ISSN2187-2015

Research Highlights for FY2014

National Institute of Agrobiological Sciences

Research Highlights for FY2014

(Apr. 2014 ~ Mar. 2015)

Major research outcomes are classified into 4 categories

- **Intellectual Contribution**
  Publications on new discoveries, theories and principles, prediction of unknown phenomena, and elucidation of unknown mechanisms contributing to the understanding of biological processes and the advancement of knowledge.
- **Advances in Technology**
  Key researches addressing innovative technological developments, advancements in existing methods, and improvement in efficiency and quality of production.
- **Agricultural Production**
  Research articles with significant impact in overall productivity in various sectors of agriculture, forestry and fisheries.
- **Bioindustry**
  Research on biotechnology and production of new materials that will contribute to the development and expansion of innovative industries.

Summary of the Third Five-year Plan (FY2011 - FY2015)

1 Enhancement of biological resources and development of research systems for improvement of innovative crops and livestock

1-1 Conservation of genetic resources for food and agriculture and intensification of their use

1-2 Development of a robust infrastructure for genomics-based approach in agricultural research

1-2-1 Enhancing the potential of the genome sequence and resources from agriculturally important organisms

1-2-2 Bioinformatics approach for advancement of agrobiological research

1-2-3 Genomics approach for advancement of research in crop improvement

1-2-4 Genomics approach for advancement of research in livestock production

1-2-5 Structural and functional analysis of biomolecules related to agriculture

2 Understanding life phenomena leading to enhancement of biological functions and developing novel technologies for their applications

2-1 Research platforms on biological functions associated with improved productivity of agricultural crops and livestock

2-1-1 Elucidation of the mechanisms involved in biomass production, growth, differentiation, and environmental response of agricultural crops

2-1-2 Elucidation of the regulatory mechanisms involved in insect growth, development and differentiation

2-1-3 Elucidation of the molecular mechanisms involved in the development and differentiation of germ cell and stem cell of livestock

2-1-4 Elucidation of the mechanisms involved in the control of the behavior and reproduction of livestock

2-2 Research platforms on biological interactions associated with improved productivity and development of novel technologies for their applications

2-2-1 Elucidation of the mechanisms involved in plant pathogenic microbe infections and development of innovative technologies for their applications

2-2-2 Elucidation of the mechanisms involved in crop response to microbial infection and development of crop strains with multiple pathogen resistance

2-2-3 Elucidation of the mechanisms involved in plant and soil microbe symbioses

2-2-4 Elucidation of the mechanisms involved in insect pest infestation and plant resistance to insects

2-2-5 Elucidation of insect-insect, insect-plant and insect-microbe interactions and their applications

2-2-6 Elucidation of molecular mechanisms in animal immune systems

3 Development of innovative technologies based on biological functions to create new bio-industries

3-0-1 Innovation of technologies for development of genetically modified crops and intensification of their use

3-0-2 Development of novel technologies for efficient use of genetically modified silkworm

3-0-3 Development of novel technologies for efficient use of genetically modified animals

3-0-4 Development of novel technologies using biomaterials based on silk proteins

3-0-5 Elucidation of insect-specific biological functions and development of novel technologies for their applications

Table of Contents

Research Highlights

Plant and Microbial Sciences

Insect and Animal Sciences

List of Publications (In order of the research themes for the Third Five-year Plan)

Intellectual Contribution

Draft sequence of the bread wheat genome

Hirokazu Handa[1], Fuminori Kobayashi[1], Jianzhong Wu[1,2], Takeshi Itoh[3], Tsuyoshi Tanaka[3], Hiroaki Sakai[3], Takashi Matsumoto[1]

[1]Plant Genome Research Unit, [2]Advanced Genomics Laboratory, [3]Bioinformatics Research Unit

In collaboration with the International Wheat Genome Sequencing Consortium (IWGSC), we have contributed in producing the first "draft sequence" of the wheat genome, which is 40 times bigger than the rice genome, and identified 124,201 sequences as genes or gene loci. The genome sequence data generated so far provide a unique resource for accelerating gene mapping and marker development in wheat breeding.

