

# National Institute of Agrobiological Sciences



http://www.nias.affrc.go.jp/index\_e.html

## Message from the President



Hirohiko Hirochika President

The world is currently facing tremendous global challenges arising from climate change and rapid population growth. Eventually, it is expected that problems associated with food supply and demand will become more and more critical in the next decades. In order to address this issue and guarantee food security in the very near future, it is necessary to establish innovative technologies that will facilitate the development of functional organisms, maximize their inherent potential, and create new industries, while at the same time, enhancing the competitiveness of Japanese agriculture.

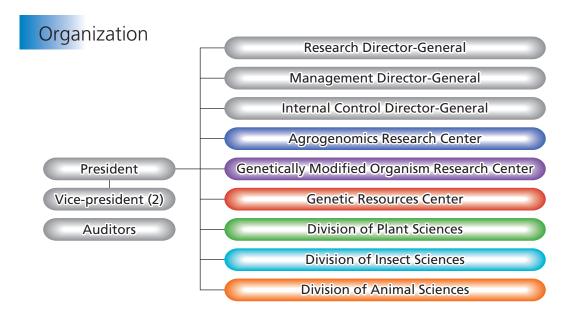
Under this scenario, the National Institute of Agrobiological Sciences is pursuing fundamental and advanced researches focusing on the biotechnology of plants, insects, and animals of agricultural importance. During the First Five-year Research Program (2001-2005), we have successfully elucidated the high-quality sequence of the entire rice genome, developed efficient genetic recombination techniques for silkworm, and produced transgenic cloned pigs. With these major achievements, we embarked on the Second Five-year Research Program (2006-2010) focusing on more efficient utilization of the rice genome sequence in crop improvement, particularly in identification of agronomically important genes and characterization of gene functions. This led to the isolation of a novel type of gene that promises resistance to blast disease and the development of a strategy for incorporating this gene in rice without degrading grain taste. In addition, we have also succeeded in deciphering the draft sequence of silkworm and pig genomes. Although it has been merely 2 years since the Third Five-year Research Program was initiated, our ongoing research platforms will further strengthen and accelerate studies in life science with implications in agriculture. In particular, we are currently pursuing the utilization of genetic resources and genome information towards the development of next-generation genome-breeding methods, sequencing the genomes of wheat and a model insect pest, and development of useful technologies to stimulate agricultural innovations. Moreover, application of the genome-breeding technology is being promoted in cooperation with other breeding institutions. We are also endorsing the utilization of gene recombination technology such as the development of rice-based vaccine against cedar pollen allergy, human-animal medicine derived from transgenic silkworm, and immune-deficient pig in collaboration with academic institutions and private enterprises to further stimulate the creation of new industries.

As a core research organization of biotechnology in agriculture, and in collaboration with the academic and industrial sectors, we are committed in pursuing large-scale research initiatives that will lead into major breakthroughs in agricultural productivity and will make significant contribution to the society at large. Towards achieving this goal, we wish to implore everyone for your understanding, full support, and continuous cooperation.

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The National Institute of Agrobiological Sciences (NIAS) is the largest basic life science research institute in the field of agriculture in Japan. Through the general understanding of biological phenomena of various agricultural organisms, NIAS plans to develop functional organisms and ways to use them in our society.

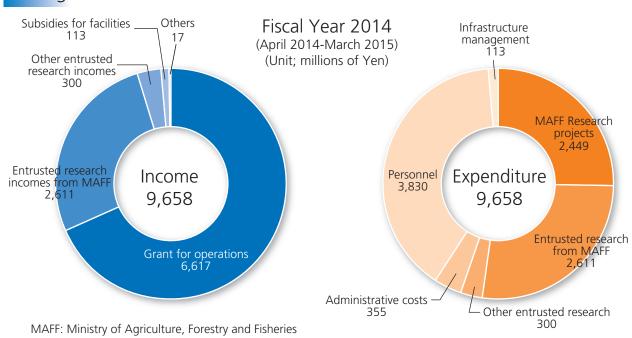


Staff (As of April 1, 2014)

Permanent staff : 348 Fixed-term staff : 483 (Researchers) : 234 (Senior researcher : 1)

(Postdoc fellow : 37)

### **Budget**



## **Agrogenomics Research Center**

### Genetic and molecular analysis of genes with agricultural and economical values by genomics approaches and development of novel breeding technologies

Genetic and molecular analyses of genes involving traits with agricultural and economical value, such as disease resistance and yield performance, are performed in plants, animals and insects. New selection methods in breeding are developed in rice, soybean and animals. To develop new biological and chemical controls of insects, genome analyses are undertaken of insect pests, such as planthoppers and moths. We develop novel methods for gene targeting, targeted mutagenesis and epigenomics research that lead to new breeding methods. Analysis of structure and functional modulation of proteins with agronomical and economical value are also performed.

#### **Research Units**

#### Advanced Genomics Laboratory

Sequencing and genetic mapping of plants, animals and insects to promote genomics research.

#### ■ Bioinformatics Research Unit

Analysis of large amounts of genome information and development of databases.

#### ■ Genome Resource Unit

Development, conservation and distribution of various resources and information derived from genomics research.

#### ■ Plant Genome Research Unit

Genome sequencing and expression profiling of crops and analysis of agriculturally important genes of rice, wheat, and sorghum.

#### ■ Insect Genome Research Unit

Genome analysis of insect pests and development of their genome databases.

#### ■ Animal Genome Research Unit

Genetic and molecular analysis of genes controlling traits with economical value in domestic animals and development of their genome databases.

#### ■ Rice Applied Genomics Research Unit

Genetic and molecular analysis of flowering time, disease resistance, yield potential and other traits in rice and development of new selection methods for breeding.

#### ■ Soybean Applied Genomics Research Unit

Genetic and molecular analysis of flowering time, disease resistance, yield potential and other traits in soybean and development of new selection methods for breeding.

#### ■ Plant Genome Engineering Research Unit

Development of novel methods for gene targeting, targeted mutagenesis and epigenomics research.

#### ■ Biomolecular Research Unit

Analysis of structure and functional modulation of proteins with agricultural and economical value.

### **Advanced Genomics Laboratory**

Supporting genome analysis of agriculturally important organisms such as rice, wheat, soybean, pig and insects

Location: Headquarters Area (Tsukuba)

Recent advances in genome analysis instrumentation, such as next-generation sequencers, have led to surprisingly fast genome DNA decoding speeds. We have isolated and analyzed in detail many genes that have an important role in agriculture.

In the current research phase, the advanced genomics laboratory continues to use the newest instrumentation for genome analyses of rice, wheat, soybean, pig, and insects. We support genome analysis based on requests from within NIAS and from outside NIAS. We have accelerated the processes for preparation of genomic BAC libraries, genome decoding of new organisms, gene isolation, and development of new plant varieties by the use of precision DNA markers. These processes facilitate genome breeding of new varieties of various crops and it is expected that development time will be reduced.



Fig. 1. Short read type sequencer (1 machine)
We obtain about 40 giga (=40 billion) bases each run.



Fig. 2. Long read type sequencer (2 machines)
Each machine produces 500 mega (=500 million) bases each run.



Fig. 3. Sanger sequencer (5 machines)
We use these machines to determine valuable sequences for gene isolation and to detect the SNPs (Single Nucleotide Polymorphism) through DNA fragment analysis.

#### **Bioinformatics Research Unit**

#### Construction and management of integrated genome databases

Location: Headquarters Area (Tsukuba)

With rapid advancement of life science technologies, enormous amounts of data have been produced. For example, today's DNA sequencers can decode billions of nucleotides within one week. In addition, further computational analyses of raw sequences generate significantly larger data sets that are difficult for laboratory workers to manage. The Bioinformatics Research Unit aims to lay the foundation for an efficient computer system that processes a large amount of biological information so that laboratory workers will analyze their own data with ease and comfort. So far, we have used large-scale computers for bioinformatics data processing to precisely examine the rice genome of 380 million bases. We have predicted regions of biological significance, such as protein-coding genes, which will be useful for future breeding and development of new agricultural products. We also construct databases of all the outcomes from our system. The databases are freely available for any researchers to use through the Internet.



Fig. 1. A considerable amount of data is processed by large-scale computers.

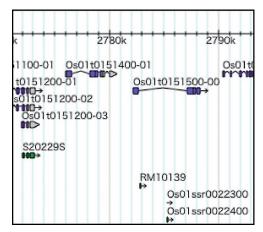


Fig. 2. Positions and functions of genes are predicted for genomic DNA. The data can be retrieved through our databases.



Fig. 3. We provide annotation databases of rice and other related cereals. Gene prediction services are also available.

#### **Genome Resource Unit**

#### Development and management of genomics resources

Location: Headquarters Area (Tsukuba)

Biological resources are indispensible components for promoting and accelerating basic research in life sciences. Among the many genomes being investigated worldwide, the Ministry of Agriculture, Forestry, and Fisheries (MAFF) is focusing on genome analysis of agriculturally important crops, insects and livestock, thereby generating resources that contribute to the advancement of a wide range of research aimed at improving agricultural productivity. The Genome Resource Unit is undertaking initiatives for collection, preservation, and access to biological materials and information derived from these projects. New genomics resources are also being developed to facilitate functional characterization of all annotated genes in rice. In addition, a support system for gene expression profiling by microarray analysis has been established to serve the scientific community.

