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MISSION OF THE GRADUATE SCHOOL OF AGRICULTURAL SCIENCE, KOBE UNIVERSITY (GSAS-KU) "FROM FARM TO FORK (TABLE)" FOR FOOD, ENVIRONMENT AND HEALTHY LIFE

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WHAT ABOUT RESEARCH AND INDUSTRY

MISSION OF THE GRADUATE SCHOOL OF AGRICULTURAL SCIENCE, KOBE UNIVERSITY (GSAS-KU) "FROM FARM TO FORK (TABLE)" FOR FOOD, ENVIRONMENT AND HEALTHY LIFE

- For a better future for Bulgaria and Japan -

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Introduction

It is our great pleasure to present the mission of GSAS-KU to our friends and colleagues in the Bulgarian science community. We hereby give thanks to DIAGNOSIS PRESS for providing us with this opportunity to publish, in "BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT", a special note explaining our research and educational activities.

Agricultural science is a multidisciplinary science well suited to finding solutions to the crises of food, energy, health and environment, all of which now confront mankind. GSAS-KU is made up of three Departments: Agricultural Engineering and Socio-Economics; Bioresource Science; Agrobioscience. There are three affiliated Centers (Food Resources Education and Research Center, Center of Regional Cooperation, Research Center for Food Safety and Security) plus one collaborative Center (Research Center for Environmental Genomics) associated with the Organization of Advanced Science and Technology. While planting our feet firmly in the main and traditional fields of food production, we endeavor to meet the various needs of our society by venturing into the innovative fields of environmental science, biotechnology and health/life science. The mission of GSAS-KU, therefore, is to create the basic and applied knowledge and technology that are required, not only for the effective utilization of living organisms and their products as resources of food and energy, but also for the development and improvement of resources with novel functions, which may offer potential seeds to agriculture and bio-industry.

We pay a great deal of attention to the needs of both the local and international communities. Collaboration with the local community is an important part of our mission, and we promote a variety of active collaboration projects with various local sectors. The promotion of educational, academic and technological exchange projects and collaboration with overseas universities and institutions also hold a great deal of importance for us. More than 80 international students and postdoctoral research fellows are currently studying in GSAS-KU. We have signed academic agreements with many overseas BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3

universities and institutions in Asia, Africa and Europe, promoting innovative educational and research programs like "International Strategy for Agricultural Education" and "International Training Program (ITP)". Thanks to the kind understanding of Bulgarian Academy of Science, particularly Prof. Atanas Atanassov, director of the AgroBio Institute (ABI), Sofia, and Acad. Blagovest Sendov, Bulgarian Ambassador of Japan, and three Bulgarian Institutes including ABI, Trakia University and Russe University, we have agreed to jointly promote ITP from October 2007 to further strengthen Bulgaria-Japan collaboration. ITP is aimed at matchmaking between EU and Asian countries for finding effective methods to avert a food crisis by providing young researchers with overseas training opportunities. This is in the course of our longlasting efforts of collaboration with JICA (Japan International Cooperation Agency) to put forward our programs including Training Courses in Biotechnology (Biotechnology Course) and Environmental Science and Animal/Plant Disease Control (Integrated Pest Management Course) for young researchers and managing officers from developing countries, working in the fields of agriculture and bio-industry.

Agricultural science, through collaboration with other science disciplines, is to feed people by producing and providing safe, nutritious and adequate foods as well as bioresources for energy and bio-functional chemicals in a way that does not destroy but rather conserves our environment. With the motto, "From Farm to Fork (Table)", we will direct our attention to all aspects of science and technology related to "Food, Environment and Healthy Life." In this Kobe Note, we will introduce to you our ongoing research and educational activities, which includes a special program ITP and six special research projects "Health Bioscience", "Breeding and Genetics of Bioresources", "Integrated Pest Management", "Environmental Genomics", "Signaling Mechanisms by Protein Modification Reactions" and "Research for Recycling Strategy of Humans, Materials and Resources for Sustainable Rural Environment". Research and educational activities in two Centers, "Food Resources Education and Research Center" and "Research Center for Food Safety and Security", are also reported. We hope this Kobe Note can inform you of our ideas and activities, which will contribute to further stimulation of our Bulgaria-Japan collaboration.

I-1 International Training Program (ITP): Practical Training Program of Young Researchers for Strategic Bioresource Utilization and Preservation Aiming at Match-Making between EU and Asia to Avoid Food Crisis

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1. Purpose of Kobe ITP

This program is the one of ten International Training Programs for young researchers adopted by the JSPS (Japan Society for the Promotion of Science) in 2007. This ITP aims at training promising young researchers including doctoral course students, post-doctoral researchers and assistant professors to make them professionals acting as the support and driving force of future Japan with a rich global viewpoint. They will acquire extensive, fundamental and innovative academic information, skills and technology, and more advanced capabilities for designing and conducting research through research experiences in overseas research institutions. This program started from the beginning of October 2007 and will continue to the end of September 2012.

2. Outline of Kobe ITP

In order to avoid a global food crisis, one of the most pressing requirements is the training of talented young researchers with international collaboration among numerous research institutions. They will be trained to be able to preserve the bioresources of animals and plants, and environmental resources required for stable food production, and to acquire knowledge and capabilities for solving food safety problems such as BSE (Bovine Spongiform Encephalopathy) and GMO (Genetically modified organisms) and for international food security. To achieve these objectives, it is a requirement that the specialists in all three fields of 1) food, 2) environment, and 3) health/life should collaborate in a complex manner.

Accordingly, this program is characterized by the following three-step training of the promising young researchers to make them specialists, who can contribute to food crisis prevention from a global viewpoint (**Fig. 1**).

- 1) Asian Preliminary Survey (10 days in June to August): To survey suitable and appropriate study objectives through discussion with Asian institutions.
- 2) EU Training (5 to 6 months from September to March in the following year): To learn international agricultural strategies and academic information to develop technological skills through training in EU institutions, because they assume responsibility for the latest research in all three fields, and they are historically well acquainted with Asian countries.
- 3) Asian Training (30 days in next June to August): To transfer achievements obtained through the EU Training into practice for the agricultural education and research in Asian institutions.

The participating institutions in the EU are as follows:

- 1) Faculty of Agricultural Sciences in University of Hohenheim, Stuttgart, Germany.
- 2) Agro Bio Institute, Sofia, Bulgaria.
- 3) Faculty of Biology, Sofia University, Sofia, Bulgaria.
- 4) Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.



Fig. 1. Kobe International Training Program (Kobe ITP)

5) University of Rousse, Rousse, Bulgaria.

The participating institutions in Asia are as follows:

- 1) Hanoi Agricultural University, Hanoi, Vietnam.
- 2) Nong Lam University, Ho Chi Minh City, Vietnam.
- Vietnamese Academy of Agricultural Science, Hanoi, Vietnam.
- College of Agriculture, University of the Philippines, Los Banos, Philippines.
- 5) Chinese Agricultural University, Beijing, China.

This program is also characterized by its collaboration with the Graduate School of Bioresource and Bioenvironmental Sciences of Kyushu University. It is believed that the achievements of this program can be doubled by this collaboration of the two Japanese universities, each having strong relationships with the EU and Asian partners.

3. Objectives of Kobe ITP

This program aims at gradual training of the following young researchers in Kobe University:

- Junior Doctoral Course Student (2nd Grader): To study international agricultural strategies and acquire academic information, skills and technology, and to develop advanced capabilities for designing and conducting research and presenting research results through the EU Training.
- Senior Doctoral Course Student (3rd Grader): To transfer achievements obtained by EU Training into practice for agricultural education and research in Asian institutions, and to complete a doctoral thesis.
- Post-doctoral Researcher and Assistant Professor: To improve educational and research capabilities as team leaders in international joint research. To act as mentors for graduate students.

In this program, two doctoral course students and one postdoctoral researcher/assistant professor from Kobe University, and one post-doctoral researcher/assistant professor from Kyushu University will take part every year, from 2008 to 2012.

4. Original features and characteristics of Kobe ITP

This program has four unique features and characteristics as follows:

- 1) Young researchers who achieve outstanding evaluation in this program, their career path is strongly promoted to universities and international organizations.
- This program is composed of the three-step training as mentioned above. It is expected that Asian Preliminary Survey should help increase the efficiency of EU Training and succeeding Asian Training.
- The cycle of PDCA (Plan Do Check Action) is carried out thoroughly. The performance of this program is improved continuously by presentation, evaluation

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and feedback at each step, and by internal and external evaluation.

4) The cycle of PDCA performed by participating young researchers is set out on the homepage of Kobe ITP with a blog-type bulletin board. This board has the original feature of keeping a record of the development process as portfolio as well as to receive advice from instruction teachers, family members and friends.

5. Request for EU and Asian collaborators

There are five each collaborators both in EU and Asia. When our collaborators accept young Japanese researchers, they will be eligible to receive a reasonable amount of study and research fees. This payment covers the charge for accommodation service in their institutions (student dormitory or apartment) for young researchers. When mentor researchers take young Japanese researchers along for research excursion, they will be eligible to receive a reasonable amount of recompense paid from the Kobe ITP.

