries were invited to Japan for joint research or training for technology transfer. A variety of interesting results were gained in the research projects.

The JBA secretariat, in cooperation with its counterparts, worked out mutually acceptable, transparent and practical procedures for handling biological resources. For example, the Japanese and Southeast Asian scientists jointly developed inventories of specimens collected during the course of the projects. They also developed Material Transfer Agreements.

Thailand

- Feeding strategies of primates:
  1. The dusky lutungs and pig-tailed monkeys were selected as target animals, and their group composition, forest use, feeding habits and other basic data were collected.
  2. Chemical analyses of primates' food plants were conducted to gain knowledge useful for future research.
- Improvement of microbial culture collection system:
  1. Japanese and Thai scientists worked together to separate and identify lactic and acetic acid bacteria, yeast, filamentous fungi and Basidiomycetes from Thai samples to transfer these techniques. A workshop was given on acetic acid bacteria and actinomycetes.
  2. An inventory was developed for the strains that were separated and identified in the collaborative research and was deposited in the culture collection of the National Centre for Genetic Engineering and Biotechnology (BIOTEC). The Japanese knowledge and experience about culture collection and data management were transferred to Thai scientists.
- Interactions among different organisms within a man-made ecosystem:
  1. Permanent plots were set up within selected natural, pseudo-natural and artificial forests to measure their stand density, the number of species, average basal area and diameter, average height, etc. A database system was developed to save the collected data on all individual stands.
  2. For major tree species in the permanent plots, the dynamics of photosynthesis, and the correlation between endogenous hormones and soil water content were studied and basic data were accumulated.
  3. The sugar and starch contents in the leaves of major epiphytes and their host plants were examined to

elucidate host selection and invasion mechanisms of the epiphytes.

4. The levels and distribution of selected chemical elements in soils and trees in the permanent plots were measured to obtain the basic knowledge required to examine the soundness of an ecosystem from the viewpoint of soil environment.

- Genetic diversity analysis of artificial ecosystem:

1. For major trees in the natural, pseudo-natural and artificial forests, the spacer regions of chloroplast tRNA were sequenced to prepare a phylogenetic tree. A comparison of the newly developed sequence data with the existing data for native varieties showed that no genetic mutation has occurred in these trees. A specific part of the nuclear DNA were sequenced for the major trees in the selected three type forests to study genetic variability.
2. For the major trees in the selected three type forests, the basic data were developed and samples were collected to construct a gene library.
3. Through these research activities, basic knowledge was gained to formulate models for maintaining and improving genetic diversity.

- Socio-economic and ethnological analysis of an artificial ecosystem:

1. For the villages newly constructed in Chachoengsao province by the Thai government, selected farmers were surveyed for their economic situation and the use of the forest community system and resources to study the current state of the economic base and resource management of these villages.
2. In the villages covered by the survey, education and training was given on how forest community system and resources should be managed.

- Use of bioresources:

1. The techniques based on the K-ras-NRK assay system to screen anti-ras substances were transferred to the Thai scientists invited to Japan.
2. At the BIOTEC, training was imparted on the same screening techniques.

- Study of traditional use of plant resources: Through interviews with ethnic minorities in northern Thailand, studies were conducted on traditional knowledge, including the names, uses and cultivation practices of plants they use as medicines.

Indonesia

- Microbial culture collection systems:

1. Lactic and acetic acid bacteria were screened, separated and identified. Many interesting acetic acid bacteria strains were separated and new knowledge was gained. In addition, Indonesian researchers were trained to improve their skills in classification and identification of microorganisms and microbial database development.
2. Separated strains were deposited in an Indonesian culture collection to expand its collection.