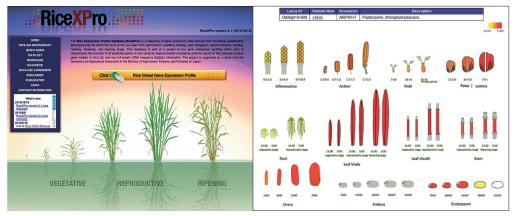


Fig. 1. RiceXPro: Rice Expression Profile Database

A comprehensive database of the gene expression profile of all the genes in rice encompassing the entire growth cycle from germination to seed maturation can be used as a tool to clarify where, when, and how each and every gene of rice is expressed. Data derived from microarray analysis of rice tissues and organs at various stages of development from transplanting to harvesting are used for construction of the database which is now open to the public. (http://riceexpro. dna.affrc.go.jp/)



Fig. 2. Access to genomics resources

Biological materials derived from MAFF-supported projects are maintained to high level of quality and reliability, and are shared to the research community. (http://www.rgrc.dna.affrc.go.jp/index.html)

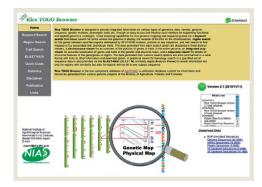


Fig. 3. AgriTOGO Database

Integration of information from various databases constructed as part of MAFF-supported projects into a unified platform to facilitate efficient utilization of genomics data. (http://agri-trait.dna.affrc.go.jp/)

#### Plant Genome Research Unit

#### Analysis of agriculturally important genes of rice, wheat, and sorghum

Location: Headquarters Area and NIAES Area (Tsukuba)

The Plant Genome Research Unit is discovering agriculturally important genes and investigating their functions in gramineaeous plants such as rice, wheat, and sorghum. By simultaneously monitoring the expression of thousands of genes in rice, systematic responses to several biotic/abiotic stresses such as high-salt, virus infection, and poor nutrition, are investigated. For the Triticeae crops, sequencing of part of the wheat genome has been completed as a member of an international project. Also, basic investigations of genes for flowering and for determining row shape of barley are being performed for the breeding of future innovative varieties. Sorghum, which had long been considered forage in Japan, is now being evaluated as a resource for biomass and conversion to bioethanol. We are studying a gene for resistance against a devastating disease of sorghum that severely damages biomass production.



Fig. 1. Analysis of agriculturally important genes of gramineaeous plants is performed.

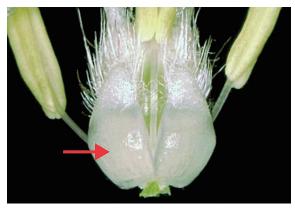


Fig. 2. Mechanism of barley flower opening Red arrow indicates swelling.



Fig. 3. Disease-infected sorghum

These plants will not yield high biomass. A disease resistant variety of sorghum is required for breeding of high biomass plants.

#### Insect Genome Research Unit

## Genome sequencing of crop pests and development of integrated databases for broad agricultural pests

Location: Owashi Area (Tsukuba)

Agricultural pest control technology is one of the most important keys for the stable supply of agricultural products. In Insect Genome Research Unit, we are targeting research that will lead to environmentally friendly and efficient insect pest control methods. For this purpose, we are conducting the following research. Using silkworm as the model organism for other Lepidopteran agricultural pests, we sequenced the silkworm genome and acquired gene expression information. The accumulated data are stored in the integrated silkworm genome database, KAIKObase, which is available world-wide. Based on the silkworm genome information, we are investigating the genes in diamondback moth, the major cruciferous vegetable pest to control pests in the Brassica family. We are also analyzing the genome of the brown planthopper, one of the major rice pests, and investigating the genes related to pesticide resistance which will contribute to development of new pesticides that show low or minimum resistance.

We will construct a crop pest database that accumulates a great deal of crop pest genome information and has a user friendly interface. Using the system, one can access integrated insect genome data sets with high-speed processing.



Brown planthopper, the major rice pest over the world



Diamondback moth, the major brassica vegetable pest (left) adult, (right) larva

Fig. 1. Examples of the insects used in our study



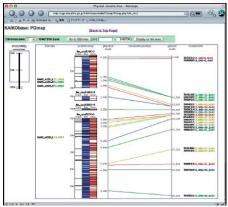


Fig. 2. Integrated silkworm database, KAIKObase

Genome sequence, predicted genes and map information can be obtained. Using keywords and BLAST search, one can access a specific interest area. (http://sgp.dna.affrc.go.jp/KAIKObase/) (left) Top screen, (right) Physical map and genetic map of a chromosome

#### **Animal Genome Research Unit**

## Construction of animal genome database and gene analysis for animal improvement

Location: Ikenodai Area and Owashi Area (Tsukuba)

In Animal Genome Research Unit, we are developing the genomic resources of livestock animals and providing information to contribute high-quality, safe and secure livestock products. We have decoded more than 10,000 expression genes (full length cDNAs) and cooperated in the International Swine Genome Sequencing Consortium. We also developed the methods for discrimination of breeds or individuals by using DNA polymorphisms, and identified the genes responsible for the number of vertebrae and for meat color in pigs. Now, we are increasing the accumulation of the genomic information in livestock animals such as pigs. We are also developing methods for detecting the association between gene polymorphisms and phenotype. With the effective utilization of genomic information, we are aiming to identify the genes responsible for meat quality, growth rate, disease resistance, reproductive performance, and other traits. We can use genomic information to develop gene markers that support rapid breed improvement for meat quality, productivity, and disease resistance. Management of production with the gene markers enables us to supply the variety of products corresponding to various consumers' needs.



Fig. 1. From the genome project to determination of gene polymorphism

Mammalian cells contain chromosomal (genome) DNA in which approximately 25,000 genes are located. Gene markers are developed by decoding the genome sequence and elucidating the polymorphisms affecting individual differences.



Fig. 2. Database of expression genes in pigs (PEDE: Pig Expression Data Explorer)

Information from the analysis of full-length cDNA libraries is located in PEDE. Sequence information and cDNA clones are distributed. (http://pede.dna.affrc.go.jp/)

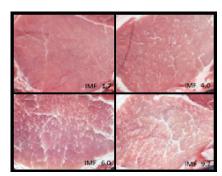


Fig. 3. Pork loins with various levels of intramuscular fat content

Gene markers will allow the effective production of pork traits to meet a variety of consumers' needs such as low fat and healthy pork, or fat-marbled and rich-tasting pork.

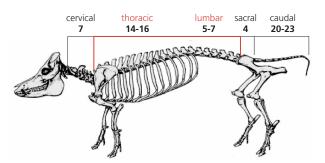


Fig. 4. The gene affecting the number of vertebrae in pigs The number of vertebrae (thoracic and lumbar vertebrae) varies and is associated with productivity, such as meat amount. We identified two genes (*NR6A1* and *VRTN*) responsible for variation in the number of vertebrae.

#### Rice Applied Genomics Research Unit

Genetic and genomic characterization of rice genes controlling useful agronomic traits and development of a novel rice breeding strategy

Location: Headquarters Area and NIAES Area (Tsukuba)

Rice shows numerous morphological and physiological differences that are observed as phenotypic variations in rice grown around the world. Some variations have been used as genetic resources to improve rice plants to satisfy human needs. Phenotypic variations are considered to be genetically controlled by the collective functions of a large number of genes in the rice genome. However, the genetic basis and biological functions of most of these genes are still unknown, which limits the wider practical use of rice germplasm variability.

The Rice Applied Genomics Research Unit exploits useful phenotypic variations from different germplasm and, with the aid of recent advances in genome information technology, elucidates the relationships between the genes involved and their biological functions. Also, the Unit tries to develop new breeding materials and to propose more effective breeding methodology. Through these research activities, the Unit aims to establish a continuous pathway from genome information of rice germplasm to actual breeding.



Fig.1. "Tomohonami", a new blast-resistant cultivar with high eating quality

Tomohonami, carrying the resistant *pi21* allele, has slight damage that supports normal panicle growth, while the leading cultivar Koshihikari has no panicles due to severe infection by blast disease.



Fig. 2. Genetic recombination experiments for clarification of rice genes potentially involved in natural phenotypic variation

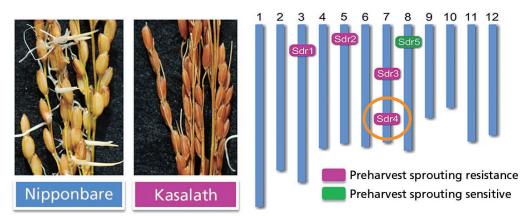


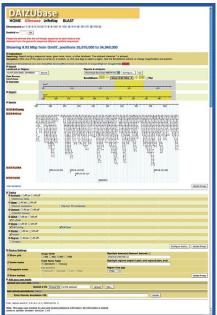
Fig. 3. We have found several genetic loci related to preharvest sprouting (PHS) resistance. Mapbased cloning of one gene, *Sdr4*, revealed that it was a key regulator of a gene network controlling seed germination and preventing PHS.

### Soybean Applied Genomics Research Unit

## Identification of important soybean genes and development of new strategies for effective breeding

Location: Headquarters Area (Tsukuba)

Soybean is one of the most important grain crops as a staple source of nutritious vegetable protein and oil for humans and livestock. Additionally, it provides industrial materials and biofuel. In Japan, soybean is an important source of traditional foods such as tofu, natto, miso and soy sauce. We have developed new strategies for effective soybean breeding through 1) analysis of the genome structure of Japanese (domestic) soybean, 2) development of DNA markers serving as landmarks of agronomically important traits, 3) development of research resources for the identification of important genes in soybean, and 4) isolation of genes controlling traits of importance such as disease and pest resistance, productivity, and regional adaptability through maturity control.



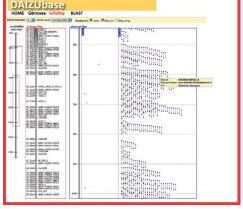


Fig. 1. Soybean genome database

DAIZUbase (http://daizu.dna.affrc.go.jp/) is a comprehensive and integrated database for a Japanese soybean cultivar 'Enrei'. DaizuBase includes "Unified map", which indicates the relationship between the linkage map and the physical map, and "Gbrowse", which exhibits aligned 'Enrei' BAC clones on the genome assembly of a U.S. soybean cultivar 'Williams 82', and facilitates the comparative genome analysis.

Fig. 2. Soybean lines with different flowering time Flowering time is one of the most important characters relating to the seed productivity of soybean through the adaptation to different cultivation areas and growing seasons. In addition, the growth habit such as plant shape is largely affected by the genes controlling flowering time.