Keywords:wheat, draft genome sequence, gene information, information of chromosomal location

Background

Wheat is one of major cereal crops with the second biggest production in the world, and together with rice and maize, is a major dietary component for many populations across the world. The development of novel varieties with desirable traits such as high yield and tolerance to biotic/abiotic stresses is therefore highly anticipated as a solution to food shortage due to an ever-increasing population. Deciphering the genome sequence and characterization of gene structure and function will accelerate our understanding of its biology with implications in agriculture. The IWGSC makes efforts to obtain the high quality reference sequence of the17GB bread wheat genome. As a member of IWGSC, we are working to sequence the wheat chromosome 6B and contributed to the release of the draft genome sequence of wheat.

Results and Discussion

1. The IWGSC is an international, collaborative consortium, established in 2005 by a group of wheat scientists in the world. The collaborators from Japan, which consist of research teams from the NIAS, Kyoto Univ., Yokohama City Univ. and Nisshin Flour Milling Co., are in-charge of wheat chromosome 6B, which corresponds to 2.5 times the size of the rice genome (Fig. 1).
2. Using the wheat variety "Chinese Spring", each chromosome of wheat was picked up by flow cytometry technique and then DNAs were isolated from individual sorted chromosomes. Sequence information was produced using high throughput 'Next Generation Sequencing' technology (Fig. 2).
3. The sequences were assembled into contigs for each chromosome covering a total of 10.2 Gb, or approximately 61% of the wheat genome.
4. From the sequences represented in the assemblies, we have annotated 124,201 gene loci distributed nearly evenly across the homologous chromosomes (Fig. 3).
5. We have sequenced 508 Mb of chromosome 6B, corresponding to 56% of the estimated size (914 Mb), and identified 4,798 gene loci. Using this information, we carried the comparative analyses between wheat and other Gramineae plants, such as rice, sorghum and Brachypodium (Fig. 4).

Future prospects

1. The draft sequence and gene information are useful for isolation and functional analysis of wheat genes. Due to the complexity of the wheat genome, a hexaploid with AABBDD genome composition derived from three ancestral species (Fig. 5), it was difficult to distinguish the homoeologous gene copies which resemble each other. Although the draft sequence was able to identify and assign genes to individual chromosomes it does not provide information that will allow breeders to identify the differences between genes that lie on the chromosomes within the A, B and D subgenomes that will make it easier and more rapidly to localize specific genes for DNA marker assisted breeding.
2. The draft genome sequence will enable breeders to accelerate the improvement of wheat through genomics assisted breeding and biotechnology, which will result in new wheat varieties with higher yield, better

resistance to diseases and pests, and tolerance to abiotic stresses. The sequence information of chromosome 6B is useful to improve wheat grain quality, resistance to *Fusarium* head blight, etc.

3. The ultimate goal of the IWGSC is to produce the complete wheat genome sequence, or the so-called reference sequence with 85% coverage of the 21 wheat chromosomes.