We wish to develop good human relationships through exchanging students and researchers bilaterally, and to conduct collaborative research between our institutions to resolve global agricultural problems. We strongly hope to reach an agreement for exchanging graduate students through a doubledegree program in the future.

II-1 Health Bioscience

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It is well known that foods have not only a physiological function associated with health promotion but also a prophylactic function. Food functions include immunomodulation, infectious disease prophylaxis, anti-hyperglycemia, antiadipositas, and anti-carcinogenesis. Representative factors of these functions are polysaccharides as fibers and polyphenols, which are recognized as non-nutrients. Recently, however, they have come to be referred to as sixth or seventh nutrients.

Foods are digested in the mouth and stomach after ingestion, and the food factors are further modified by several bioprocesses during gastrointestinal absorption and transportation. To perform physiological functions, these food factors have to arrive at specific organs or cells without modification, or have to exert their activities in modified forms. However, our current knowledge is insufficient to explain their functional mechanism, even though numerous researchers have studied these fields diligently. To understand the mechanism, we have to not only find and evaluate functional food factors but also elucidate the problem including the cross-talking between food factors, bio-molecules and intestinal flora, and the bioactive forms and bioavailable concentrations in target tissues. The purpose of this project is to elucidate the health promoting activities of food factors by multiple approaches including development of functional food factors and their evaluating methods, and elucidation of the cross-talking which takes place between food factors, bio-molecules and intestinal flora.

1. Development of tailor-made probiotics

Studies of so-called tailor-made medicines designed to suit individual patients are progressing rapidly, not only in Japan but also in other developed countries. This is because the therapeutic and preventive effects of certain proprietary medicines have been found to vary from individual to individual, sometimes with adverse side effects on certain individuals. The need for tailor-made drugs that are both effective and side effect-free for any individual is thus becoming increasingly apparent. In this context, we have extended such a "tailor-made" perspective to the sphere of functional foods and embarked on development of so-called tailor made probiotics, with specific focus on bifidobacteria. The bifidobacteria are one of the most predominant groups in the set of human intestinal microflora and known to have an important role in maintaining or promoting the general health of the host. Bifidobacterial cultures are therefore often used as probiotics in pharmaceuticals and foods. We have so far observed that each individual harbors so-called "indigenous" bifidobacteria over a long period of time, and suggested that it is not "exogenous" ones available from commercial probiotics but the "indigenous" ones from individual microflora that will remain stable life-long in the host. On this basis, we are currently studying structural variations in fimbriae of *Bifidobacterium* longum strains that bind oligosaccharides of mucin secreted on the human intestinal walls, in order to elucidate the mechanism involved in the host specific colonization of the "indigenous" bifidobacteria. The future outcome of our study will be a novel tailor-made functional food that uses bifidobacteria capable of colonizing any individual intestine to ensure maximum health benefits from genuine probiotics.

2. Food factors suppressing hyperglycemia

Metabolic syndrome is becoming increasingly common in the developed countries, including Japan. People with metabolic syndrome are at increased risk of coronary heart disease and other diseases related to plaque buildups in artery walls (e.g., stroke and peripheral vascular disease) and type 2 diabetes. The dominant underlying risk factors for this syndrome appear to be connected to chronic hyperglycemia and often insulin resistance, a generalized metabolic disorder in which the body is not able to use insulin efficiently.

Insulin is a hormone with extensive effects on both the metabolism and several other body systems, and mainly it enables cells to take up glucose from the blood. Skeletal muscles and adipose tissues are mostly responsible for insulin-regulated glucose disposal, and the former play a more important role in maintaining whole body glucose homeostasis. When the control of insulin levels fails, diabetes mellitus

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results ultimately. It is well established that insulin stimulation of glucose uptake in skeletal muscle cells is mediated through translocation of glucose transporter 4 (GLUT4) from the endoplasmic reticulum to the plasma membrane. It is also known that a defect in glucose transport efficiency and GLUT4 activity results in insulin resistance. Thus, the action of insulin on muscle cells for glucose uptake depends on the induced translocation of GLUT4. However, the mechanism regulating the translocation of GLUT4 has not yet been well elucidated. Attempts have been made to develop efficient model systems to study in-depth on the glucose uptake involving GLUT4 in muscle cell lines and in animals such as mice and rats.

On the other hand, these cell lines and animal models are useful tools for us to study food factors that can improve the translocation of GLUT4 and action of insulin on muscle cells. So far, some of the inositol derivatives, such as pinitol from soybeans, and flavonoids, such as epigallocatechin-3-gallate from green tea, have been shown to possess an insulin-mimetic activity. The model systems allow us to understand how these useful food factors function in the body, and we aim to establish promising ways to apply them efficiently in health promotion and disease prevention.

3. Research and development of new functional food materials in waste parts of agricultural products

This topic focuses on the profitable uses of waste parts of agricultural products as new resources of functional food materials. For example, horseradish (Cholearia armoracia) is one of the cruciferous plants, and its root is popularly consumed as a condiment called 'wasabi' in Japan and 'raifort' in Europe. However, most of its leaves are wasted, and there are few reports on its functional properties. We have recently demonstrated that the oral administration of hot water extract of horseradish leaves to mice activated hepatic drug-metabolizing phase II enzymes glutathione S-transferase and quinone reductase. These findings suggested that dietary consumption of horseradish leaves would contribute to cancer prevention by enhancing hepatic detoxification and could also be used as functional food materials. Thus, we are continuing to screen the waste parts of various agricultural products for their usefulness as functional food materials.

4. Polysaccharides suppressing Type I allergy

Allergy is a common health problem in developed countries. The incidence of type I allergic disorders has been increasing worldwide, particularly, hypersensitivity to food and airborne allergens. The mechanism of Type I allergy includes a series of events leading to production of antigen-specific immunoglobulin E (IgE). IgE synthesis is considered to be caused by development and activation of T helper-2 (Th2) cells and B cells. This specific Th2 cell produces predominantly interleukin (IL)-4. In contrast, the T helper 1 (Th1) cells mainly secrete cytokines such as IL-2 and interferon (IFN)- γ that inhibit IgE and IgG1 secretion and enhance IgG2a secretion. Thus, it would seem that shifting the balance from Th2 to Th1 dominance is a rational strategy to prevent IgE-mediated

allergic diseases. We have focused on the food factors, which possess the immunomodulatory activity and shown that mushrooms are candidates to have such activity. Particularly, *Agaricus brasiliensis* S. Wasser et al. possesses the highest activity in IL-12 (a key role in Th1 differentiation) and IL-18 (a proinflammatory cytokine in enhancing Th1 immune response) production from macrophages and the suppression of allergy sensitized with ovalbumin. Now, to clarify how mushroom can activate the immune cells, we are investigating using co-culture system mimicking an enteric canal. The coculture system is made up of an intestinal epithelial cell line, Caco-2 cells and a murine macrophage cell line, RAW264.7 cells.

5. Dietary fiber reducing risks of absorbing harmful heavy metals

It is well known that heavy metals, such as mercury (Hg) and cadmium (Cd), have a bad influence on our health and cause serious illness, the so-called Minamata- and Itai-Itai diseases. respectively, by ingestion of even a very small amount of each element. If we accidentally ingest a trace amount of these harmful heavy metals contained in foods and beverages, they must be excreted immediately to outside of the body. A food factor, the dietary fiber such as alginic acid in algae and pectin in fruits and vegetables, possibly has the function to remove the harmful elements by specific interactions between its acidic groups (-COO⁻) and the metal ions, and can reduce the risks of absorbing the harmful metals into our bodies. From this point of view, we have studied the relation between functions and structures of dietary fibers on adsorptions of several metal ions by using vibrational (Raman and IR) and atomic absorption spectroscopies, and theoretical calculations. For example, the results of Raman study suggest that at least two types of ligation structures were involved in the interaction between the carboxylate groups and some M2+ cations in alginic acid. In addition, we have started investigations into other functional biological molecules, not only the dietary fibers (polysaccharides) but also some kinds of proteins that can effectively interact with harmful metal cations.

6. Non-invasive biological measurement technology

This section is working on various applications and new developments in the field of near infrared spectroscopy (NIRS) as non-destructive technology for bio-monitoring and diagnosis. Qualitative and quantitative analysis in biological and food related research and technology and understanding new phenomena related to water in biology have been the main areas of interest. NIRS has been successfully applied for in-vivo diagnosis of mammary gland inflammation (mastitis) in dairy cows followed by identification of the bacterial pathogen. Oxidative stress, prion disease in rats and mosaic virus in soybean plants are other successful examples of noninvasive diagnosis based on *in-vivo* tissue spectra acquisition and analysis. Various studies at the molecular level have been related to food functionality and on-line monitoring of a fermentation process proved to be a good tool for feedback BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3

in the food production line. Recently, a new scientific area, Aquaphotomics, has been proposed. The aim is to describe and understand biological systems through the multidimensionality of water–light interaction expressed as a spectrum.