### Plant Genome Engineering Research Unit

#### Towards precision engineering of the plant genome

Location: Headquarters Area (Tsukuba)

In conventional mutation breeding technology using chemical mutagens and ionizing radiation, it remains quite difficult to select mutants harboring target genes modified exactly as required, because mutations occur randomly. Site-directed mutagenesis via gene targeting (GT) is the ultimate mutation breeding technology. However, GT efficiency is still low due to lower frequency of homologous recombination in higher plants. We are trying to establish an efficient and reproducible GT system in higher plants based on the understanding of basic mechanisms controlling homologous recombination and non-homologous end joining in higher plants.

The development of technologies to enhance/repress expression of genes of interest is important for research on gene function. For this purpose, we seek to develop technologies to efficiently introduce and express genes of interest efficiently in higher plant cells. Furthermore, we discover basic mechanisms of genome shuffling for efficient production of unprecedented crop lines with favorable characteristics.

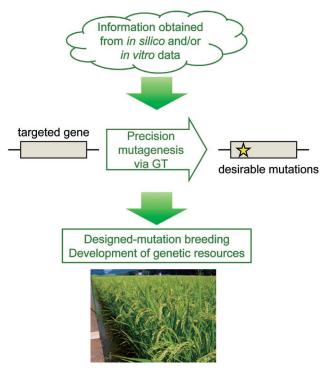


Fig.1. Schematic representation of designed-mutation breeding in plants via homologous recombination-mediated gene targeting (GT)

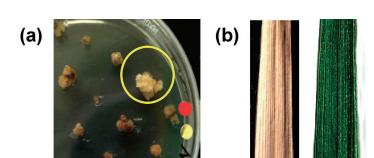


Fig. 2. Production of herbicide tolerant rice via gene targeting

- (a) Herbicide tolerant callus emerged after GT.
- (b) Herbicide tolerant GT rice (right) and sensitive original rice (left). Introduction of the two point mutations confers herbicide tolerant phenotype in rice.

#### Biomolecular Research Unit

#### Structure-based drug design and protein modification

Location: Headquarters Area (Tsukuba)

Organisms perform life activities on the basis of sophisticated biomolecular systems composed of proteins, nucleic acids, sugars, lipids and other molecules. These biomolecules exert their functions through interactions with other biomolecules, which are modulated primarily by three-dimensional structures of the interacting molecules. Hence, understanding of biomolecular functions and life systems requires a detailed knowledge of molecular structures and interaction mechanisms. At the Biomolecular Research Unit, we analyze structures and functions of biomolecules involved in important life phenomena related to agriculture and thereby discover substances and strategies that can control functional mechanisms of these biomolecules. We also perform proteome analysis to understand the temporal and spatial behaviors of biomolecular systems.

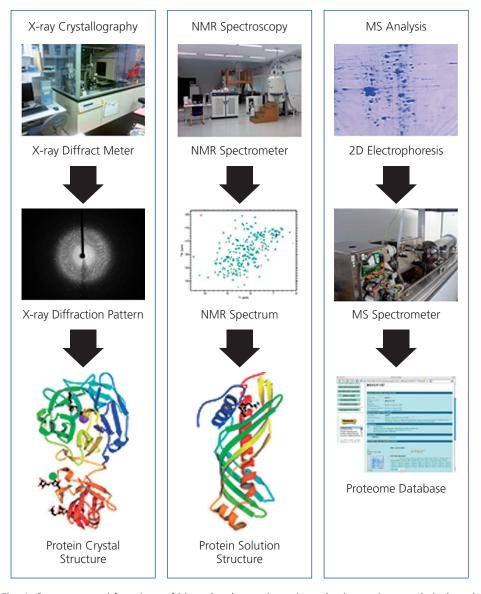


Fig. 1. Structures and functions of biomolecules are investigated using various analytical methods.

## **Genetically Modified Organism Research Center**

## Research and development for creating new industries and new demands using genetic engineering technologies

As an approach to obtain maximum benefits from agricultural organisms, genetic engineering technologies hold great potential. Our center is doing research and development to create new industries and new demands in the field of agricultural and medicinal industries by applying genetic engineering technologies to plants (rice), insects (silkworm and others) and animals (swine). In plants, for example, we are developing new rice varieties resistant to major diseases including blast. Moreover, we will include non-clinical and clinical research trials of rice-based edible vaccines for curing cedar pollinosis. In our research on insects, we are developing medicinal materials from silk protein and medicines for humans and animals using transgenic silkworm. Furthermore, in our animal research, we are developing transgenic pigs to produce immune-deficit pigs and animal models for human diseases.

#### **Research Units**

■ Research Promotion Section for Genetically Modified Organisms

Promotion of research for development of genetically modified organisms by following various relevant laws.

- Functional Transgenic Crops Research Unit Development of new functional crops to produce high levels of useful materials such as functional components and medicines for human health.
- Disease Resistant Crops Research Unit Clarification of the molecular level mechanisms of

disease resistance and development of new varieties resistant to various rice diseases by genetic engineering technology.

■ Transgenic Silkworm Research Unit

Implementation of a wide range of research and development from analysis of gene functions to the production of useful materials using transgenic silkworm.

■ Transgenic Pig Research Unit

Research and development to produce and utilize transgenic pigs with immune deficiency and animal models for human diseases.

■ Silk Materials Research Unit

Research and development to produce silk protein materials, to develop utilization technologies, and to improve silk materials using transgenic silkworm technology and chemical modification.

■ Insect Mimetics Research Unit

Clarification of molecular mechanisms of insect genes and proteins related to specific insect functions and their utilization to improve human life.

## Research Promotion Section for Genetically Modified Organisms

## Promotion of the research and development of genetically modified organisms by keeping up to date with various laws

Location: Headquarters Area (Tsukuba)

The GMO research promotion office is in-charge of the following tasks:

- 1. Acceleration of development of novel genetically modified crops, silkworm, and livestock according to several domestic laws, such as Cartagena law, food sanitary law and Pharmaceutical Affaires Act.
- 2. Research for efficient science communication methods with the public.
- 3. Accumulation of information related to biosafety and providing the information to the public.

### **Functional Transgenic Crops Research Unit**

## Development of transgenic crops including health promoting ingredients or pharmaceuticals

Location: NIAES Area (Tsukuba)

Targets of our unit are to generate health promoting functional transgenic crops by introducing functional peptides or proteins having functions such as anti-hypertensive or hypocholesterolemic activities, and to establish a production system for high-value products such as pharmaceuticals using transgenic crops as bioreactors. We have developed a rice-based allergy vaccine against Japanese cedar pollen allergy by introducing its modified Cry j 1 and Cry j 2 antigens with reduced IgE reactivity. As one step to put this rice-based allergy vaccine to practical use, a clinical trial will be conducted using the pollinosis patients according to rules of GCP to examine safety and efficacy of rice-based vaccines.

To establish a system to enhance accumulation levels of recombinant products in transgenic rice seed, we are elucidating the network system of transcription factors that regulate seed protein genes. We also are developing a targeting system (site directed integration) via homologous recombination at the highly transcriptionally active site of the desired target gene. Furthermore, we will solve the problems of gene silencing and ER stress that sometimes occur when recombinant proteins are highly expressed.



Fig. 1. GM rice producing allergy vaccine against cedar pollen allergy

- (a) Cultivation of GM rice in closed field
- (b) Practical scheme

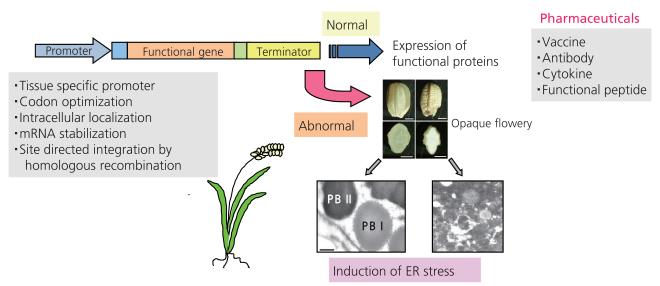


Fig. 2. Strategy for high-level accumulation of valuable products in plant

#### Disease Resistant Crops Research Unit

## Elucidation of disease resistance mechanisms in plants and development of transgenic disease resistant crops

Location: Headquarters Area (Tsukuba)

Infectious diseases due to various fungal and bacterial pathogens can severely damage crop production, and the controls of those pathogens have been costly. Plants have innate defense mechanisms to resist pathogen infection. However, plants become diseased when such defense mechanisms fail to function properly or when pathogens have developed infectionus strategies to overcome the defense mechanisms. With rice as a model system, we are investigating plants' responses to the infection of agronomically important pathogens, such as *Magnaporthe oryzae*, which causes rice blast disease, and the plants' mechanisms to resist the diseases at the molecular levels. We have been searching for important genes that play roles in the defense mechanisms and that can enhance plant resistance to the diseases. By using these genes and information, we will develop transgenic crops that are resistant to multiple diseases.

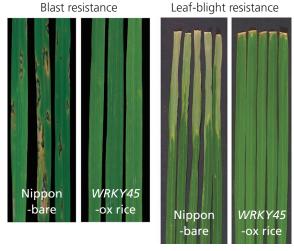


Fig. 1. Rice plants overexpressing *WRKY45* are resistant to blast (left) and leaf-blight (right) diseases.

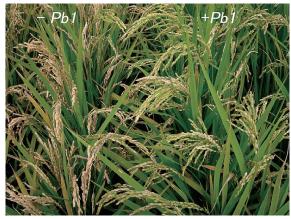


Fig. 2. Rice gene *Pb1* confers panicle-blast resistance to rice

Rice cultivars with (+Pb1) and without (-Pb1)

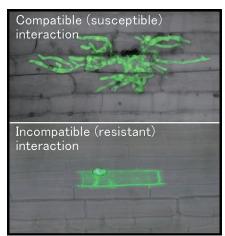


Fig. 3. GFP-expressing blast fungi invading leaf sheath cells of rice

### Transgenic Silkworm Research Unit

## Development of transgenic silkworm for analysis of gene function and productions of recombinant proteins/silks

Location: Owashi Area (Tsukuba)

We developed a system for stable germline transformation in the silkworm *Bombyx mori* in 2000. Since then, we have been developing transgenic silkworms for fundamental research and applications. In 2008, we succeeded in producing recombinant silks with new functions in collaboration with companies, universities and public research institutes.