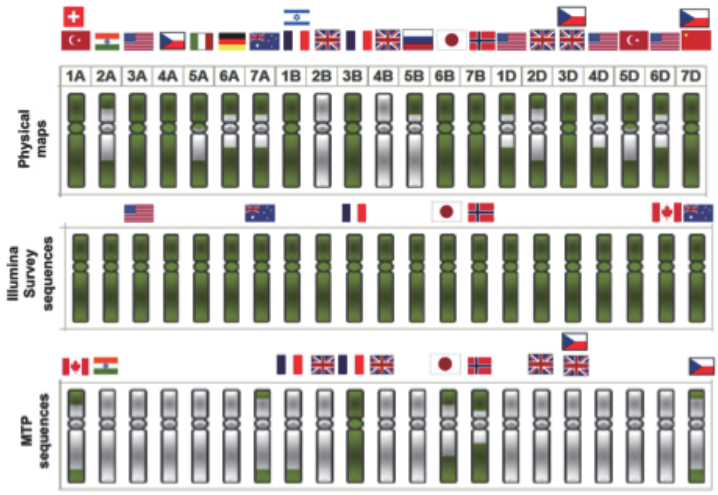


Fig. 1. Current status of genome sequencing in terms of physical map construction (top panel), draft genome sequencing (middle) and reference genome sequencing (bottom). The state of progress is shown in green

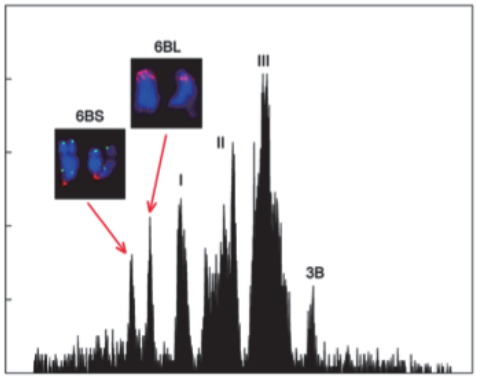


Fig. 2. Isolation of wheat chromosome 6B by flow cytometry.

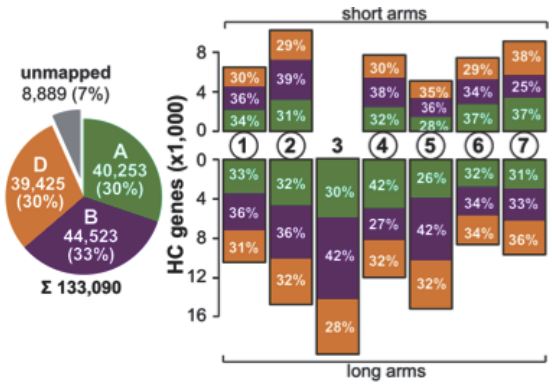


Fig. 3. Distribution and total number of HC (high confidence) bread wheat genes identified on the A (green), B (purple), and D (orange) subgenomes.

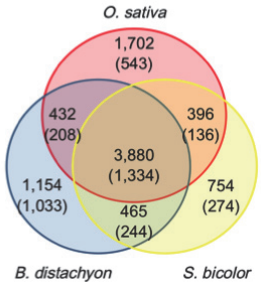


Fig. 4. Distribution of chromosome 6B genes with significant similarity to *O. sativa, B. distachyon* and *S. bicolor.*

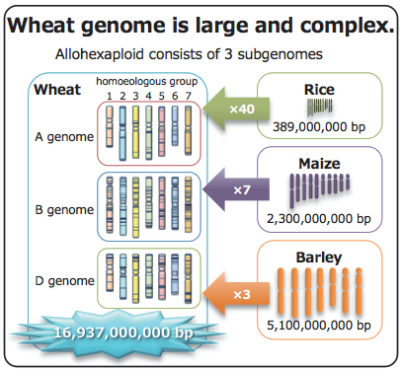


Fig. 5. Comparison of wheat and other cereal crop genomes.

References

1. International Wheat Genome Sequencing Consortium (2014) A chromosome-based draft sequence of the hexaploid bread wheat genome ***Science*** 345:1251788
2. Tanaka T, Kobayashi F, Joshi G.P, Onuki R, Sakai H, Kanamori H, Wu J, Šimková H, Nasuda S, Endo T.R, Hayakawa K, Doležel J, Ogihara Y, Itoh T, Matsumoto T, Handa H (2014) Next-generation survey sequencing and the molecular organization of wheat chromosome 6B ***DNA Research*** 21 (2):103-114

Intellectual Contribution

Establishment of an efficient CRISPR/Cas9 mediated genome editing system in rice

Masaki Endo, Seiichi Toki

Plant Genome Engineering Research Unit

We compared the mutation frequency in various Cas9, sgRNA constructs under the same experimental condition and established an efficient targeted mutagenesis system via CRISPR/Cas9 system in rice. Using the sgRNA designed on the conserved region, multiple paralogous genes were mutagenized by single sgRNA by on- and off-target cleavage. In addition, we revealed that extension of the culture period of rice calli expressing Cas9 and sgRNA is an effective approach for increasing mutation efficiency.