II-2 Breeding and Genetics of Bioresources

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Every single organism, including animals and plants, has a genome that contains all of the genetic information needed for its building and maintenance. The genetic information contained in a genome is encoded in its deoxyribonucleic acid (DNA). Through the modification of genomes, mankind has domesticated and made improvements to animals and plants for approximately 10,000 years. The roles of the team "Breeding and Genetics of Bioresources (BGB)" are, 1) clarification of genetic information stored in genomes, 2) development of future food resources and novel breeding strategy, and 3) provision of valuable biological resources for food production and novel bio-industry (**Fig. 2**). BGB has the following three major projects targeted at animal, crop and fruit genomes.

Animal Genome Project

Japanese Black cattle are valued for the excellent quality of their beef. Genetic conservation and improvement of productive traits is important for economic impact. The project team has attempted to produce novel technologies, which integrate molecular biology and statistical genetics, and conducted research focused on genetic improvement of Japanese Black cattle.

The aims of our project are 1) identification and utilization of functional genes associated with fatty acid composition, 2) development of DNA markers for breed identification, 3) development of single nucleotide polymorphism (SNP) markers for individual identification and parentage testing, and 4) achievement of breeding in Wagyu cattle in a sustainable manner.

1. Identification and utilization of functional genes associated with fatty acid composition

Japanese Black cattle are valued for their highly marbled beef with lower fat melting points than other breeds. The lower fat melting points reflect the higher proportions of unsaturated fatty acids. Besides contributing to the softness of fat, the unsaturated fatty acid components may also contribute positively to the favorable beef flavor. We have demonstrated that stearoyl-CoA desaturase (SCD) and sterol regulatory element binding proteins (SREBP) are key enzymes responsible for conversion of saturated fatty acids into mono-unsaturated fatty acids (MUFA) and the polymorphisms in these enzymes affect the fatty acid composition. We aim at estimating the correct genetic effects of the genes for bovine MUFA and to identify novel functional genes for this valuable trait.



Fig. 2 Contribution to sustainable food production by development of future biological resources and novel breeding strategy for animals and plants

2. Development of DNA markers for bovine breed identification

In the meat industry, correct breed information is required in food labeling to assure beef quality. Genetic markers provide corroborating evidence to identify the breed. Our aim is to develop DNA markers to discriminate among breeds in the Japanese beef market. We have developed six DNA markers to distinguish between Japanese Black and F_1 (Japanese Black × Holstein) breeds based on the amplified fragment length polymorphism (AFLP) method. Development of DNA markers to discriminate between Japanese and Australian beef has also been achieved using mitochondrial DNA, SRY gene, MC1R and AFLP-SNPs. We are now developing DNA markers for USA beef.

3. Development of SNP markers for individual identification and parentage testing

Individual identification and parentage testing in Japanese cattle are carried out using microsatellite markers. However, microsatellite markers have the disadvantage of misjudgment of alleles by slippage during PCR amplification and a fairly high mutation rate. Our purpose, therefore, is to develop SNP markers. Using AFLP technique and DNA chip, we have determined 44 novel bovine SNPs available for testing. We will increase the number of SNP markers usable for this purpose.

4. Achievement of sustainable breeding in Wagyu cattle

Breeding of Wagyu cattle including Japanese Black should be directed to improve production efficiency and preservation of genetic diversity. One of the ways to improve feed utilization efficiency is through residual feed intakes. They are the feed intakes, which consider both energy requirement for production and maintenance. Our team have already estimated genetic parameters of the intakes during performance testing of Japanese Black. We should then estimate genetic relationships between the intakes and carcass traits to examine the efficiency of the intakes as selection criteria. Our team also developed a measure for evaluating reproductive ability and number of calves produced, and found a certain genetic variability. Its effect on other economically important traits is another interesting objective. We also conduct basic research studies on the genetic structure and diversity of Japanese Black and Japanese Brown cattle. It has been shown that the genetic diversities of both breeds are in a critical state. Our important mission is to find a suitable method to strike a balance between genetic improvement and diversity.

Crop Genome Project

The aim of our project is to clarify genetic diversity, evolution and adaptation of major crops in the world, and contribute to the improvement of crops not only for Japan but also for world food production. Three major crops, wheat, rice and potato, are intensively studied in this project.

1. Wheat genetics

Wheat is one of the most important founder crops in Western Asia and still plays a major role in the world food economy. Our research is directed towards understanding of the genetic mechanisms that underlie polyploidy evolution and function, domestication and adaptation to various environments.

We focus on the following aspects in polyploidy wheat genetics and genomics:

- 1) Functional role of organellar (chloroplast and mitochondrial) genomes under abiotic stresses and in different stages of plant development, particularly the transition from quiescence to imbibition and germination and from vegetative to reproductive stages. Special interest is taken in the nucleus-cytoplasm interaction including male sterility and fertility restoration system, which is required for F_1 hybrid production.
- 2) Signal transduction systems that are induced and operate during acclimation under abiotic stresses including low temperature, dehydration and high salinity. Regulation of *Cor/Lea* regulon including *CBF/DREB* transcription factor genes and downstream *Cor/Lea* genes are studied together with genes involved in ABA (abscisic acid)

signal transduction pathways. A core collection of *Aegilops squarrosa*, a D genome donor to bread wheat, is used for clarifying the role of D genome in abiotic stress tolerance.

- 3) Clarification of the genetic mechanism of reproductive isolation. A study of the hybrid necrosis is conducted through molecular genetic and histochemical analyses. An important correlation has been found between reactive oxygen species and cell death associated with hybrid necrosis.
- 4) Organellar genome polymorphisms and the domestication process. A large-scale investigation on the maternal lineage is now under way by taking advantage of the organellar genome polymorphisms. By DNA fingerprinting technique based on organellar microsatellites, it has been shown that two independent maternal lineages were involved in the domestication of emmer wheat.

2. Rice genetics

Rice (*Oryza sativa*) is an important crop and a major source of food for more than one-third of the world's population. To improve rice varieties, we focus on the following topics in rice genetics:

- Domestication process from wild forms. Cultivated rice varieties are likely to have domesticated from a wild Asian species, *O. rufipogon*, about 10,000 years ago. To clarify the key characters leading to rice domestication, morphological differences between wild and cultivated forms are compared. Of these, the closed panicle shape might have been one of the major triggers in the shift to the cultivars.
- 2) Clarification of genetic variation among cultivated and wild rice species. Since both cultivated and wild rice species are widely distributed under variable environmental conditions, a broad genetic differentiation is observed in these species. To provide basic information for rice breeding, Asian local varieties (more than 1,000 varieties from Vietnam, Laos, Cambodia and Thailand) and wild accessions (more than 1,000 individuals from Myanmar, Vietnam and Cambodia) are under examination using microsatellites.
- 3) Identification of useful genes from rice genetic resources. To identify useful genes from wild rice relatives, an accession of *O. rufipogon* was backcrossed with two typical rice cultivars, *O. sativa* Japonica Nipponbare and Indica IR36. In their backcrossed generation, several wild chromosomal regions were detected that might further improve agronomic characters of elite rice varieties.

In addition to the above research, the following research projects are being carried out; mechanisms of abiotic stress tolerance, resistance mechanisms against brown planthopper, and improvement of Japanese sake-brewing rice cultivars.

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3. Potato genetics

The potato is the fourth most important crop in the world, which contains the largest number of closely related wild species (over 200 spp.) and has the longest history of using these wild species in breeding among the top four crops. The potato is an autotetraploid and a vegetatively propagated crop differing greatly from the other crops in its transmission genetics. Complicated segregation in progeny is always expected and manipulation of ploidy levels must be undertaken for efficient utilization of wild germplasms in potato breeding.

Among our broad interests in potato genetics, current research projects are focused on:

- 1) The genetic mechanism of heterosis using diploid inbred lines produced by taking advantage of a function of *S*-locus inhibitor gene (*Sli*). It was found that genetic heterozygosity/homozygosity of methylated DNA might play an important role in the regulation of heterosis.
- 2) To investigate the mechanism of diploidization, the genomes of a Mexican hexaploid wild species *Solanum demissum* (2n=6x=72) are being dissected by back-crossing to a tetraploid potato. Chromosomal behavior and their transmission to the following generation, and male and female factors controlling endosperm development in hybrid seed formation are specific interests.
- Finally, development of practical methods for DNA marker-assisted selection is being pursued for Japanese potato breeding programs.

Fruit Genome Project

Research interests are focused on the preservation and evaluation of the genetic resources of the pear as donors of desirable agronomic traits, e.g. self-incompatibility, aroma components, acid and sugar contents, and resistance to diseases.

1. Gene responsible for self-incompatibility of Japanese pears

Our present aim is to clone the pollen S gene that determines self-incompatibility in the Japanese pear. A bacterial artificial chromosome (BAC) library has been constructed from an S_q -homozygote. Chromosome walking initiated from pistil S gene, S_q -RNase, was used to assemble a BAC contig spanning from 395 kb upstream to 615 kb downstream of the S_q -RNase locus. It was found that S_q^{sm} -haplotype derived from a self-compatible cultivar, *Osa-Nijisseiki*, lacks a 236 kb region spanning from 48 kb upstream to 188 kb downstream of S_q -RNase. Our result suggests that the pollen S_q allele is localized outside the deletion of 236 kb.