Our aims are to promote fundamental research and application by identifying gene functions, by producing useful recombinant proteins for tests as drugs and medicines, by developing a human disease model for drug discovery, and by producing new high-performance silks.

Our near term research goal is to contribute to the creation of new industries by achieving practical uses for recombinant proteins and silks using transgenic silkworms.



Fig. 1. An adult of transgenic silkworm expressing fluorescent proteins

Green fluorescent protein is expressed in wings and other body parts. Red fluorescent protein is expressed in eyes.



Fig. 2. Green fluorescent protein expressed, extracted and purified from transgenic silkworm.

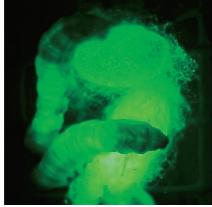


Fig. 3. Transgenic silkworms producing recombinant silk with green fluorescent protein



Fig. 4. Trial product to study the properties of fluorescent silks with green and red fluorescent proteins. (in collaboration with Yumi Katsura International Co., ltd.)

## Transgenic Pig Research Unit

## Development of transgenic pig by advanced gene manipulation for practical use

Ikenodai Area and Owashi Area (Tsukuba)

Swine are markedly similar to humans regarding anatomy, physiology and genetics and offer substantial advantages as a human model. It is very important to establish highly beneficial human models, such as a disease model as a primary objective, to increase the utilization of pigs in biomedical research. The final goal of our unit is to develop advanced technology for production of transgenic pigs via genetic engineering combined with cloning for practical applications. Severe combined immunodeficiency (SCID) pigs for human cell transplantation, human lifestyle-related disease models, and human cancer models are our objectives.

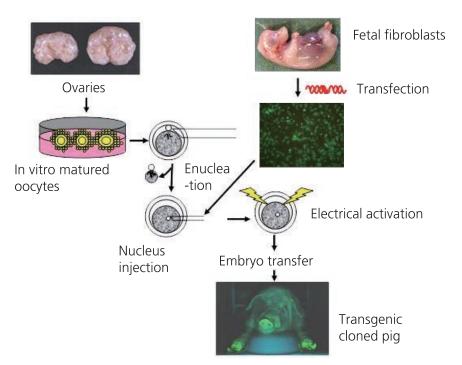


Fig. 1. Production of transgenic pigs by nuclear transfer

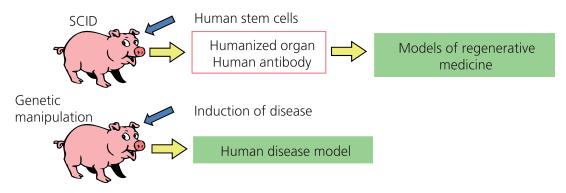


Fig. 2. Transgenic pigs in biomedical research

#### Silk Materials Research Unit

### Development of technologies for utilization of silk proteins and for modification by transgenic techniques

Location: Owashi Area (Tsukuba)

The Silk Materials Research Unit is studying the development of new materials based on silk proteins as a natural resource in order to commercially use the silk materials in medical and cosmetic fields in addition to textile fibers. We focus on fibroin and sericin produced by silkworm, and hornet silk from Vespa, as the silk proteins. Several novel fabrication processes have been developed to form a variety of materials with different characteristics, such as a film, gel, sponge, gel-film, tube, and resin, from the silk proteins. To improve the properties and functions of the silk materials we are analyzing the structure and properties of the silk proteins and are investigating modification technologies using chemical and transgenic techniques.

At the present time, we are proceeding to develop: a scaffold for cartilage regeneration using fibroin sponges; materials for cornea regeneration and wound dressing using gel-films from sericin and hornet silk; hepatocyte culture substrates using lactose-binding fibroin; a high strength and modulus silk fiber using transgenic fibroin containing spider silk protein; an affinity silk using a single-chain antibody-fused fibroin; artificial silk proteins by introduction of non-natural amino acids; and other products.

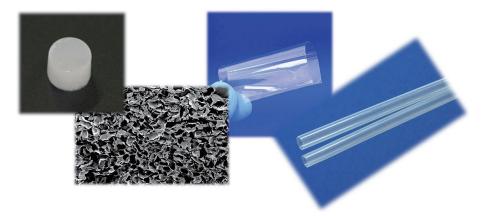


Fig. 1. Sponge, film, and tube from 100% silk proteins

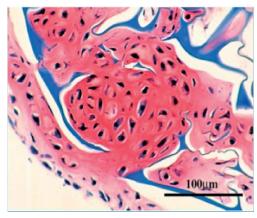


Fig. 2. Regenerated cartilage in fibroin sponge (Collaborated with Kyoto University)





Fig. 3. Anticoagulant effect of sulfated fibroin

### **Insect Mimetics Research Unit**

#### Analysis of genes and proteins involved in specific insect functions

Location: Owashi Area (Tsukuba)

During the 400 million years of their evolution, insects have acquired a broad variety of specific biological functions that allowed them to colonize almost all types of environments with remarkable adaptation. Insects now constitute the most flourishing group of creatures on earth. The characterization of specific molecular mechanisms discovered in insects and the development of technologies mimicking such mechanisms recently became a focus of attention, because these technologies potentially generate new fields in bioindustry.

For example, we characterized new enzymes used by termites to digest wood, which degrade cellulose very efficiently. In silkworms and beetle larvae, we also discovered unique antimicrobial peptides that protect those insects against bacterial or viral infection.

Larvae of the sleeping Chironomid can survive complete desiccation. Revival of dry larvae upon rehydration was even observed after a long-term exposure to the outer space environment on the international space station. We expect that characterization and utilization of the molecular mechanisms underlying such specific insect functions will lead to evaluation of these new bioresources for industrial purposes.

The Insect Mimetics Research Unit is devoted to the characterization of genes and proteins involved in specific insect functions, and to the mass production of such useful proteins via recombinant DNA technology for their introduction into the market of new biotechnologies.

## New enzymes from termites



## New drugs from insects



## Application of anhydrobiosis

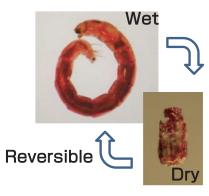


Fig. 1. Our research is devoted to the analysis of specific genes and proteins from insect, and to the mass production of such useful proteins.

#### **Genetic Resources Center**

## Implements NIAS Genebank Project for conserving, managing and distributing plant, microorganism and animal genetic resources

Human being have selected useful organisms from biological diversity and have bred better varieties through the long history of agriculture. At present, the importance of diverse genetic resources as materials for research and development has become even more evident with advances in science and technology. The Genetic Resources Center established the NIAS Genebank Project to collect and introduce valuable genetic resources from domestic and overseas sources, to maintain passport and evaluation data, and to provide them widely for research and development for food and agriculture. The Project also carries out diversity studies and develops preservation technology to conserve genetic resources, and creates new genetic and breeding materials using irradiation mutagenesis and other methods. To date, the Project's conserved genetic resources include about 215,000 plant accessions, about 28,000 microorganism accessions, and about 1,000 animal accessions.

#### **Research Units**

■ International Relations Section for Genetic Resources

Keeps up with changing international agreements and coordinates with overseas research institutes to promote conservation of genetic resources and their utilization.

- Genebank Project Promotion Section
- Receiving, preserving, and distributing genetic resources and their information as the secretariat of NIAS Genebank Project.
- Biodiversity Research Unit

Analysis of genetic diversity accumulated through evolution and domestication processes as the plant research section of the NIAS Genebank Project.

- Classification and Evaluation Research Unit Classification, identification, characterization, evaluation and field survey as the microorganism research section of the NIAS Genebank Project.
- Genetic Resources Conservation Researcrh Unit

Introduction and conservation of genetic resources as the animal research section of the NIAS Genebank Project, and research for preservation technology and for information management and disclosure.

#### ■ Institute of Radiation Breeding

Development of useful experimental lines, new breeding materials and new varieties using irradiation mutagenesis and other methods.



#### International Relations Section for Genetic Resources

Coordination of cooperation in relation to conservation and use of genetic resources with overseas research institutes and international organizations

Location: Headquarters Area (Tsukuba)

The Convention on Biological Diversity (CBD) came into force in 1993. It's objectives are: (1) conservation of biological diversity, (2) sustainable use of biological resource, and (3) sharing of benefits arising from the utilization of genetic resources in a fair and equitable way. It reaffirmed the sovereign rights of states over their natural resources. In the 10<sup>th</sup> Conference of the Parties of CDB held in Nagoya in 2010, the Nagoya Protocol on access to genetic resources and benefit sharing to the CBD was adopted. The Japanese government is a correspondent to the Nagoya Protocol and is preparing to join the International Treaty on Genetic Resources for Food and Agriculture (ITPGR).

We have 4 major missions to promote conservation and use of genetic resources:

- (1) Promotion and support for international cooperation in the context of international treaties and conventions (for example CBD, ITPGRFA);
- (2) Collaboration on international genetic resources activities in Japan, such as training;
- (3) Arrangement of bilateral cooperation and joint research activities on genetic resources;
- (4) Promotion of survey, collection, introduction, evaluation, conservation, regeneration, preservation and exchange with national and international research institutions.



Fig. 1. Training in collecting crop wild relatives



Fig. 2. Participant asking question during NIAS-FAO conference on plant genetic resources

### **Genebank Project Promotion Section**

### Conservation, studies, management and distribution of plant, microorganism and animal genetic resources

Location: Headquarters Area (Tsukuba)

Genebank project promotion section serves as the secretariat of NIAS Genebank Project on promoting collaboration with sub-banks and related organizations and receives, preserves, and distributes genetic resources and manages their information.