- Plant conservation techniques:

1. *Conservation of plant diversity*
Techniques were explored to develop data for use in molecular systematics and to process authenticated genetic data to construct gene banks. Indonesian botanists were given training in these techniques. The approach of molecular systematics was applied for the first time in Indonesia to the study of selected plants of the families *Anacardiaceae* and *Aspleniaceae*.
2. *Tissue and cell culture of tropical plant species*
The applicability of the photoautotrophic micropropagation (i.e. using sugar-free medium) system to tropical plant species was confirmed.
Acasia (*Acasia mangium*) as an important tropical plant, mangostin (*Garcinia mangostana*) and pineapple (*Ananas comosus*) as two representative tropical fruits, and peppermint (*Mentha piperita*) as a medicinal plant, among others, were selected as model plants, and it was shown that the photoautotrophic micropropagation system can be used to produce these useful plants in large quantities.
3. *Development of DNA techniques for evaluation of biological diversity*
Using crab-eating macaques, cockatoos, soft-shelled turtles and bullfrogs, the basic DNA techniques for biodiversity evaluation were developed and transferred to Indonesian scientists.
2. The DNA techniques identified for the first time in the world a colourblind macaque.
3. The entire sequence of the mitochondrial DNA of a soft-shelled turtle was determined.

- Utilization of microbial bioresources:

1. Tropical plants (including endangered species) were collected to elucidate the roles of the microorganisms (endophytes) that live in symbiosis with them and to screen the endophytes' ability to produce useful substances.
2. Microorganisms were screened for their ability to produce new oils and degrading cyanides.

- Utilization of plant resources:

1. A study of the feeding habits of silver lutungs found a substantial difference in their preferences in food plants depending on plant species and items. Such differences in feeding habits may be used to screen the plants' secondary metabolites. Using this approach, nine plant species were identified as those that silver lutungs might be eating for their medicinal activities.
2. Food and medicinal plants used by mankind as well as those eaten by primates were investigated for their biological activities. In this programme, candidate plant species were shortlisted quite efficiently through extensive screening. Also studied were several interesting substances active as pesticides, anti-inflammatory and anti-cancer agents.

- Promoting the establishment of Tropical Bioresources Industrial Development Centre in Indonesia:

1. The initiative was originally proposed by the Agency for the Assessment and Application of Technology (BPPT) of Indonesia with its strong need for scientific and technological information valuable for the sustainable use of the nation's tropical bioresources.
2. After a series of meetings between Indonesian and Japanese person

nel, and visits by BPPT officials to related organizations in Japan, it was finally proposed to implement a Tropical Bioresources Industrial Development Centre (TroBIDEC) project that focuses on industrial development of Indonesia's bioresources.

Malaysia

- Biodiversity database and gene banks:
  1. Gene diversity evaluation techniques were transferred to the country's scientists in respect of Malaysian plants with commercial values such as durian;
  2. The techniques involved in the separation and identification of microorganisms were also transferred to Malaysian scientists. A total of 89 strains of *Pseudomonas* and other species were separated from the samples collected in Malaysia and *Pseudomonas oryzihabitans* was identified. These separated strains were deposited in a Malaysian culture collection.
- Evaluation and monitoring of marine ecosystems:
  1. The techniques for separating and storing marine life forms were transferred to Malaysian scientists. A field survey was conducted in Sabah, and methodologies to monitor the degradation of river, coastal and oceanic environments were developed.
  2. Joint research was conducted on porifera and symbiotic microorganisms and separated a total of 435 microbial strains. The analysis of the useful substances produced by these microorganisms found, among others, a biodegradable polymer and an anti-microalga active substance.
- Ecosystem evaluation and inventory development based on advanced technologies:
  1. In a collaborative effort, a biodiversity inventory was developed. The joint team also developed a database system with online data search and exchange capabilities.
  2. As a tool to analyze genetic variations in tropical trees, advanced technologies and automatic sequencers were transferred to Malaysia. The *fagaceae* and *dipterocarpaceae* trees were analyzed and a substantial accumulation of genetic variations was found.
- Screening and separation of bioactive compounds produced by microorganisms and plants:
  1. Effective anti-tumour promoter substances were found in Malaysian food plants and one was successfully identified.
  2. A total of 354 endophytes separated from Malaysian plants were screened for their capabilities of producing antibiotics, high-molecular polysaccharide-degrading enzymes and oligosaccharides. A strain was found to produce an antibiotic that selectively attacks the *alternaria* species.
- Evaluation of therapeutic and toxic potentials of natural products:
  1. Techniques to evaluate therapeutic and toxic potentials such as antitumour, neuro-protective and anti-allergic activities and cell culture techniques for medicinal plants were transferred to Malaysian scientists.
  2. A tumor-suppressing substance was found in the bark extract from a *simaroubaceae* plant. The substance was separated as a triterpene biosynthetic compound and the structure was determined.
  3. A standard quality evaluation method was established based on HPLC for the Malaysian traditional folk medicine "*Tongkat Ali*".
  4. Neurotoxity prevention activity was found in compounds from Malaysian palm oil.
  5. Extracts from 15 Malaysian plants screened were found to exhibit promising anti-allergic activities.
  6. *Araceae* rhizome propagation techniques were established and developed to improve their efficiency.