2. Evaluation of wild pears as genetic resources

'Iwate-yamanashi' (*Pyrus ussuriensis var. aromatica*) is one of the pears grown in the wild and is an endangered species in Tohoku region in Japan. Its population has been decreasing and therefore, conservation is urgently needed. A set of 650 trees including 'Iwate-yamanashi' has been conserved as a core collection of wild pear germplasm. Current interests are focused on discrimination by molecular markers (microsatellite and chloroplast DNA), and evaluation of unutilized 'Iwateyamanashi' and their derivatives, some of which produce fruit with desirable agronomical traits, e.g. certain aroma and higher contents of acids and sugars. Aroma compounds contributing to 'Iwate-yamanashi' flavor were evaluated by the Aroma Extract Dilution Analysis method (AEDA). Eighteen key odorant compounds such as esters and aldehydes have been identified by the AEDA method.

II-3 Integrated Pest Management

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The history of food production has been a history of struggles against diseases, insect pests, and weeds. The most common and widely used method for pest control has been the use of chemicals. However, the heavy application of pesticides during the 20 years from 1950 to 1970 caused serious pollution of foodstuffs and environment in Japan, leading to the proposal for Integrated Pest Management (IPM). The objective of this project is to elucidate mechanisms of interactions between plants and their enemies (pathogens, insect pests, weeds, and parasitic plants) using genetic, cytological, molecular biological, biochemical and ecological approaches, and to develop new methods for IPM.

1. Toward the development of a system for predicting durability of resistance genes

Blast is one of the most serious diseases which affect rice. Many resistance genes have been introduced into commercial rice cultivars, and new cultivars with these resistance genes have been released to farmer's fields. A few years after the release, however, new strains of the rice blast fungus appeared, and rendered those resistance genes ineffective. On the other hand, some of the resistance genes have been effective over a long period. The problem is that we do not know which gene is durable when we start the breeding program. The objective of this study is to develop a system for predicting durability of given resistance gene. Our hypothesis is that the durability of a resistance gene. We are now trying to clone avirulence genes corresponding to known resistance genes.

2. Cytological and ultrastructural analyses combined with biochemical and molecular analyses for elucidating cellular responses in disease-stressed tissues

Living organisms are exposed to various stresses, which include salt, drought, temperature, ultraviolet rays, chemicals, toxins, and microbes. Even when stressed organisms appear intact, they operate mechanisms to cancel out cell damage due to the stressors and then have continued to survive by making changes to the function and structure of stressed cells. Cell responses have been analyzed in the stressed plants, especially in the interaction between plants and phytopathogenic fungi, with cytological techniques involving electron microscopy. A variety of electron microscopy methods with highly developed techniques can be used for the analysis as follows:

- 1) skillful electron microscopic preparation for scanning and transmission electron microscopy,
- 2) cytochemical methods for detecting reactive oxygen species, lignin, polysaccharides, and cellular ions,
- 3) immunohistochemical or immunoelectron microscopy,
- 4) autoradiography,
- 5) stereology,
- 6) element analysis with energy filtering electron microscopy, and
- 7) rapid-freezing and freeze-substitution electron microscopy.

Additionally, we can analyze the stressed tissues with biochemical and molecular biological techniques combined with cytochemical analysis to profoundly understand the biological significance of cell response changes. Our final goals are the production of stress-resistant plants with high quality.

3. Insect physiology and molecular biology; the other side of IPM

We focus on three aspects of pest management;

- 1) molecular mechanisms that underlie photoperiodism by which pests regulate their life cycle,
- 2) search for new targets for disturbing pest physiology such as digestion, reproduction, neural transmission, metabolism and metamorphosis, and
- 3) target-oriented screening for new agrochemicals based on the expressed protein.

We have cloned batteries of cDNAs for circadian genes, neuropeptides and neurotransmitters genes in a cockroach, crickets, moths, and sawfly. Temporal patterns of transcription have been analyzed. Some of them have been expressed as proteins in vitro and antibodies are produced with which neuroanatomical architectures of these insects are clarified and temporal expression patterns are analyzed. We isolated several peptides from the cockroach midgut and cloned cDNAs for growth factors and apoptosis genes from the midgut of a noctuid moth. We are now analyzing the function of these factors. Vitellogenin and lipophorin gene mechanisms are being studied in cockroaches, a planthopper and bugs. A species-specific neurotransmitter receptor has been cloned for screening for species specific chemicals. For example, we cloned the GABA receptor (GABAR) of the two-spotted spider mite, Tetranychus urticae. This species has a unique amino acid sequence in a transmembrane domain. By reconstructing this GABAR, it is possible for screening of specific acaricides. It was found that N-acetyltransferase (NAT) is involved in numerous important physiological functions in insects. Using the reconstructed target protein, we found chemicals with good potential for insect pest control. NATs in insects are grossly different in the structure from their mammalian counter-parts

and therefore this system carries little risk of disturbing human enzymes.

4. Toward the conservation biological control of agricultural and forestry pest insects

For the conservation and wise use of native natural enemies in order to control agricultural and forestry pest insects in IPM, we are studying the species diversity, behavior, genetic characteristics, and landscape population structure of parasitoid wasps and predatory ground beetles. Application of the DNA bar-coding system is on trial for the identification and storing biological information of highly diversified parasitoid wasps. The behavior and genetic system of *Meteorus pulchricornis*, a polyphagous and parthenogenetic parasitoid of lepidopteran larvae, is now being intensively studied as a promising biocontrol agent of vegetable pests (**Fig. 3**). Also, the life history, behavior, and habitat requirements of predatory ground beetles (*Carabus, Chlaenius*, etc.) are being investigated for their conservation and use for pest control in rural woodlandfarmland landscapes.



Fig. 3. A braconid parasitoid (*Meteorus pulchricornis*) attacking the common cutworm (*Spodoptera litura*). The photo was taken by Yamamoto and Itokawa.

5. Adaptation and ecology of the weed seeds from tropical area to temperate zone

The germination temperature and water condition of water lettuce (*Pistia stratiotes*) seed, a floating tropical weed in Yodo River, Osaka Prefecture, and Lantana (*Lantana camara*) seed, a tropical weedy shrub of the volunteer seedling vegetation in Kobe City, Japan, are analyzed. Recently, both species have been introduced into Japan because of the heat island phenomenon of cities and global warming. They set seeds in the summer and autumn seasons in the temperate zone. The adaptation, intra-specific variation and bottleneck of the species into the temperate zone from tropics are now observed.

6. Toward the elucidation of host-parasite recognition mechanisms

Broomrapes (*Orobanche* spp.) and witchweeds (*Striga* spp.) are obligate root parasitic weeds, which are the scourge of BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3

agriculture and food security in many parts of the world. The ultimate control method for parasitic weeds lies in the development of high yielding resistant crop varieties with acceptable qualities. Accordingly, reactions of *Lotus japonicus*, a model legume for functional genomics, to *Striga* spp. and *Orobanche* spp. have been studied at cellular, chemical and molecular levels in order to obtain greater insight of host-parasite interactions. Attachment of the incompatible parasite *S. hermonthica* induced host tissue browning and expression of genes related to phytoalexin biosynthesis, whereas such responses were not observed in a compatible relation with *O. aegyptiaca*. These findings suggest that the host plant recognizes the incompatible parasite as an unfavorable intruder like pathogenic fungi. Host responses are also analyzed in more detail to probe host resistant mechanisms.

II-4 Environmental Genomics

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Three main groups are studying the environmental genes in the aspects of signal transduction, sex differentiation and nanobio-technology (**Fig. 4**).

1. Plant signal transduction

Research in our group is focused on environmental signal transduction in plants. Plants are frequently exposed to unfavorable environmental conditions, due to their sessile life cycle. Extreme temperature, drought, salinity, pollution, and pathogens greatly affect plant growth, development, and productivity. To survive, plants must respond and protect themselves from all forms of environmental biotic and abiotic stresses. Plants have developed a complex signaling network that senses and protects them from an ever-changing environment. We are interested in the elucidation of intracellular signal transduction pathways controlling plant development and defense. Protein phosphorylation is one of the major mechanisms for controlling cellular functions in response to external signals. The modules rapidly amplify and transduce extracellular signals into various appropriate intracellular responses. The mitogen-activated protein kinase (MAPK) cascade is one of the signal modules that connect the perception of these external environmental stimuli to physiological cellular responses and are important mechanisms for stress adaptation by control of gene expression. MAPK cascades are composed of three protein kinases: MAPKs, MAPK kinases (MAPKKs) and MAPKK kinases (MAPKKKs). Activated MAPKKK phosphorylates MAPKK, thus activated MAPKK in turn activates MAPK through phosphorylation. In Arabidopsis thaliana, there are 20 MAPK genes, 10 MAPKK genes and 60 MAPKKK genes, suggesting complicated signal networks of this cascade. Recently, investigation of each set of plant MAPK cascade has begun. We carry out research projects on Arabidopsis MAPKK (AtMEK1) and have shown that AtMEKK1-AtMEK1-AtMPK4 cascade becomes active and works on a wounding stimulus. Combination of the molecular biology, molecular genetics and biochemical approach, we will clarify the molecular events in environmental stress signaling in plants, which will lead to the creation of new tools for agricultural and environmental improvement.