Seed storage facilities for the active collection at -1°C and 30%RH

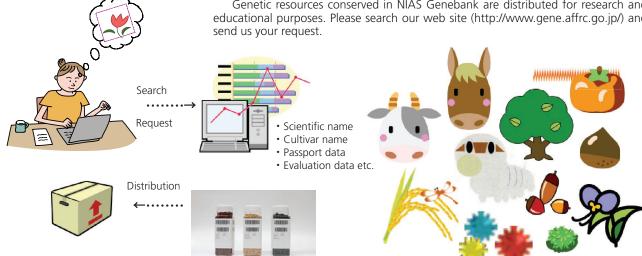


Fig. 2. Preparation of seeds for distribu-

#### Provision of Genetic Resources ···

Full details of available information concerning the genetic resources (i. e., plant, microorganism, animal, DNA, etc.) conserved in the NIAS Genebank are provided

Genetic resources conserved in NIAS Genebank are distributed for research and educational purposes. Please search our web site (http://www.gene.affrc.go.jp/) and send us your request.



## **Biodiversity Research Unit**

### Analysis of evolution, domestication and biodiversity

Location: Headquarters Area (Tsukuba)

The objectives of the Biodiversity Research Unit are to analyze and use plant biodiversity produced by the processes of evolution and domestication. Our tasks also include collection and conservation of plant genetic diversity.

At present, target plant species include *Oryza sativa, Sorghum bicolor, Glycine max, Vigna angularis* and other *Vigna* cultigens. Wild relatives of the target crop species are also being studied.



Fig. 1. Diversity of rice grains



Fig. 2. Diversity of sorghum spikelets

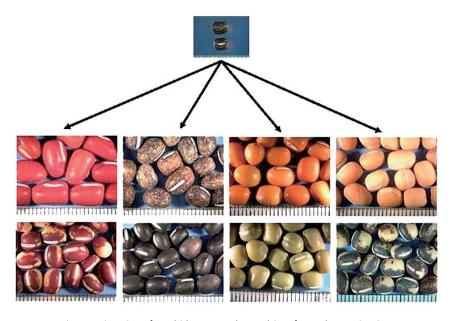


Fig. 3. Diversity of azuki bean seeds resulting from domestication

#### Classification and Evaluation Research Unit

## Classification, identification and character evaluation of microbial genetic resources

Location: Headquarters Area (Tsukuba)

The Classification and Evaluation Research Unit is a research division dealing with microbial genetic resources within the NIAS Genebank Project. In cooperation with the sub-bank laboratories in other research organizations, the Unit conducts taxonomic studies and character evaluation of microbial genetic resources, including field investigations. Agriculture-related microorganisms, such as plant pathogens that infest crops, are the main research target of the Unit and they are collected and accessions added to the Project as new microbial genetic resources. Based on the recent rapid progress in taxonomic studies, the system for classifying microorganisms has changed remarkably. To ensure the taxonomic reliability of microbial genetic resources distributed from the Project, taxonomic re-evaluation of stock microorganisms, such as anthracnose fungi and crown gall bacteria, is being performed. Phenotypic character data for the stock microorganisms have been accumulated and are being prepared for public access via the internet. DNA nucleotide sequence data useful for species identification are also being added for the accessions. Together with safe and stable preservation of microbial genetic resources, strain sets approved for distribution are being selected from stock microorganisms that were taxonomically re-evaluated. Accessibility to the microbial genetic resources of the Project is being improved for users.



Fig. 1. Acervulus of an anthracnose fungus (needle-like setae and falcate conidia)

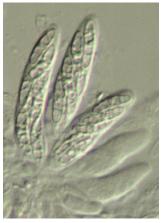


Fig. 2. Asci of an anthracnose fungus (a sexual organ)



Fig. 3. Disease symptom of crown gall of apple (a gall formed on a root)

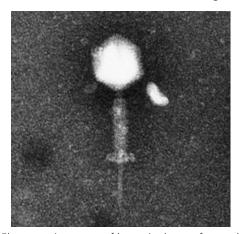


Fig. 4. Electron microscopy of bacteriophage of natto bacillus



Fig. 5. Culture colony of an anthracnose fungus

#### Genetic Resources Conservation Research Unit

## Introduction and conservation of animal genetic resources, and research on conservation methods and information systems

Location: Headquarters Area and Owashi Area (Tsukuba), Hokuto

As a research section of the NIAS Genebank, our unit introduces and conserves animal genetic resources such as livestock, poultry, and insects in collaboration with sub-banks. We also conduct research on conservation management of genetic resources and related information. The safe and certain conservation of genetic resources such as seeds, plants, animal germ cells, and microorganisms is one of the most important roles of Genebanks. The most suitable forms and methods for preserving genetic resources are investigated to keep them vital. Advanced conservation methods for recalcitrant genetic resources are being investigated, for example improvement in cryopreservation techniques for vegetative propagation. Regarding the information system, the database of passport, evaluation, and storage control has been established and data systems available through the internet have been developed. Various preservation techniques that have been developed so far ensure that valuable genetic resources will be handed over to the next generations.



Fig. 1. Conservation of animal germ cells Animal germ cells are preserved for the long-term in liquid nitrogen at -196°C (right figure: outward appearance of the liquid nitrogen tanks).



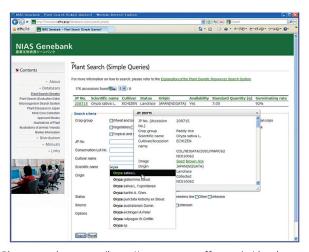


Fig. 2. Plant search system (http://www.gene.affrc.go.jp/database\_en.php)

## Institute of Radiation Breeding

Development of experimental lines, breeding materials with new traits and new varieties using gamma-ray irradiation

Location: Hitachiomiya

The Institute of Radiation Breeding (IRB) has two irradiation facilities, namely, the gamma field and the gamma room, for mutation breeding of various crop species. The gamma field is the world's biggest circular irradiation field with a radius of 100 m and an 88.8 TBq <sup>60</sup>Co source at the center. The gamma room is a shielded building with a 44.4 TBq <sup>60</sup>Co source used for high-dose rate irradiation of seeds, bulbs, tubers, and other plant materials.

The IRB contributes to the peaceful use of radiation through the development of new crop varieties by mutation breeding. In addition, the IRB is also conducting research to create new mutants, to develop crop varieties with new traits, and to elucidate the function of genes by constructing mutant resources useful for genome analysis.



Fig. 1. Aerial view of the gamma field



Fig. 2. "IRBIi light blue", an *Ipomoea indica* variety developed with gamma ray irradiation (left) and the original variety (right)





Fig. 3. New tartary buckwheat varieties developed with gamma ray irradiation

"Darumadattan" is a dwarf buckwheat variety with lodging resistance (left) and "Ionnokosai" is an ornamental yellow buckwheat variety (right).

### **Division of Plant Sciences**

#### Understanding of plant life for technology application

Crops are important for human diets and biomass energy. Food shortage in the near future is a serious concern, considering ongoing population growth and loss of potential cultivation areas in the world due to climatic instability and environmental destruction as a consequence of human activities. To achieve a stable food supply, it is essential to maintain and improve crop productivity even under changing and stressful environments. For this purpose, a systematic understanding of plant life and functions is necessary. The Division of Plant Sciences aims to elucidate mechanisms of how plants produce biomass and respond to environments and to understand the interactions between plants and microbes including pathogens and symbionts. Further, we will develop new technologies that lead to improved productivity of crops with high quality and safety in a sustainable way by understanding and utilizing diverse potentials of plants.

#### **Research Units**

■ Functional Plant Research Unit

Analyses of carbon and nitrogen metabolisms involved in plant biomass production and mechanisms of plants to respond to environmental signals.

Plant Symbiosis Research Unit

Analysis of symbiosis between plants and beneficial soil microbes (rhizobia and mycorrhizae).

■ Plant-Microbe Interactions Research Unit Analysis of interactions between plants and pathogens (fungi, bacteria, and viruses).

#### **Functional Plant Research Unit**

## Analyses of carbon and nitrogen metabolisms involved in biomass production and mechanisms of responses to environmental signals of plants

Location: Headquarters Area and NIAES Area (Tsukuba)

Plants assimilate inorganic carbon through photosynthesis and inorganic nitrogen absorbed from roots to produce organic compounds we use as food and energy resources. Plants are also capable of acclimating to fluctuating environments so as to optimize their own biological functions. We investigate molecular mechanisms of these fundamental processes of plants, aiming at improving their productivity and environmental adaptability. The food and energy production by plants depends on assimilation of carbon and nitrogen, and production is repressed if either process is limited. Assimilation and subsequent metabolism of carbon and nitrogen are not independent but they interact with one another in response to carbon/nitrogen supply and product demand. We are investigating mechanisms of metabolic interactions, translocation of metabolites, and starch accumulation in grains. The growth of plants is governed by their own developmental program, which is highly influenced by a variety of environmental factors, such as temperature, the quality and intensity of light, and the day length. Although environmental conditions vary every hour, day or season, plants have mechanisms to recognize and acclimate to surrounding environments. We are investigating mechanisms of plant development and how plants recognize and respond to environments.

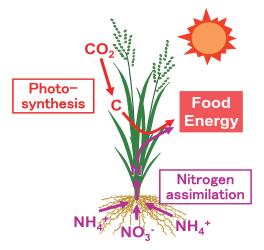


Fig. 1. Assimilation of inorganic carbon (photosynthesis) and inorganic nitrogen supports production of food and energy by plants

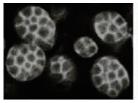




Fig. 2. Starch in amyloplasts of the rice grain Images observed with a confocal microscope (left) and scanning electron microscope (right) are shown.

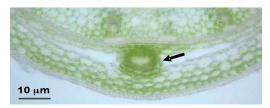


Fig. 3. Ectopic development of chloroplasts Overexpression of a key factor for the chloroplast development makes green chloroplasts accumulate in vascular bundles which normally have a few chloroplasts (arrowhead) in rice.