Keywords:rice, CRISPR/Cas9, genome editing

Background

The clustered regularly interspaced short palindromic repeat (CRISPR)-associated endonuclease 9 system (CRISPR/Cas9) has been demonstrated to be a robust genome engineering tool in a variety of organisms. There have also been several reports of successful CRISPR/Cas9-mediated targeted mutagenesis in rice. However, comparison of each result doesn't make sense because target genes, tissues used for transformation, and evaluation methods of mutation frequency differ in each report. So we compared the mutation frequency in various Cas9, sgRNA expression constructs under the same experimental condition and selected the one that showed predominant expression. Furthermore, we attempted to disrupt multiple paralogous genes by using off-target mutation of CRISPR/Cas9, which is often regarded as a disadvantage in using the CRISPR/Cas9 system. In addition, we analyzed the effect of culture period of calli expressing Cas9 and sgRNA to determine the factors affecting mutation frequency.

Results and Discussion

1. We introduced Cas9 and sgRNA expression cassettes separately and sequentially into rice calli, and assessed the frequency of mutagenesis at the same endogenous targeted sequences (Fig. 1A). As a result, the best combination of Cas9, sgRNA was determined and all-in-one vector of these constructs was confirmed to be useful for target mutagenesis of rice with high-efficiency (Fig. 1B).
2. CRISPR/Cas9 system has some propensity for causing off-target mutations and it is often regarded as a disadvantage. We attempted to use this off-target mutation for inducing mutations in paralogous genes in rice. When sgRNA was designed on consensus sequence of 4 CDK genes, mutation frequency of on-target gene (CDKB2) and the most strong candidate (CDKA2) with 1-nt mismatch in 20bp target sequence were almost the same. However, as the number of mismatch increased and the position of mismatch come close to PAM sequence (NGG), mutation frequency was decreased (Fig. 2, CDKB1 and CDKA1).
3. Mutation frequency largely depends on the target sequence (data not shown). However, extension of the culture period of calli expressing Cas9 and sgRNA was effective for increasing the ratio of mutated cell (Fig. 3).

Future prospects

1. Because vector construction of CRISPR/Cas9 system is much easier than ZFNs and TALENs and mutation frequency of CRISPR/Cas9 is relatively high, CRISPR/Cas9 system will be the major artificial nuclease used for genome engineering. In plant, direct delivery of RNA to plant nuclei is difficult. Thus, utilization of appropriate expression constructs for both gRNA and Cas9 is important. The CRISPR/Cas9 vector established in our study has been proven in many laboratories to induce targeted mutagenesis efficiently in rice and will contribute molecular breeding of rice.
2. Off-target mutation is often referred to as a disadvantage in using the CRISPR/Cas9 system. However, we showed that such off-target mutations can be used for knockout of multiple genes with high homology by

single sgRNA. In case of the gene families in polyploid or diploid plants, disruption of multiple genes is necessary to obtain the desired phenotype. Our study showed that off-target mutations could be utilized for plant molecular breeding.