Signal transduction

Fig. 4. Research of environmental genomics and environmental problems

2. Molecular morphology in animals

It is well known that numerous chemicals released into the environment potentially disrupt the endocrine system, and that some of them exhibit estrogenic activity by binding to estrogen receptors. Recent reports on the possible disruption of development and reproductive functions caused by a variety of environmental contaminants with estrogenic or anti-androgenic properties have heightened public concerns about the adverse health consequences of these chemicals for various species, including homo sapiens. These compounds include some polycyclic aromatic hydrocarbons, chlorinated organic compounds and pharmaceutical agents. Mice exposed prenatally to these compounds provide a model for exploration in humans, because the genital development of newborn mice corresponds approximately with that of the human fetus at the end of the first trimester. The fetal and neonatal period has a clinical window that is particularly sensitive to exposure to exogenous estrogenic compounds. Furthermore, long-term changes, including molecular alterations, have been observed after exposure to endocrine disrupting chemicals during these early developmental periods. In addition, it has been reported that neonatal exposure of animals to estrogenic compounds caused disorders of the male reproductive tract, reduction of testosterone, testicular atrophy and carcinoma, increased rate of breast cancer, uterine adenocarcinoma and various cervicovaginal lesions. The fetus and neonate are highly sensitive to the chemicals, and therefore mammalian neurodevelopment may be disrupted by these exogenous chemicals that are lower than NOAEL (No Observed Adverse Effect Level) or LOAEL (Lowest Observed Adverse Effect Level). However, there are few reports that assess the risk of exposure to low dose of multiple environmental chemicals, which is the most practical problem. In this point, we investigate the effects of in utero and lactational exposure to three types of representative environmental chemicals (bisphenol A: BPA, di-(2-ethylhexyl)-phthalate: DEHP, 2,3,7,8-tetrachlorodibenzo-pdioxin: TCDD) which act as endocrine disruptors via different mechanisms, and compare the effects between sole and multiple administrations.

We also attempt to meld an enormous quantity of morphological insights with molecular science, and provide comprehensive explanations on molecular functional morphology that encompass a spectrum ranging from gene to body, along with introducing relevant advanced research and topics. Techniques frequently used in our investigation are a combination of the molecular biology, molecular-cytogenetics, epigenetics, immunohistochemistry and ultrastructural approaches. The main topics of our research are classified into the following three fields:

- 1) Difference between testis and ovary What is the sex difference in the brain and reproductive system.
- 2) Mechanisms of sex determination and differentiation and their abnormality in mammals.
- 3) Working mechanisms in environmental chemicals acting as endocrine disruptors.

3. Nanobio-technology

The cytochrome P450s play an important role in the oxidation of xenobiotics, including drugs and environmental pollutants, and therefore represent a primary focus of toxicological and drug metabolism research. A majority of P450s and NADPH-P450 reductases is located in a hydrophobic environment in the endoplasmic reticulum of cells. Some P450 species are known to hydroxylate substrate molecules (e.g. steroids) at various positions, and one P450 species also can metabolize a variety of substrates. In addition to the hydroxylation, P450s catalyze a wide range of reactions, including dealkylation, epoxidation, desaturation, and oxidative ester and ether cleavage. A complete set of P450 genes from many species is now available. However, the molecular information on P450 species is quite limited because these enzymes are expressed at low levels and are unstable. To analyze the enzyme function of P450s, we are trying to use the heterologous expression systems of bacteria and yeast cells. The importance of P450s

for toxicological and drug metabolism researches has prompted the development of methods to assay their enzymatic activities. Currently available assay tools utilize the multi-well plate format, in which suspensions of purified microsome fractions containing P450s or bacteria cells expressing P450s are mixed with the enzyme substrates. Although the use of suspended microsome fractions is convenient, it has some limitations. Immobilization on solid substrates should be advantageous for sensitive analytical techniques. However, direct immobilization of microsomes onto solid substrates very often results in the loss of P450 enzymatic activities, mainly because of the unfavorable interactions between the substrate surface and membrane-bound enzymes.

Recently, we have developed a methodology for immobilizing membrane-bound P450 on patterned lipid membrane substrate for bioassays. The enzymatic activity was significantly higher on lipid bilayers compared with direct adsorption on glass. Furthermore, the competitive assay experiments between two fluorogenic substrates demonstrated the feasibility of bioassays based on immobilized P450s. The polymeric bilayer plays multiple roles in this configuration, i.e. stabilization of the fluid bilayers containing fusogenic lipids (the binding agent), suppression of non-specific binding to the surface, and patterning of P450s. Further developments of the detection methods should open up new avenues for the highthroughput screening of P450 activities.

II-5. Signaling Mechanisms by Protein Modification Reactions

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This group is based on the 21st Century Center of Excellence (COE) Program entitled "Signaling Mechanisms by Protein Modification Reactions" that was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan 2002-2006, aiming to form an international research center for the study of signal transduction in Kobe University. The members took over the objectives of the program and reorganized the group with additional young members into the present group in 2006. Now 14 faculty members in Kobe University from the Biosignal Research Center (8), the Research Center for Environmental Genomics (1), the Graduate School of Science (2) and the Graduate School of Agricultural Science (3) join this group and are studying the roles of protein modification reactions in order to clarify the outline of the signal transduction mechanisms. The members hope that the cooperation with different backgrounds and approaches will not only give rise to advances in the research but also contribute to the higher education of young students based on rich human resources and good traditions in this research field in Kobe University for signaling mechanisms.

One of the protein modification reactions, protein phosphorylation/dephosphorylation reaction has been accepted BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3

to play a critical role in the regulation of cellular functions by controlling the activity of various proteins through the reversible modification processes. During the 1970s and 1980s, key molecules and cascades were identified that follow the binding of an extracellular signal to receptors on the cell surface, and transfer the signal to the cell nucleus to elicit a response. The activation of G proteins, which are associated with hormone receptors inside the cell, leads to the activation of protein kinase A (PKA). Activated G proteins also trigger the cleavage of phospholipids, phosphatidylinositol-4,5-bisphosphate (PIP2) in the cell membrane, into inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) that in turn activates protein kinase C (PKC). The activated PKA and PKC phosphorylate other proteins, initiating cascades of phosphorylations to induce the correct cellular response. PKC is the enzyme discovered in Kobe University in the late 1970s. In the present group, various protein modification reactions and signaling mechanisms are investigated using different organisms such as worm, fly, frog, mouse, and domestic animals as the experimental models.

From the Graduate School of Agricultural Science, 3 members in the Laboratory of Reproductive Biology and Biotechnology join this group. The main targets are oocytes, spermatozoa and early embryos in mammals including domestic species. Oocytes and spermatozoa are highly specialized cells for fertilization. In mammals, oocytes mature in the ovary, and then they are ovulated to the oviducts where they meet spermatozoa. Spermatozoa are ejaculated into the female genital tract where they become capable of fertilization of the oocytes. Here, we introduce our recent findings of oocyte maturation and sperm activation. They activate specific signaling cascades and modify some specific proteins before fertilization.

1. Oocytes

In the mammalian ovary, gonadotrophic hormones from the pituitary trigger the oocyte maturation. When oocytes start maturation, they undergo well-ordered events including chromosome condensation, nucleolus disassembly, breakdown of nuclear membrane, spindle formation in metaphase I, separation of the homologs, extrusion of the first polar body, and spindle formation in metaphase II. These events are induced by the fluctuation of oocyte MPF (maturation-promoting factor or M-phase promoting factor), now known as Cdc2 kinase or CDK1 (cyclin-dependent kinase 1). Actually, nuclear lamins are phosphorylated by CDK1, and nuclear envelope breakdown occurs concomitantly with the activation of oocyte CDK1. On the other hand, oocyte chromosomes start to condense and the nucleolus disassembles before the CDK1 activation. The chromosome condensation correlates the modification such as phosphorylation and deacetylation of the DNA-binding protein histone H3 on the specific amino acid residues.

The oocyte nucleolus is a prominent organelle, although its role and disassembly/reassembly mechanisms during oocyte maturation and fertilization have not been elucidated. Even after removal of the nucleolus by microsurgical manipulation, mammalian oocytes mature normally with regular spindle formation and subsequent polar body extrusion, and activities of CDK1 and mitogen-activated protein kinase (MAPK) fluctuate normally. However, after fertilization, the oocyte nucleolus is essential for nucleolus assembly in zygotes, and is thus also essential for normal embryonic development.