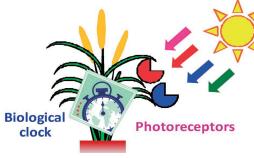


Fig. 4. Plants have an internal biological clock to track the time of day and recognize the daylength from light environments. Flowering of rice, a shortday plant, is induced when days grow shorter than the threshold length.

### Plant Symbiosis Research Unit

### How do plants establish symbiosis with rhizobia and mycorrhizae?

Location: NIAES Area (Tsukuba)

We are investigating symbiosis between plants and beneficial soil microbes. Legumes such as soybean establish symbiosis with soil bacteria - rhizobia. Rhizobia can fix nitrogen in the air into ammonium which is delivered to host plants. In turn, plants provide rhizobia carbon compounds that are made by photosynthesis. Most land plants, including major crops such as rice and soybean, establish symbiosis with soil fungi - mycorrhizae. Mycorrhizae absorb water and nutrients such as phosphorus and deliver them to host plants.

Our research focuses on the molecular genetic mechanisms by which plants establish symbiosis with rhizobia and mycorrhizae. As a result, we can improve the efficiency of symbiosis for agriculture. Much of current agriculture is highly dependent on chemical fertilizers which are produced from natural gas, phosphate rock, etc. that will be scarce in the future. By effective utilization of symbiosis between plants and soil microbes, we can realize low-input sustainable agriculture.



Fig. 1. Invasion of rhizobia (green) in plant cells (red)

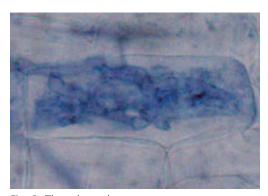


Fig. 2. The arbuscule
The arbuscule (blue) is the structure where
mycorrhizae exchange nutrients with plant cells.



Fig. 3. Symbiosis-defective mutants (left) fail to grow under nitrogen-poor condition. Upon symbiosis of rhizobia with plants (wild type, right) nitrogen fixation takes place which results in proper plant growth.

#### Plant-Microbe Interactions Research Unit

#### Analysis of interactions between plant cells and pathogenic microbes

Location: Headquarters Area (Tsukuba)

Most plant diseases are caused by fungi, bacteria and viruses. These pathogens interact with plants in complex manners. For example, some microbes invade plant tissues by recognizing specific surface structures of plants and multiply using specific plant factors. On the other hand, plants resist microbes by eliciting resistance responses to which some microbes further develop counter-defense mechanisms. Our research unit aims to understand how microbes and plants interact with each other, and to establish sustainable strategies to protect crops from pathogen infection.

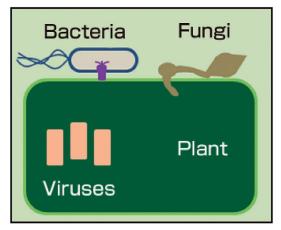


Fig. 1. Interactions between a plant cell and microbes

Our research unit aims to understand how microbes and plants interact with each other.



Fig. 2. Rice plants infected with the rice blast fungus

We have revealed how the rice blast fungus evades the host innate immune recognition response.



Fig. 3. Tomato plants inoculated with a virus TMGMV

We have revealed the mechanism by which a TMGMV mutant became infectious to a non-host plant, tomato.

### **Division of Insect Sciences**

### Studies on insects for integrated pest management

Insects live in almost all terrestrial and freshwater habitats and more than 800,000 species are currently known. Because they can adapt to various environments and have high reproductive potential, insects often cause serious damage to crops. The Division of Insect Sciences investigates insect hormones and pheromones, and interactions between pest insects and their host plants, predators and microbes to develop new insect growth regulators and basic technologies for Integrated Pest Management.

#### **Research Units**

- Insect Growth Regulation Research Unit Studying the mechanisms of molting, metamorphosis, reproduction and diapauses.
- Insect-Plant Interaction Research Unit Studying plant defense chemicals and proteins against insects, and sap sucking mechanisms by sucking insects.
- Insect Interaction Research Unit

Studying the chemicals and communication mechanisms controlling behaviors of pest insects and their natural enemies.

■ Insect-Microbe Research Unit

Studying the interaction between arthropods and associated microorganisms, and transmission mechanisms of plant pathogenic viruses.

### Insect Growth Regulation Research Unit

## Elucidation of the mechanisms of molting, metamorphosis, reproduction, and diapause in insects

Location: Owashi Area (Tsukuba)

While insects cause a great deal of damage to crops as agricultural pests, silkworms and honeybees provide useful products for human life. Therefore, development of a new technology that enables artificial control of insect growth, development and reproduction is an important subject for agricultural productivity.

The Insect Growth Regulation Research Unit performs the identification and functional characterization of genes involved in growth, development and reproduction in serious insect pests and beneficial insects such as brown planthopper and silkworm using genomic and biological information and develops practical applications of these genes. In particular, we focus on the identification and functional characterization of the genes involved in the actions of insect-specific hormones such as juvenile hormone (JH), ecdysteroids, and neuropeptides and the genes causing insecticide resistance. We also analyze the function of genes involved in development, regeneration, and diapause using RNA interference (RNAi) techniques. Based on the knowledge obtained in such studies, we aim for the development of novel insect pest control methods and the countermeasures for the insecticide-resistant pests.

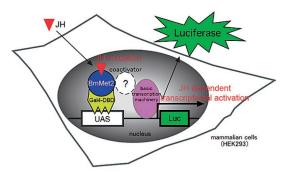


Fig. 1. Development of screening method for new JH agonists and antagonists using a candidate gene for JH receptor in *Bombyx mori* (*BmMet2*)

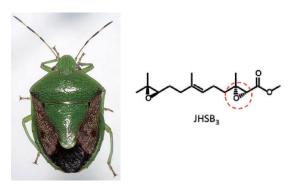
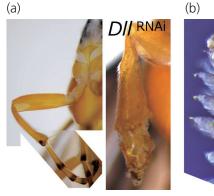


Fig. 2. Structure of heteroptera-specific JH JHSB<sub>3</sub> (right) isolated from the brown-winged green bug, *Plautia stali* (left). This new JH is expected to serve as a target for the development of novel heteroptera-specific insecticides.







- Fig. 3. Knockdown effects engendered by RNA interference (RNAi) in adults and larvae of the sawfly, *Athalia rosae*
- (a) Distal portions of thoracic legs are lost by interfering the *Distal-less (DII)* gene that is essential to form wings, legs, antennae and mouthparts at pupal stage (right).
- (b) Knockdown of the *DII* gene during embryonic development caused deficiencies of distal portions of thoracic legs and mouthparts in newly hatched larvae (right). On the other hand, abnormality was not seen in the abdominal legs (prolegs) where the *DII* gene function is dispensable.

## Insect-Plant Interaction Research Unit

# Analyses of the mechanisms of plant resistance to herbivorous insects and adaptive feeding in insects

Location: Owashi Area (Tsukuba)

Plants have evolved chemical or physical features as resistance mechanisms to protect against the feeding damage caused by insects. On the other hand, herbivorous insects that overcome plant defenses have also evolved to be able to utilize the food plants. We are conducting fundamental research on these insect-plant interactions with a view for developing new insect-resistant crops and innovative sustainable methods for insect-pest control. A primary study in our research unit focuses on the brown planthopper, *Nilaparvata lugens*, and the green leafhopper, *Nephotettix cincticeps*. These insects suck nutrient fluids from sieve tubes of rice, *Oryza sativa*, and occasionally transfer the disease-causing microbes. We are trying to identify substances from the saliva or other body parts of the insects, which have key roles in the sucking procedure and in utilization of nutrients. We also are attempting to isolate insect-resistance genes from some rice strains. However, it is recognized that an insect "biotype" can arise which breaks the barrier of the resistant rice. Thus, it is also important to know the adaptive mechanisms of the insects overcoming the rice defense as well as the function of the resistance genes of rice. Our research covers defense mechanisms of various plant species and adaptive feeding strategies of herbivorous insects, such as identifying novel plant secondary metabolites and their functional effects on insect feeding, and clarifying mechanisms determining host-plant range that could cause appearance of novel insect pests for agricultural crops.

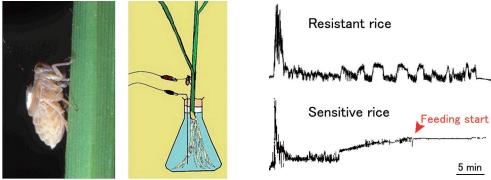


Fig. 1. By using a current circuit conducting trough, a brown planthopper and a rice plant, different wave patterns of the current can be recorded depending on each stage of sucking process. Attainment of sucking fluids from the rice sieve tube could be observed in an insect feeding on the insect-sensitive rice strain but not the resistant one.

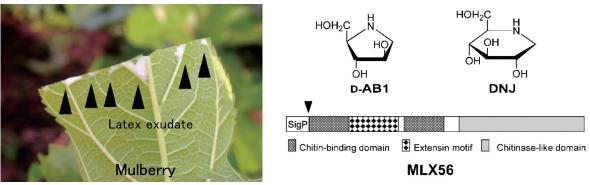


Fig. 2. More than 20,000 plant species exude latex from the wounded site and the latex contains various plant secondary metabolites. For example, we identified that the latex exudate of mulberry leaves (arrows) contains sugar-like alkaloids, D-AB1 and DNJ, which inhibit sugar metabolism and digestion, and a novel protein, MLX56, which binds chitin; thus inhibiting insect growth.

## **Insect Interaction Research Unit**

## Elucidation of the mechanisms controlling pest and natural enemy behavior

Location: Owashi Area (Tsukuba)

Many pests, their natural enemies, and other insects live together in agroecosystems which leads to various interactions such as competition for resources and/or habitats, predation, parasitism and mating behavior. These interactions are essential for insects to survive, but conversely we can utilize these relationships to control agricultural insect pests.

In the Insect Interaction Research Unit, we analyze insect behavior and inter- and/or intra-specific interactions by identifying semiochemicals, or chemical substances that act as signals. We also develop the genetic markers to conduct molecular phylogenetic analysis among species and strains and apply them for population genetics.