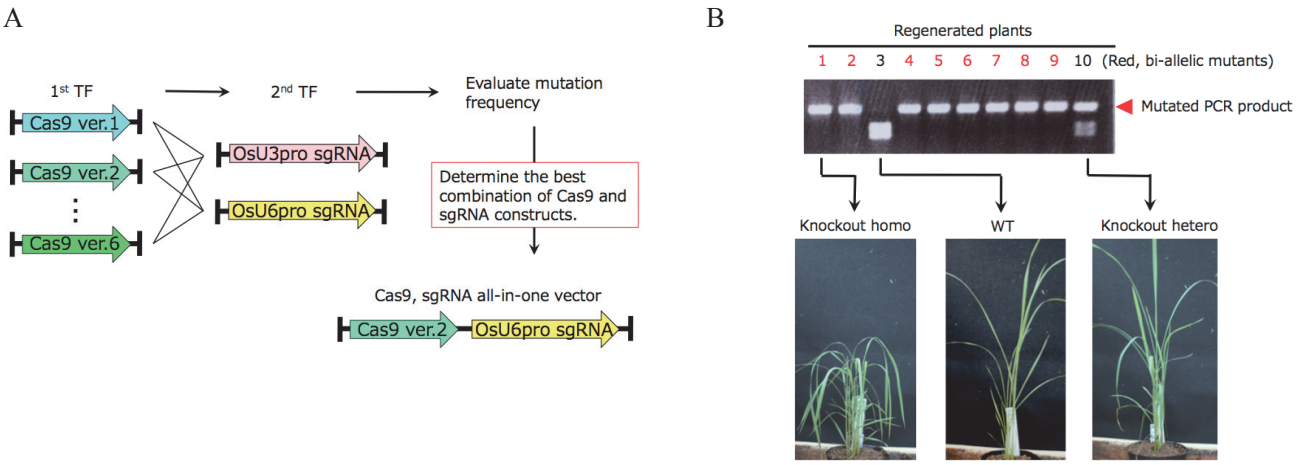
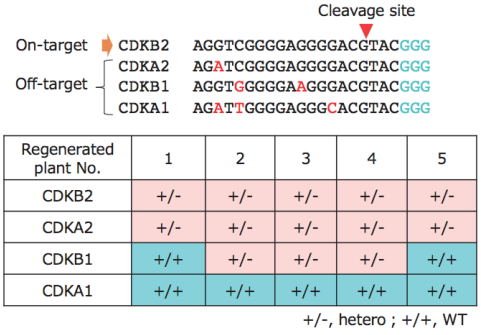


Fig. 1. Selection of appropriate Cas9 and sgRNA expression construct in rice. (A) Six Cas9 constructs and two sgRNA constructs were transformed into rice calli separately and sequentially. The best combination of Cas9 and sgRNA construct was determined and an all-in-one vector was constructed. (B) Using an all-in-one vector, targeted mutagenesis of drooping leaf (*DL*) gene was conducted. As a result, bi-allelic mutants with the drooping leaf phenotype were obtained efficiently.



Regenerated plant No.	1	2	3	4	5
CDKB2	+/-	+/-	+/-	+/-	+/-
CDKA2	+/-	+/-	+/-	+/-	+/-
CDKB1	+/+	+/-	+/-	+/-	+/+
CDKA1	+/+	+/+	+/+	+/+	+/+

+/-, hetero ; +/+, WT

Fig. 2. Multigene knockout utilizing off-target mutations.
sgRNA was designed on the consensus sequence of 4 CDK genes. Mutation frequency in CDKA2 gene, which has 1-nt mismatch at 18-nt from PAM sequence (NGG) was almost the same as that in the on-target gene, CDKB2. When the number of mismatch increases and the position of mismatch comes close to PAM sequence, mutation frequency decreased (CDKA1, CDKB1).

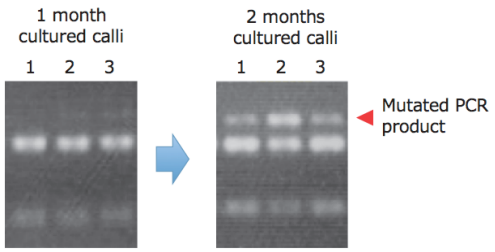


Fig. 3. Increment of mutation frequency by extension of culture period of Cas9 and sgRNA transformed calli. Mutation in DNAs extracted from one and two months cultured calli with the Cas9 and sgRNA transgenic expression construct was detected by CAPS analysis. In one month cultured calli, a few mutated PCR products were detected whereas in 2 months cultured calli, mutated PCR product was significantly increased.