During maturation, oocytes arrested at prophase I resume meiosis and progress to metaphase II. At the end of meiosis I, homologs separate from each other, while each chromosome is still composed of two sister chromatids held together at their centromeres. The sister chromatids do not separate until anaphase II, which begins after sperm penetration. Our group have revealed that mammalian shugoshin Sgo2 is expressed in oocytes and is solely responsible for the protection of cohesin Rec8, a meiosis-specific cohesin component regulating sister chromatid cohesion, to ensure that the sister chromatids remain connected.

2. Spermatozoa

Mammalian spermatozoa are differentiated in the testicular seminiferous tubules and then transferred through the long duct of epididymis. At the arrival of the terminal portion of epididymis, they have the potential to move progressively and fertilize oocytes but are temporally quieted there. After ejaculation into the female genital tract, the spermatozoa quickly initiate flagellar beating and gradually undergo a series of changes in the intracellular space as well as on the cell surface (capacitation). These changes are terminated after several hours and only fully-capacitated spermatozoa are capable of undergoing the acrosomal exocytosis and exhibiting flagellar hyperactivation which are both required for successful fertilization with the oocytes.

Our recent research has been focused on the disclosure of the signaling cascades that regulate flagellar hyperactivation. Specifically, flagellar hyperactivation is characterized by the larger-magnitude and asymmetric beating of the principal and middle pieces. This unique movement generates a great driving force that enables the spermatozoa to penetrate through the extracellular matrix of oocytes. One of the key reactions for the induction of flagellar hyperactivation is protein tyrosine phosphorylation which is regulated via the complicated network of sperm-specific signaling cascades. In the sperm connecting piece, for instance, the cAMP/PKA-dependent tyrosine kinase Syk activates phospholipase C (PLC)y1 by the tyrosine phosphorylation at the activation loop. The activated PLCy1 generates IP3 that can directly open the calcium channels of the internal store. Subsequently, the increased cytoplasmic calcium ions induce the activation of sperm PKC in cooperation with DAG that is derived from PIP2 by the PLCy1. Coincidentally, this protein kinase is also phosphorylated by the PKA-regulated kinases. These capacitation-related changes apparently promote flagellar hyperactivation. In contrast, the cAMP/PKA-dependent phosphatidyl-inositol-3 kinase (PI3K) signaling cascade suppresses precocious protein tyrosine phosphorylation in the principal piece leading to flagellar hyperactivation before the sperm access to the oocytes. However, this signaling cascade is likely shut off by the inactivation of target molecule (phosphoinositide-dependent protein kinase 1) of PI3K during the capacitation process, and fully-capacitated spermatozoa consequently initiate flagellar hyperactivation.

The accumulation of these new findings regarding the molecular nature of oocyte maturation and sperm activation is important for improving the reproductive technologies in domestic animals as well as in humans.

II-6. Research for Recycling Strategy of Humans, Materials and Resources for **Sustainable Rural Environment**

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This research project is aiming at planning novel strategies for sustainable rural environment by fusing the concepts of agricultural engineering, agricultural economics, and environmental science.

1. Serious differentials in humans, materials, and resources between rural and city areas

The imbalance in humans, materials, and resources between rural and city areas has become a serious problem. This imbalance works as a brake on a wholesome recycling rural environment. Rural areas have so far supplied manpower in the form of seasonal migrant laborers, material supply such as food and water, and fuel resource supply such as firewood and charcoal to big city areas. On the other hand, big city areas have re-distributed the funds obtained by industrial production and exports founded on the resource transferred in the form of subsidies and public works to rural areas. This social structure supporting postwar economic development in Japan has been unable to keep going because of the progressing depopulation and aging, non-growth of industries, and insufficiency of supporters in rural areas. Furthermore, it has resulted in further increase in the imbalance between rural and city areas with developing international free trading of agricultural products and oil dependence in society.

Many rural areas are troubled by depopulation and aging to be exhausted to the extent that they could not maintain even agrarian society culture including festivals, and that they have concern about marginal colonies in near future. Due to this exhaustion of rural areas, an increase in abandoned cultivated land and deterioration of forest management are caused, which markedly reduces the multiple functions in agricultural and rural areas. As a result, environmental deterioration including human and ecosystems in rural areas is taking place rapidly. On the other hand, in city areas, population has concentrated and environmental deterioration leading to global warming are

caused by the heat island effect. This global warming as well as lowering of the multiple functions carried out in rural areas has increased the danger of flood damage and declining water quality to bring about worsening of the local environment in city areas.

2. Vision for a wholesome and sustainable recycling rural environment

The postwar social structure mentioned above caused destruction of the intermediate and mountainous areas forming the principal part of the country structure in Japan. The destruction of secondary nature such as cultivated lands and forests in the intermediate and mountainous areas resulted in poorer circulation of water and materials and in the degradation of the ecosystem and biodiversity. These factors have caused a decline of the soundness of natural environment, and a degradation of the tolerance to a natural disaster. In order to solve such problems, considering the decrease in population, reduction of energy supply, and increase in the frequency of natural disasters, city areas need to be miniaturized, and rural areas need to develop agricultural industries for construction of the recycling environment. That is, conservation of sustainable agricultural land and construction of a recycling and natural symbiosis type environment are needed with collaboration of rural and city areas to maintain wholesome humans, materials and resources. This will serve as a driving force which produces a low carbon society with continuity. The basin area is essentially an organism balancing nature and a social structure surrounded by forest, river and ocean. Therefore, the local unit centering on the basin with a new symbiosis of nature and humans have various reasons for the existence bearing each role in the country formation, and this collateralizes the diversity and the toughness to withstand environmental changes.

3. Research for recycling strategy of human and material resources for sustainable rural environment

The direction that our country should take from now on has "a national commitment to the environment" which can respond flexibly to change of nature or social environment. That is, the appropriate population arrangement between city and rural areas is proposed as a fundamental direction by development of characteristic environmental industry with a high-valueadded knowledge base, development of efficient agricultural production systems, and improvement in the foodstuffs selfsufficiency ratio, etc. Thus, in order to manage the independent basin area with natural symbiosis, it is an appropriate strategy to reconstruct a society which is resilient to disaster caused by the local and global environmental changes with a wholesome water recycle and ecosystem preservation as well as sustainable water, energy and food supply in each basin unit.

To realize a symbiotic environment of all processes from agricultural production processes in rural areas to circulation and consumption processes in city areas, research projects are in progress to study scientific technologies and strategies with a global standard. The characteristic of this research is performed as research from a bird's-eye view of wholesome BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3 and sustainable circulation of humans, materials and resources. In this research project, the following scientific technologies and strategies are conducted (**Fig. 5**);



Fig. 5. Recycling strategy of humans, materials and resources for sustainable rural environment

- Planning of migration strategy from city regions to rural areas with a healthy and safe generous living environment;
- Planning of international agricultural strategy including domestic and Asia to attain a stable supply of safe food;
- Development of maintenance technologies for sound biomass energy resources, water resources, soil resources, and ecosystem resources by integrated management of forests, farmlands, irrigation reservoirs, etc.

Last year, the following outcomes in this research project were obtained;

- Engineering preservation technologies for wholesome water, soil and ecosystem resources by integrated management of forests, agricultural lands, and reservoirs;
- 2) Stable technologies for supplying safe food while considering sustainable environment;
- Evaluation and effective use of green resources mainly on the forest in a recycling society;
- Food production and distribution system in a recycling society;
- 5) Analysis of domestic food, agriculture, and rural promoting and activating policy.

III-1. Food Resources Education and Research Center

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The Food Resources Education and Research Center (hereinafter referred to as the Center) is a field-oriented institution subsidiary to the Graduate School of Agricultural Science (GSAS). The Center features real-scale agricultural production carried out as a base for educational and research activities. To assure our continuing food supply and accomplish sustainable agriculture, research projects are conducted primarily through genetic approaches such as variety improvement in plants and prediction of genetic merits in animals.

1. Mission

The mission of the Center is to conduct education and research on subjects ranging from development of food resources to actual food production (**Fig. 6**). In particular, activities to accomplish sustainable agriculture and continuing utilization of diverse biological resources are aimed to develop and expand the area of agrobiosciences for contributing to local and global communities.



Fig. 6. "From Farm to Fork (Table)", practiced in the Food Resources Education and Research Center

2. Location and environments

The Center is located in the central area of the Banshu Plains in Kasai City. It is approximately 60 km west of the Rokko-dai campus where GSAS is located. The Center has 40 ha of land areas including 28.3 ha of field areas for cultivation (paddy rice fields, orchard, grass and forage field, etc.). The yearly average temperature is 14.4°C (max. 33.4°C and min. -6.2°C), and the precipitation is 1287 mm.

3. History

The Experimental Farm was founded primarily as an attached educational facility in 1967 when the Faculty of Agriculture was founded in Kobe University by absorption of Hyogo University of Agriculture in 1966. In 2003, from the Experimental Farm as a base, the Food Resources Education and Research Center was established by reorganization and by addition of several concurrently working GSAS faculty members.