It is expected that this fundamental research will lead to the development of new insect pest control methods in the future.



Fig. 1. Field trapping of the white grab beetle, *Dasylepida ishigakiensis*, by a trap baited with synthetic sex pheromone



Fig. 2. An adult pirate bug, *Orius sauteri*, preying on *Thrips palmi* 



Fig. 3. A white-spotted longicorn beetle, *Anoplophora malasiaca*, feeding the branch of willow

## Insect-Microbe Research Unit

## Functional analysis of insect-associated microorganisms and viruses

Location: Owashi Area (Tsukuba)

Insects are the most diversified group in the animal kingdom. Various microorganisms including viruses infect insects and affect agricultural production. Our goal is to understand the interactions between insects and microorganisms and to integrate the knowledge in pest management strategies.

Bucillus thuringiensis, a species of entomopathogenic bacteria, is known for a source of Bt toxins which are widely used for microbial pesticides and Bt crops. However, development of insect resistance threatens continued success of the utilization of Bt toxins. Understanding the mechanism of the insect resistance to Bt toxins is important to plan strategies against the resistant insects. We are identifying lepidopteran resistance genes for Bt toxins. The brown planthopper, Nilaparvata lugens, is one of the most destructive pests for rice. This insect not only causes severe sucking damage, but also transmits phytopathogenic viruses. We intend to find crucial components for the virus transmission in insect vectors to facilitate the next stage of research for plant virus—insect vector interactions.

Some maternally-transmitted symbiotic bacteria can subtly manipulate the reproductive systems (including sex determination) of their host insects for their own benefit. Understanding the mechanism of this striking ability of such bacteria may allow us to develop a novel strategy to regulate demography of pest insects.

A pair of salivary glands locates in head part of the insect. Viruliferous insects inject viruses into plant with saliva.

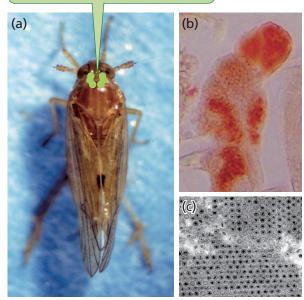


Fig.1. A reovirus replicating in the salivary gland of the brown plant hopper, *Nilaparvata lugens* 

- (a) The location of salivary glands (marked by green spots) in the brown plant hopper
- (b) Immunohistochemical detection of reoviruses in a thin section of a salivary gland
- (c) Electron microphotograph of multiplicated reoviruses in salivary gland of the insect

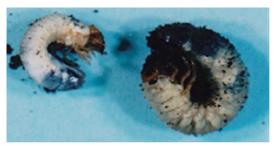


Fig. 2. Anomala cuprea (Coleoptera) larvae infected with an entomopoxvirus show the symptoms of the infection such as a whitish appearance and underdevelopment (left, infected larva; right, healthy one).

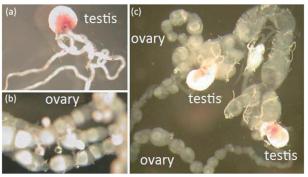


Fig. 3. Gonadal abnormalities induced by the suppression of *Wolbachia* population

In the butterfly *Eurema mandarina*, males are feminized by *Wolbachia*. Suppression of the *Wolbachia* density during larval development results in generation of intersexes (i.e., individuals having both male and female characters).

(a) Normal male; (b) Normal female; (c) Intersex

## **Division of Animal Sciences**

## Research to improve production and health of livestock animals

Animal products, which are nutritionally better balanced and abundant in essential amino acids, are important for human health. The Animal Science Division focuses on research on improving livestock production and health to deliver sufficient high-quality animal proteins to consumers at reasonable prices. In particular, we conduct the following research: 1) Development of a new usage of germ cells and pluripotent stem cells for both effective improvement and reproduction of livestock, 2) Mechanism by which livestock react to stressors such as light, temperature, and rearing environment, 3) Mechanism by which brain, ovary and uterus adjust the reproduction of livestock, 4) Cellular and molecular mechanisms of innate immune systems, and 5) Development of novel cell culture systems.

## **Research Units**

■ Animal Development and Differentiation Research Unit

Research and development of new germ cell and pluripotent stem cell technologies in domestic animals and poultry.

■ Animal Physiology Research Unit

Development of novel techniques that raise physiological functions to increase productivity in domestic animals.

■ Animal Immune and Cell Biology Research Unit

Cellular and molecular research on the immune responses of livestock animals and development of novel cell culture systems.

## Animal Development and Differentiation Research Unit

# Research and development of new germ cell and pluripotent stem cell technologies in livestock

Location: Headquarters Area and Ikenodai Area (Tsukuba)

It is necessary to develop new technology which utilizes genome information for effective improvement and reproduction of domestic animals and poultry. Therefore, our research unit focuses attention on developing a new usage of germ cells and pluripotent stem cells. We have reported for the first time that oocytes fertilized with sperm from ectopic xenografts of the testis tissue from immature pig have the ability to develop to viable piglets. As the next step in developing a more practical technology, we introduced the vitrification (non-freeze super low temperature preservation) method to preserve immature testis tissue as the donor source. In addition, we have developed a method to establish and culture embryonic stem (ES) cells of livestock. The study of pluripotent stem cells such as induced pluripotent stem (iPS) cells is advancing rapidly in humans, but the study in livestock is lagging behind. We have established ES cells from rabbit, pig and bovine and now we start studies to develop a method to induce the differentiation of ES cells into the germ cells.



Fig. 1. The piglet was produced by ICSI (Intracytoplasmic sperm injection) with sperm from ectopic xenograft.

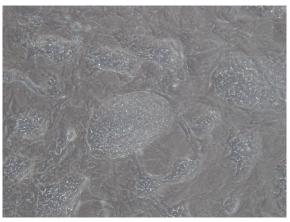


Fig. 2. Phase contrast image of the bovine ES cell colonies

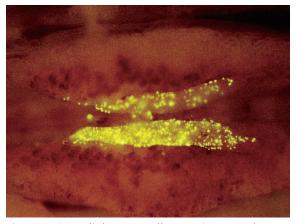


Fig. 3. Primordial germ cells carrying GFP (Green Fluorescent Protein) gene were homing to the gonad after intravenous injection into the chick embryo.

## **Animal Physiology Research Unit**

## Development of novel techniques that improve physiological functions to increase productivity in domestic animals

Location: Ikenodai Area (Tsukuba)

To increase quality and quantity of animal products such as meat and milk, it is necessary to raise domestic animals under less stressful conditions and to breed them efficiently.

Therefore, to elucidate physiological functions underlying stress responses and reproduction, we are working on 1) development of methods in which the degree of stress and positive emotion in domestic animals are objectively estimated, 2) analysis of effects of day light length, temperature and raising environment on the stress response, 3) analysis of central mechanisms that control ovum development and ovulation in response to environmental changes inside and outside of the body, such as nutritional conditions and pheromone, 4) analysis of mechanisms involved in the periodic changes of ovarian morphology and functions, and 5) analysis of processes for blastocyst implantation and placenta formation, and placental functions.

Knowledge obtained through these studies will fundamentally contribute to develop novel techniques that improve physiological functions to increase productivity in domestic animals.

When thinking about mechanisms in the body,



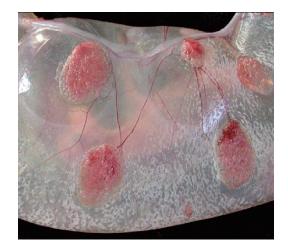


Fig. 1. Bovine fetus and placenta on the day 65 of gestation

Bovine blastocyst (0.3mm in diameter) is implanted in maternal uterus and grows up to 30 kg in weight during 280 days of gestation. The placenta mediates the dramatic growth of newborn. Our unit works on the elucidation of implantation and the placental function to promote the healthy growth of the fetuses in the cow.



Fig. 2. Kids and their mother

The goat is a member of domestic ruminants as a cattle. Because the goat is smaller than cattle, its nature is gentle and thus is easy to handle, it is an ideal experimental model to investigate physiological functions in domestic animals. In this unit, we are working on research to elucidate the central mechanism controlling reproductive functions using the Shiba goat.



## Animal Immune and Cell Biology Research Unit

## Cellular and molecular research on the immune responses of livestock animals and development of novel cell culture systems

Location: Owashi Area and Ikenodai Area(Tsukuba)

To improve disease resistance in livestock animals, the Animal Immune and Cell Biology Research Unit is involved in basic research on the cellular and molecular mechanisms of innate immune systems - mainly on the macrophage-lineage cells. We have established immortalized cell lines of microglia (brain macrophage) and Kupffer cells (liver macrophages), which reliably retain their original immune functions. We are investigating the molecular mechanisms of immune signal transduction and the production and release of inflammatory cytokines from these cells. In addition, we are using the pig genome database to investigate genetic variations in specific pig populations for their pattern recognition receptors that are possibly related to the inherent resistance to infectious diseases. Furthermore, we are developing novel complex cell culture systems by using a collagen vitrigel membrane (composed of high-density collagen fibrils) to validate chemical efficacy or toxicity *in vitro*. This technique may possibly be applied as an alternative to classical drug tests using experimental animals.

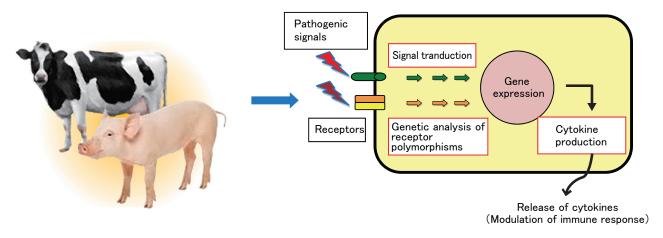


Fig. 1. Animal Immune and Cell Biology Research Unit is involved in basic research on the cellular and molecular mechanisms of innate immune systems to improve disease resistance in livestock animals. Infections by the pathogenic microorganisms are detected by specific receptors in innate immune cells, such as macrophages. Then, the information is transmitted through the signal transduction pathway to activate specific gene expressions for immune responses, such as production and release of inflammatory cytokines. There are several polymorphisms in the pattern recognition receptors, which affect genetic resistance to the disease.