Reference

1. Endo M, Mikami M, Toki S (2015) Multigene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice ***Plant and Cell Physiology*** 56 (1):41-47

The universal pinpoint mutagenesis system in rice

Ayako Nishizawa-Yokoi, Masaki Endo, Namie Ohtsuki, Hiroaki Saika, Seiichi Toki

Plant Genome Engineering Research Unit

We have succeeded in establishing a technique that allows pinpoint genome editing in rice. This is made possible by gene targeting with positive-negative selection and subsequent marker excision via *piggyBac* transposon.

Keywords:rice, gene targeting, positive-negative selection, marker excision, *piggyBac* transposon

Background

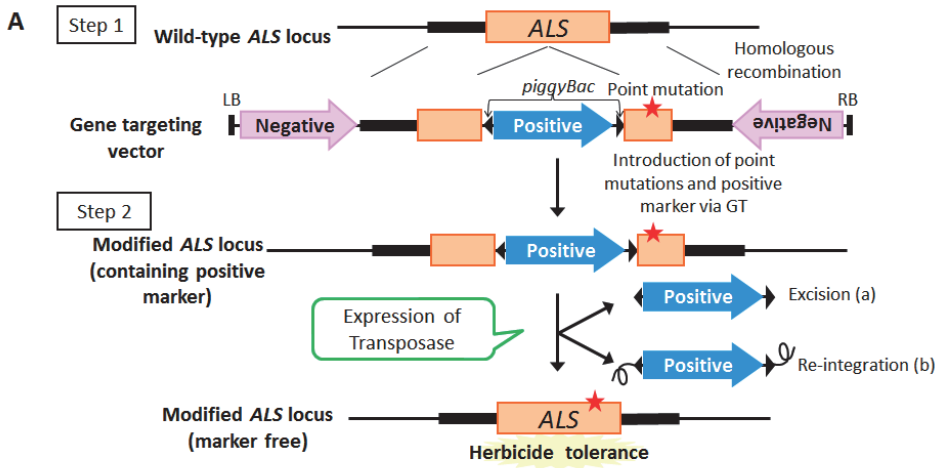
Gene targeting (GT) is a technique for accurate genome editing. Using GT with positive-negative selection, the positive selection marker gene should be removed completely from GT locus. However, no such system has been established so far. Here, we have succeeded in accurate excision of marker without leaving a footprint at the excise site using insect-derived *piggyBac* transposon.

Results and Discussion

1. We attempted to introduce targeted two point mutations conferring resistance to herbicide into the rice acetolactate synthase (*ALS*) gene via GT with positive-negative selection (Fig. 1A:Step1). Subsequently, the positive marker gene was excised by *piggyBac* transposition leaving targeted two point mutations on the *ALS* gene (Fig. 1A:Step2).
2. The transgenic calli harboring a modified *ALS* locus containing point mutations and positive selection marker were then selected and subjected to Step2. PCR analysis revealed that the marker gene was excised completely from *ALS* locus by *piggyBac* transposition in 99 out of 100 regenerated plants from five independent calli lines (Fig. 1B). Sequencing analysis demonstrated transposition of *piggyBac* without leaving a footprint at the excise site.
3. We confirmed that the transcripts of modified ALS locus were comparable to that of wild-type *ALS* gene in T_1 progenies obtained from marker-free regenerated plants (Fig. 2A and 2B). Furthermore, T_1 progenies containing modified *ALS* locus showed the herbicide-tolerant phenotype (Fig. 2C).
4. This approach was also applied successfully in pinpoint mutagenesis of several genes in rice.