4. Organization

The Center consists of three core divisions: Division of Field Production, Division of Food Resources Development, and Division of Collaboration. Nine professors (five Center-based and four GSAS-based professors) are arranged into divisions. To the Division of Field Production, three professors are assigned, and also within this division, all 13 technical staff are organized into three agricultural production sections; Staple Crop Section (dealing with all annual crops including rice and vegetables), Fruit Tree Section and Livestock Section. The Division of Food Resources Development is subdivided into the Animal Resources and Plant Resources Sections to which five professors are assigned. One GSAS-based professor works to arrange and promote collaboration in the Division of Collaboration. The administrative office is operated by four staff including one part-time member as of April 2008.

5. Educational activities

The primary responsibility for undergraduates is to provide students with farming practices. The importance of practical training never decreases for students to understand all processes "from farm to table" because nowadays we can observe a wide gap between food production and consumption. Our aim is to provide an opportunity to capture the essence of agriculture and deepen the thoughts for agricultural science by experiencing real production scale agriculture. Five subjects are provided as follows:

Farming Practice I (2nd grade students of the Division of Plant Science, 2 credits)

Farming Practice II (3rd grade students of the Divisions of Plant Science and Food and Environmental Economics, 2 credits)

Farming Practice (3rd grade students of the Division of Agroenvironmental Biology, 2 credits)

Farming Practice (3rd grade students of the Divisions of Agricultural Engineering and Applied Chemistry in Bioscience, 1 credit)

Practice in Livestock Farm (3rd grade students of the Division of Animal Science, 2 credits)

Two research fields "Animal Genetic Resources" and "Plant Germplasm Enhancement" of GSAS are stationed in the Center, which provide opportunities for undergraduate and graduate students to earn Bachelor's, Master's and PhD degrees.

6. Research activities

Research outlines of the Center-based professors are summarized below.

1) Potato

The potato has a large genetic reservoir containing more than 200 wild species. DNA marker analysis revealed species relationships and disclosed the evolutionary pathway of cultivated potatoes. A specific chloroplast DNA type (T-type) was found in the common potato, and its maternal wild ancestor *Solanum tarijense* was discovered. A single dominant gene (*Sli*), which alters self-incompatible diploid potatoes to self-compatible ones, was identified. Using the function of this gene, diploid inbred lines were developed, which offer useful materials to investigate the mechanism of inbreeding depression and heterosis. Current interests are focused on the genetic factors controlling chromosome paring behavior and those regulating endosperm development in interspecific hybridization using naturally successful polyploid species such as *S. demissum* (6x), *S. stoloniferum* (4x) and *S. acaule* (4x).

2) Wagyu

The Wagyu stock consists of four Japanese domestic beef-cattle breeds. Among them, the Japanese Black cattle is a major breed and famous for its high ability to produce marbling in the beef. One of the aims is to genetically improve the economic traits of Wagyu especially Japanese Black through the evaluation of genetic merits. The examples of target traits are reproductive traits, feed utilization traits, carcass traits, etc. The procedures are mainly based on restricted maximum likelihood and best linear unbiased prediction. The aim includes both the development of new measure for evaluation and exploring effective ways to use the result of evaluation. In order to achieve more efficient Wagyu production, examinations of appropriate rearing, milking ability, and behavior during estrus are currently investigated.

3) Pear

'Iwate-yamanashi' (*Pyrus ussuriensis var. aromatica*) is one of the *Pyrus* species grown in the wild and is now endangered in Japan. The origin of the Japanese pear (*P. pyrifolia*) is uncertain but it has been suggested that 'Iwate-yamanashi' is the possible progenitor of the modern Japanese pear. Its population size has been decreasing and therefore, conservation is urgently needed. A set of 650 trees including 'Iwate-yamanashi' have been conserved as a wild core pear germplasm collection at the Center. Population genetics approaches using morphological traits and DNA markers (simple sequence repeat markers and chloroplast DNA markers) revealed a wide range of genetic diversity in *Pyrus* species. This diversity is caused, not only by high heterogeneity in 'Iwate-yamanashi' itself, but also by the BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3 coexistence of 'Iwate-yamanashi', *P. pyrifolia* and their hybrid progeny in the collected areas. Current interests are focused on the selection and evaluation of unutilized 'Iwate-yamanashi' and their derivatives, some of which produce fruits with desirable agronomical traits, e.g. certain aroma, acids and/or sugars.

4) Rice

Based on the genetic/phenotypic diversity among Asian rice (*Oryza sativa*) accessions/varieties cultivated in the world and their crossed populations, recent researches have been focused on, 1) identification of genes and quantitative trait loci (QTLs) for agronomic traits (e.g. heading date, plant height and biotic resistance), 2) identification of genes explaining phenotypic variation by genetic/QTL and linkage disequilibrium/ association analysis, 3) development and improvement of the methodology to detect genes/QTLs efficiently, 4) study of Asian rice by population genetics approach, and 5) development of a series of chromosomal segment substitution lines and near isogenic lines for valuable genes/QTLs by marker-assisted selection. As in the case in maize (*Zea mays*), results obtained by these research and rice plant materials developed are expected to contribute to future breeding of rice.

5) Genetic variability in domestic animals

Rapid reduction of genetic variability in the domestic animal populations has caused some undesirable phenomena such as inbreeding depression, reduced long-term genetic responses, and random fluctuation of selection responses. Genetic variability is also essential for adaptation to unexpected changes of economic and environmental conditions, such as change of consumer's preference and prevalence of a novel disease. Research interests are primarily in the investigations of genetic structure and genetic diversity of the livestock populations through pedigree analysis, and also in the theoretical developments of selection and mating schemes for the avoidance of inbreeding and the maintenance of genetic variability.

7. Agricultural production

Agricultural products as outcomes of education and research activities are commercialized through various channels and earn a yearly income of approximately 30 million yen. For example, approximately 40 tons of rice is sold, mostly to the university staff. The other products are sold at market or department stores, known as Kobe University's "Beef", "Potato", "Onion", "Pear", "Grape", etc. In particular, Kobe University Beef sold in Mitsukoshi, the most famous department store in Tokyo, enjoys a highest-ever price of over 10,000 yen per 100 g this year. Potatoes are produced using a new variety "Ran-ran Chip", bred and released in 2005 by National Agricultural Center for Hokkaido Region, NARO, and processed to chips and merchandized by Calbee Konan Co., Ltd. A new sakebrewing rice variety "Toji no Yume (dream of a sake-brewer)", bred and released from Hyogo Prefecture in 2004, was grown in the Center and processed by a local sake-brewing company Fukunishiki Co., Ltd. to high quality Japanese sake with a brand name of "Fragrance of Kobe".

8. Further information

More information about the Center and research details can be obtained from the website of the Center (http://www.edu. kobe-u.ac.jp/ans-foodres/) and from Center-based professors' websites linked from the Center website, respectively.

III-2. Research Center for Food Safety and Security (RCFSS)

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As voiced by Dr. F. Fischler of the European Commission responsible for Agriculture at the beginning of this century "We have to opt for an integrated approach towards agriculture and food: from stable to table, or from farm to fork. Modern agriculture has to be demand-oriented and meet the expectations of consumers and citizens alike", food safety has been identified as a key component in meeting the challenges of the ever changing agriculture environments and the agrifood market both in Japan and internationally.

Now, Japan is heavily dependent on imports for her food supply and there are growing public concerns about not only the safety aspect of foods and agricultural products available in the market but also their stable production. The public concern includes contaminations by infectious agents (i.e. unusual prions, avian influenza, SARS, EHEC O157, cholera), genetically modified elements (i.e. GM foods), and toxic chemicals (e.g. dioxins, pesticides, herbicides, antibiotics). We must recognize the importance of food safety and security and the need to work collaboratively with other agricultural institutes within and outside of Japan to meet these challenges.

Equally challenging is development of foods and dietary components that may provide a health benefit beyond basic nutrition since the recent consumers' interest in the relationship between diet and health has markedly increased the demand for better and safer products. As many of us are well aware, there are countless numbers of so-called "health foods & supplements", "probiotics", "prebiotics", and "synbiotics"



Fig. 7. The intra- and extra-cooperative system of Graduate School of Agricultural Science, Kobe University for food safety and security

available in markets worldwide. Some of them are reportedly to be beneficial to our health and others are alleged to be counter-beneficial, causing much confusion and skepticism in the public. Therefore, it is of paramount importance for us to have ways to establish a solid scientific basis to support and further validate claims for their functionality and safety. In this context, the RCFSS has been established at the Graduate School of Agricultural Science of Kobe University since April 2006 as the first on-campus research center for food safety and security ever established in Japan. The aim of RCFSS is to help develop not only new strategies and technologies for safe and stable production of foods and agricultural products but also more effective and safer functional foods and supplements. In order to accomplish this aim, RCFSS cooperates with other intra- and extra-disciplines (Fig. 7): 1) Food Safety Division puts emphasis on development of strategies and technologies to ensure "traceability" of foods and agricultural products as well as chemical and bio-hazards potentially contaminating them in the course of "from stable to table"; 2) The Food Security Division aims at development of strategies and technologies to ensure stable production or supply of safe agricultural products, with specific emphasis on controlling animal and plant diseases; and 3) Functional Foods Division aims at development of safe and effective functional foods and components to promote human health.