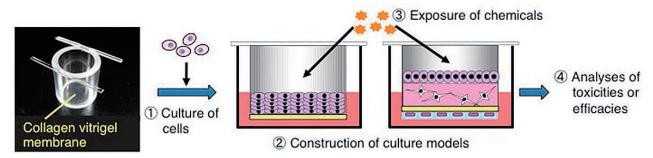


Fig. 2. Animal Immune and Cell Biology Research Unit develops novel cell culture systems to replace toxicological studies with experimental animals. Human cells can be cultured on one or both sides of a collagen vitrigel membrane to reproduce specific tissue architecture as it occurs *in vivo*. These complex culture models can be used to validate chemical toxicities or efficacies *in vitro*, which may possibly be applied as alternatives for classical drug tests using experimental animals.

#### International Research Activities

The National Institute of Agrobiological Sciences (NIAS) is promoting research cooperation, dissemination of information, and collaboration via exchange of researchers with research organizations from different countries around the world.

## Research cooperation and exchange of researchers

To promote international research cooperation and collaboration, the NIAS has established partnerships with 11 organizations from 9 countries in accordance with the Memorandum of Understanding (MOU) agreed by both parties. In partnership with the International Cooperation Agency (JICA), researchers from NIAS are sent to developing countries, and in return, researchers from those countries are accepted for training in various laboratories of the institute.

As of March 2013, NIAS has established research collaborations with the following organizations:

- Leibnez Institute of Plant Genetics and Crop Plant Research (IPK), Germany
- National Institute of Agricultural Biotechnology, Rural Development Administration, Korea
- The Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia
- Institute of Entomology, Biology Center of the Academy of Sciences (BCAS), Czech Republic
- International Center for Tropical Agriculture (CIAT)
- Tamil Nadu Agricultural University, India
- Cambodian Agricultural Research and Development Institute (CARDI), Cambodia
- Kasetsart University, Thailand
- International Barley Genome Sequencing Consortium (IBSC)
- Southwest Agricultural University, China
- Institute of Genetics and Developmental Biology (IGDB), Chinese Academy of Sciences, China
- Kenya Agricultural Research Institute (KARI), Kenya
- National Forestry, Crops and Livestock Research Institute (INIFAP), Mexico
- Plant Resources Center (PRC), Vietnamese Academy of Agricultural Sciences (VAAS), Vietnam
- National Agriculture and Forestry Research Institute (NAFRI), Lao PDR
- Foreign company



Signing of MOU with IPK



JICA trainees at work

## ◆ International Conferences

The NIAS organizes international conferences to stimulate exchange of information with foreign researchers.



The Rice Annotation Project (RAP) Workshop at the Academia Sinica, Taiwan in conjunction with the 9th International Symposium of Rice Functional Genomics (ISRFG9), Nov 26-29, 2011.



Workshop on Silkworm Genome Annotation November 9-11, 2010 - Tsukuba, Japan

### **Outreach Activities**

Various outreach activities are organized so that the general public could have a better understanding of research programs in the institute.

## ◆ Website and Publications

Through the website of NIAS (http://www.nias.affrc.go.jp/index\_e.html), we announce hot topics, press releases, and event information for the Institute. We also publish the NIAS News, a quarterly newsletter and the NIAS Mail Magazine (both in Japanese only) on a regular basis, and send short messages online via Twitter.



Top page of the website of NIAS (left) and NIAS news (right)

## Events and Exhibits

#### **NIAS Open-house**

The institute is open to the public during the 'Tsukuba Science and Technology Week' which is held on the 3<sup>rd</sup> week of April every year. This gives the general public an opportunity to view various research facilities and research outputs on display and get on-hand experience on some scientific methods such as DNA extraction.

#### **NIAS Science Communication**

The institute organizes the NIAS Open College to introduce our latest research achievements. This program is conducted in the form of lecture seminars and discussions with participants from the general public. It consists of lecture seminars held once a week from September until December.

#### **GMO Crops Field Tour**

One of our major research area focuses on the development of genetically modified organisms (GMOs). Guided tours of fields planted with GMO crops are organized to give the general public an opportunity to view GMO crops growing in the field.

#### **NIAS Exhibits**

Some of our research achievements are also available as exhibits at the Tsukuba Agriculture Research Gallery (http://trg.affrc.go.jp) located at the NARO campus. The gallery is open to the public daily (9:00 am - 4:00 pm except the yearend holidays) and admission is free.



NIAS Open-house



NIAS Open College



GMO soybean field

#### -NIAS Institutions inside Tsukuba-



#### **Contact for NIAS Institutions inside Tsukuba**

TEL: +81-29-838-7406

#### **Headquarters Area**

2-1-2 Kannondai, Tsukuba, Ibaraki, 305-8602, Japan

#### NIAES Area (in National Institute for Agro-Environmental Sciences)

3-1-3 Kannondai, Tsukuba, Ibaraki, 305-8604, Japan

#### Access

From Tokyo:

- Take the JR Joban Line from Ueno Station and get of at Ushiku Station. From the West Exit, take the Kantetsu Bus bound for Tsukuba-Daigaku-byouin, Yatabe-Shako, or Seibutu-Ken-Ohwashi and get off at Norin-Danchi-Chuo(For NIAES Area, get off at Nougyo-Kankyo-Gijutsu-Kenkyusho-Mae).
- Take the Tsukuba Express Line from Akihabara Station and get of at Tsukuba Station. Take the Tsuku Bus 'Nambu Shuttle' and get off at Norin-Danchi-Chuo.
- Take the Tsukuba Express Line from Akihabara Station and get of at Midorino Station. Take the Kantetsu Bus 'Norin-Danchi-Junkan' and get off at Norin-Danchi-Chuo.

From Tokyo International Airport (Narita):

● Take the Kantetsu or Keikyu Bus bound for Tsukuba Center and get off at Tsukuba Center (in front of Tsukuba Station). Take the Tsuku Bus 'Nambu Shuttle' and get off at Norin-Danchi-Chuo.

#### **Owashi Area**

1-2 Owashi, Tsukuba, Ibaraki, 305-8634, Japan

#### Access

From Tokyo:

- Take the JR Joban Line from Ueno Station and get of at Ushiku Station. From the West Exit, take the Kantetsu Bus bound for Seibutu-Ken-Owashi and get off at Seibutu-Ken-Owashi. \*Only 2 buses a day.
- Take the Tsukuba Express Line from Akihabara Station and get of at Tsukuba Station. Taxi is available from Tsukuba Station.

From Tokyo International Airport (Narita):

 Take the Kantetsu or Keikyu Bus bound for Tsukuba Center and get off at Tsukuba Center (in front of Tsukuba Station). Taxi is available from Tsukuba Station.

## Ikenodai Area (in National Institute of Livestock and Grassland Science)

2 Ikenodai, Tsukuba, Ibaraki, 305-0901, Japan

#### Access

From Tokyo:

- Take the JR Joban Line from Ueno Station and get of at Ushiku Station. Take the Kantetsu Bus bound for Tsukuba-Daigaku-byouin, Yatabe-Shako, or Seibutu-Ken-Ohwashi and get off at Chikusan-Souchi-Kenkyusho-Mae.
- Take the Tsukuba Express Line from Akihabara Station and get of at Tsukuba Station. Take the Tsuku Bus 'Nambu Shuttle' and get off at Makizono-chuoh.

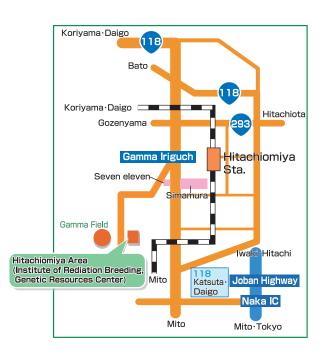
From Tokyo International Airport (Narita):

 Take the Kantetsu or Keikyu Bus bound for Tsukuba Center and get off at Tsukuba Center (in front of Tsukuba Station). Take the Kantetsu Bus bound for Ushiku Stasion and get off at Chikusan-Souchi-Kenkyusho-Mae.

## -NIAS Institutions outside Tsukuba-







#### Hokuto

6585 Kobuchizawa, Hokuto, Yamanashi, 408-0044, Japan TEL: +81-551-36-2046

#### Access

From Tokyo:

 Take the JR Chuo Line from Shinjuku Station and get off at Kobuchizawa Station. Taxi is available from Kobuchizawa Station.

#### Hitachiomiya

2425, Kamimurata, Hitachi omiya, Ibaraki, 319-2293, Japan TEL: +81-295-52-1138

#### Access

From Tokyo:

• Take the JR Joban Line from Ueno Station. At Mito Station, transfer to the JR Suigun Line and get off at Hitachiomiya Station. Taxi is available from Hitachiomiya Station.

### \*Contact Us\*

#### **NIAS Institutions inside Tsukuba**

### Headquarters Area, NIAES Area, Owashi Area, and Ikenodai Area

2-1-2 Kannondai, Tsukuba, Ibaraki, 305-8602, Japan

TEL: +81-29-838-7406, FAX: +81-29-838-7408

E-mail: www@nias.affrc.go.jp

#### **NIAS Institutions outside Tsukuba**

### Hitachiomiya (Institute of Radiation Breeding)

2425, Kamimurata, Hitachiomiya, Ibaraki, 319-2293, Japan

TEL: +81-295-52-1138

## **Hokuto (Genetic Resources Conservation Research Unit)**

6585 Kobuchizawa, Hokuto, Yamanashi, 408-0044, Japan

TEL: +81-551-36-2046

## **National Institute of Agrobiological Sciences**



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Public Relations Section TEL: +81-29-838-8469 2-1-2 Kannondai, Tsukuba, Ibaraki, 305-8602, Japan

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