Present and future cooperation

At present, research is being conducted and development projects being undertaken on biological complex systems (bioconsortia) to understand the mechanism of ecological complexity and to find industrial applications. In this project, the Japanese side is collaborating with Indonesia and Malaysia on prevention of the basal stem rot disease of oil palm, which is very important for the palm oil industry. Among several species of the Basidiomycete fungus, *Ganoderma*, the *G. boninense* fungus has been identified and isolated through morphological observation and molecular methods. Early detection methods of this disease will be developed by further investigation on the interaction between oil palm and the soil-born fungus.

Research on conservation and sustainable use of biological diversity should be implemented consistently. On completion, project activities are being reviewed with the partner countries, in the hope of finding possible projects for future cooperation. The development of an inventory of biological diversity and the establishment of biological resource centres, including a repository of biological materials and related information, must be among the priority areas.

Future cooperation on biological diversity will also need the involvement of industry, because only industry has the practical capability to bring the benefits of biological resources to daily life.

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Rice Genome Project, Thailand

An International Rice Genome Sequencing Project (IRGSP) was established to completely sequence the genome within 5 years. Among cereals, rice has the most compact genome. The genome size of 400 mb is predicted to have more than 20,000 genes expressed differentially in space and time. Thailand has a five-year commitment to sequence chromosome 9. This work will allow our scientists to directly access the rest of the genome sequence made available by other collaborating members.

It is also intended to utilize the genome sequence data for gene discovery from wild rice germplasm. The complete sequence data physically linked to Khoa Dawk Mali 105, the Thai jasmine rice, is under way. The complete physical map based on BAC technology, extensive isolation of high polymorphic molecular markers and comprehensive genetic maps created between aromatic rice with valuable landraces and wild rices and evaluation schemes for responses to biotic and abiotic stresses are also important long-term objectives of this project. The gene-rice wide species and landraces will be selected for the construction of genetic and physical maps. Positional candidate gene cloning approaches, including the disease resistance gene analog (RGAP), are among the most efficient methods of directly utilizing the sequence information for gene identification.

This project will bring Thailand to the international scientific arena, incorporate state-of-the-art technology, and enhance the sustainability of rice production. The ultimate outcome will improve the competitive edge of Thailand in the international rice market.

IRGSP: Vision and goals

- The function and map location of cereal and ultimately all plant genes.
- Using map-based sequence information to identify and provide markers for agronomically significant genes.
- The molecular basis of plant growth and development so that fundamental questions in plant physiology, biochemistry, cell biology and pathology can be addressed.
- The relationship, if any, of genome structure to gene expression.

Rice gene discovery project

The rice gene discovery project is part of the Thailand Rice Genome Initiative Programme (TRIP). The main foci of this project are (a) physical linking of map and sequence information from Nipponbare to KDML 105 and (b) large-scale searching for map positions of economically important genes/QTLs linking KDM105, landraces and wild rices. A tenfold coverage BAC library of KDML 105 is used as a sequence tag connector with other rice lines.

Cooking quality, aroma, nutritional value, blast and bacterial leaf blight resistance, drought, salt, submergence tolerance and insect resistance are set as top priorities for Thailand to become more competitive in the world market. To determine new allelic forms of these valuable QTLs, critical mapping populations are developing from the crossing between KDM105 and landraces and wild rice. Combined with the Rice Genome Sequencing Project, this project will lay a solid foundation and provide efficient tools for Thai scientists to discover genes and QTLs shortly.

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