Various studies have been conducted and novel technologies developed by a group of researchers of different scientific disciplines that include biochemistry, animal and plant genetics, microbiology, veterinary medicine, plant pathology and agricultural engineering. Specific research topics currently being investigated at each division are as follows.

Food Safety Division

1. Traceability of various enteric disease causing bacteria that could contaminate foods and agricultural products

We aim at development of simple and rapid methods to detect pathogenic bacteria contaminating foods and agricultural products and DNA fingerprinting methods that facilitate early determination of the source or route of their contaminations. We have so far developed novel methods for detection of toxin producing *Vibrio parahaemolyticus* and enterohemorrhagic *Escherichia coli* (EHEC) O157 in foods, and are currently developing a novel selective agar media and novel DNA fingerprinting methods for toxigenic *V. cholerae* strains that are indistinguishable by conventional DNA fingerprinting methods, and a multiplex PCR method that can simultaneously detect EHEC O157 and EHEC O26.

2. Epidemiological study for *Staphylococcus aureus* in foods *S. aureus* is widely distributed in nature and is often involved in food poisoning cases as well as various infections in humans and animals. The epidemiology of the organism should be fully understood for control of the diseases. Consequently, we have carried out studies for many years on this aspect. Now we attempt the establishment of a domestic standard operating BIOTECHNOL. & BIOTECHNOL. EQ. 22/2008/3

procedure for isolation of *S. aureus* in foods in collaboration with several major institutes in Japan for the purpose of harmonization of domestic traditional methods and international methods such as those which appear in the documents of the International Organization for Standardization. The following studies are also conducted: epizootiological study for *S. aureus* in fish in commercial distribution; distribution of methicillinresistant staphylococci in foods in relation with the community acquired-MRSA.

3. Development of DNA markers for discrimination between domestic and imported beef

Recently, the problem of false sales has arisen: imported beef could be mislabeled as domestic beef, due to consumers' increasing concerns about the food safety of imported beef since the BSE outbreak. The aim of this project is to develop effective DNA markers to discriminate between imported and domestic beef for the reduction of incorrect labeling of food. We have developed novel DNA markers and system to discriminate between Japanese and Australian beef, and are currently developing novel DNA markers for discrimination between domestic and U.S.A. beef by using amplified fragment length polymorphism method and currently bovine 50K SNP chips. These markers would contribute to the prevention of falsified breed labeling of meat.

4. Development of risk-reduction technology for food safety in diverse systems of agriculture and livestock production

Hazards, such as pathogenic bacteria and antibacterial agents in foods, and livestock waste threaten food safety in various systems of agriculture and livestock production. The aim of this subdivision is to develop the technologies for instrumentation and control to reduce or eliminate the risks caused by those factors; The studies on a dielectrophoresis device to concentrate and capture microbes, an electrical impedance method for microorganism count, non-thermal sterilization by Pulsed Electric Field, removal and electrolyzation of antibacterial agent in livestock waste by the application of electrochemical and magnetic engineering have been conducted and the feasibility of these technologies is investigated in various systems.

5. Development of a simple biological method for measurement of dioxins

Environmental contaminants, dioxins are exposed to us mainly through our diet. An aim of this research is to develop a simple and rapid method for measurements of dioxins in the environment and foods instead of the complex instrumental analyses. We have developed a new simple method based on the South-Western chemistry-based ELISA (SW-ELISA) for measurement of transformation of an aryl hydrocarbon receptor, also known as a dioxin receptor. SW-ELISA is a highly sensitive method that is able to measure TCDD, which is the most toxic chemical in dioxins, and its related chemicals. Improvements of SW-ELISA with a higher sensitivity and more simple experimental steps with shorter time are in progress. Determination of PCBs and application of SW-ELISA to foods and environmental samples are important future plans.

6. Study of residual agrochemicals in foods and agricultural products by using instrumental analysis

We will try to develop a new analytical method using gas chromatography with mass spectrometry (GC-MS) and liquid chromatography with mass spectrometry (LC-MS) for the quantitative determination of residual agrochemicals, from foods and agricultural products. We have so far developed methods for detection of residual sulfonylurea herbicides in food plants and agricultural products with the GC-MS and/or LC-MS. The residual agrochemicals were extracted from food plants with a single step solid-liquid extraction by using the solvent extraction method. We are currently developing a new method to detect a small quantity of insecticide contaminating foods and agricultural products using GC-MS and LC-MS.

Food Security Division

1. Identification of genes for resistance to the wheat blast fungus, *Magnaporthe oryzae*

The wheat blast caused by *M. oryzae* is a serious problem in wheat production in South America. The development of resistant cultivars is an effective, economical, and environmentally friendly approach for controlling diseases. However, no genes are available so far for breeding of wheat cultivars resistant to the wheat blast. Our objective is to identify genes for resistance to the wheat blast fungus and to provide them to wheat breeders as materials for developing multilines. We have already identified two novel genes for resistance to the wheat blast fungus in the wheat cultivar 'Thatcher', and designated them as Rmg2 and Rmg3. We are now screening various wheat accessions for additional resistance genes.

2. Eradication of parasitic weed by induction of suicidal germination

Broomrapes (Orobanche spp.) and witchweeds (Striga spp.) are obligate root parasitic weeds that are the scourge of agriculture and food security in many parts of the world. One important aspect in the biology of these parasites that may provide options to develop control methods is their requirement for signaling molecules indicating the presence of suitable hosts. These molecules, germination stimulants, can induce germination of the seeds of these parasites in absence of or away from host roots, known as suicidal germination. We have demonstrated the importance of structural features including stereochemistry of the stimulants. Currently we are evaluating the activity of a new series of germination stimulants and their analogues for ability to induce suicidal germination of the parasites. Structure-activity relationship to each of the parasite spp. is to be clarified. Such information is important for management and/or eradication strategy based on suicidal germination.

3. Development of non-invasive technology for bio safety analysis, bio monitoring and diagnosis

We are working on various applications and new developments in the area of near infrared spectroscopy (NIRS) as a noninvasive technology for bio safety analysis, bio monitoring and diagnosis. For example, raw milk quality and composition is highly important for the dairy industry and consumers. Very often, milk from a single diseased cow spoils the quality of a large amount of milk, and bacteria that cause the disease is transferred to people. We have developed a noninvasive technology for raw cow's milk quality evaluation and milk composition measurement. In our Laboratory on Bio Measurement Technology, NIRS has been extensively used for mammary gland inflammation (mastitis) detection in dairy cows and for disease pathogen identification. Prion disease, HIV, and oxidative stress have been a target for *in-vivo* diagnosis and successful results have been reported.

Functional Foods Division

1. Development of *in vitro* IBD model for evaluating functional foods showing the anti-inflammatory activity

The intestinal epithelium is constantly exposed to antigenic and toxic products in the gut lumen. Recently cases of inflammatory bowl disease (IBD) increased in young people and one of its etiology is due to immune disorder. We aim at developments of *in vitro* IBD model to screen the food components that can inactivate the immune disorder. In this *in vitro* IBD model, the macrophage is stimulated with lipopolysaccharide, and then IL-8 mRNA level in Caco-2 is measured by RT-PCR to estimate the anti-inflammatory activity. When IL-8 mRNA level is decreased by a test compound, it indicates that this compound has the anti-inflammatory activity. Indeed, budesonide, which is used as its therapeutic agent, shows its activity. Now we are screening functional food having the anti-inflammatory activity using this system.

2. Discovery and application of functional inositols

Epimerization of the six hydroxyl-groups in inositol (1,2,3,4,5,6-cyclohexanehexol) results in the formation of nine stereoisomers, including D-*chiro*-inositol (DCI) and *scyllo*-inisotol (SI). DCI and its 3-*O*-methyl form (pinitol), that possess an insulin-mimetic activity and are useful for the treatment of type 2 diabetes and other related diseases. On the other hand, SI is a sort of chemical chaperone and reported to be effective in treating Alzheimer's disease. We aim to understand how these functional inositols exert the activities, and mechanisms underlying their function are investigated in cellular and molecular biological ways. In addition, in order to apply these inositols for functional food production, we develop possible strategies to produce them efficiently from plant resources processed by genetically manipulated microorganisms.

3. Development of functional foods and evaluation of their safety

There is considerable information available on the beneficial effects of functional foods and food factors, but little information is available on the pharmacokinetics and toxicity of these products. For example, we have recently reported on the anti-carcinogenic activity of fucoxanthin, which is one of the carotenoids in brown sea algae such as *Laminaria japonica* and *Undaria pinnatifida*, but there is little information regarding the pharmacokinetics and toxicity of fucoxanthin will be examined, and the bioavailability of fucoxanthin and its metabolites in humans will be determined by collaborative research with the Faculty of Medicine, Kobe University. Thus, our mission includes the evaluation of both the functional potency and the potential toxicity of functional foods or factors, and the distribution of our findings.