Future prospects

1. The marker-free T_1 progenies containing the two point mutations in *ALS* gene but without the expression cassette of *piggyBac* transposase were obtained from marker-free regenerated plants. These plants contained only the targeted point mutations in the ALS gene without any dispensable sequences and are therefore similar to plants generated by classical mutation breeding techniques.
2. The *piggyBac* mediated transposition system could be used not only in rice but also in other plant species. The development of an effective GT system with positive-negative selection could facilitate pinpoint mutagenesis of target gene in several plant species.



B

Line no.	No. of plants analyzed	*piggyBac* excision from *ALS* locus (%)		
		Without re-integration [(a)]	With re-integration [(b)]	Total [(a+b)]
5	20	19	1	20
6	20	20	0	20
10	20	20	0	20
13	20	20	0	20
24	20	20	0	20
		99 (**99%**)	1 (1%)	100 (**100%**)

Fig. 1. Strategy for the introduction of point mutations into the *ALS* locus via GT and subsequent marker excision from GT locus using *piggyBac* transposon. (A) Schematic diagram of GT at the *ALS* locus. Step1:The point mutations conferring resistance to herbicide (red star) and positive selection marker were introduced into the *ALS* locus by homologous recombination. Step2:GT calli were again infected with *Agrobacterium* harboring a transposase (PBase) expression vector and the positive selection marker was excised by *piggyBac* transposition. (B) The frequency of *piggyBac*-mediated marker excision in PBase-expressing regenerated plants.

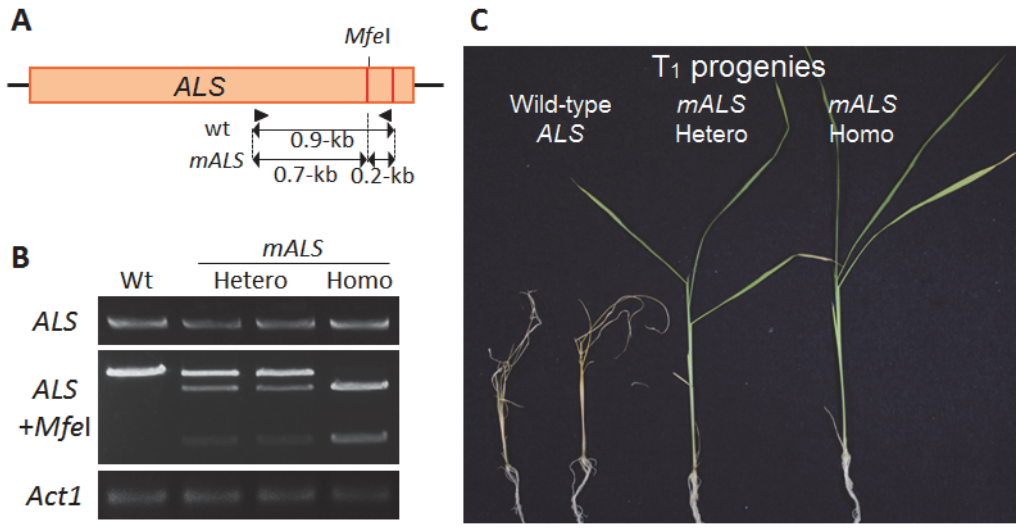


Fig. 2. Analysis of the *ALS* gene harboring mutations in T_1 progenies. (A) Diagram showing the targeted *ALS* locus. (B) CAPS analysis combining PCR analysis using *ALS* gene-specific primers (arrowheads) with cDNA and *MfeI* digestion in T_1 plants carrying the wild-type (wt) or modified *ALS* gene (heterozygous or homozygous). (C) Herbicide-tolerant phenotype of T_1 plants.

Reference

1. Nishizawa-Yokoi A, Endo M, Ohtsuki N, Saika H, Toki S (2015) Precision genome editing in plants via gene targeting and *piggyBac*-mediated marker excision ***The Plant Journal*** 81 (1):160-168

Intellectual Contribution

Structural basis for the coevolution of *Tomato mosaic* virus and the resistance protein Tm-1

Kazuhiro Ishibashi[1], Chihoko Kobayashi[2], Masahiko Kato[2], Masayuki Ishikawa[1], Etsuko Katoh[3]

[1] Plant-Microbe Interactions Research Unit, [2] Biomolecular Research Unit

We determined the crystal structures for the complex between the N-terminal inhibitory domain of Tm-1 and helicase domain of tomato mosaic virus replication proteins (ToMV-Hel). The complex contains a Tm-1 dimer and two ToMV-Hel monomers, with the Tm-1-ToMV-Hel interface bridged by an ATPγS. Residues in ToMV-Hel and Tm-1 involved in antagonistic coevolution are also found at the interface. The crystal structures provide an atomic view of step-by-step coevolutionary arms race between a plant resistance protein and a viral protein.

Keywords:tomato mosaic virus, crystal structure, protein complex, arms race, coevolution

Background

Viruses evolve so rapidly that they can escape host defense systems. As a counter strategy, the sequences of many host restriction factor genes are subject to positive selection and, consequently, evolve rapidly. Molecular evolutionary approaches in conjunction with the tertiary structures of related proteins may provide useful information on virus–host evolutionary arms races. We previously found that the resistance protein Tm-1 binds the tomato mosaic virus (ToMV) replication proteins thereby inhibiting RNA replication, and that a part of the *Tm-1* gene has been under positive selection. In this study, we aimed to clarify the atomic details of the coevolutionary arms race between them through crystal structure determination and molecular dynamics simulation.

Results and Discussion

1. The ToMV replication proteins are involved in RNA replication and harbor a superfamily 1 (SF1) helicase-like domain (ToMV-Hel). Determination of the crystal structure of ToMV-Hel revealed a novel N-terminal domain tightly associated with a helicase core. Prediction of secondary structures in other viral SF1 helicases and comparison of those structures with that of the ToMV-Hel suggested that many viral SF1 helicases have a similar fold.
2. We determined a crystal structure of a complex of an N-terminal fragment of Tm-1 (residues 1–431:referred to herein as Tm-1 (431)), which is sufficient for the inhibitory activity, and ToMV-Hel. The structure of Tm-1 (431) and ToMV-Hel complex shows a tetrameric complex, comprised of a Tm-1 (431) dimer and two monomeric ToMV-Hel. Notably, an ATPγS molecule is found in each ToMV-Hel–Tm-1 (431) interface and ATP is required for the complex formation.
3. The residues in ToMV-Hel that are changed in the resistance-breaking mutant LT1, which has Q979 to E and H984 to Y substitutions, are directly involved in the interaction. The positively selected region of Tm-1 forms the binding surface with ToMV-Hel.
4. A naturally occurring amino acid change (I91 to T) in Tm-1 makes it a stronger inhibitor of ToMV RNA replication, which enables it to inhibit the replication of LT1. We also solved the structure of the ToMV-Hel–Tm-1 (431/I91T) complex. The overall structure of this complex is very similar to that of the ToMV-Hel–Tm-1 (431) complex. In the ToMV-Hel–Tm-1 (431/I91T) structure, T91 is located at the center of the interface with ToMV-Hel, and is involved in a hydrogen bond network containing water molecules. The structural information reasonably explains how the I91 to T substitution strengthens the inhibitory activity of Tm-1.
5. Based on the crystal structure, we simulated how the resistance-breaking mutations in ToMV-Hel affect the interaction with Tm-1. Together with all above results, an atomic view of the step-by-step coevolutionary arms race between a plant resistance protein and a viral protein emerged.

Future prospects

1. The structures revealed here provide useful information in developing new anti-viral drugs.
2. Although co-evolution between ToMV-Hel and Tm-1 has been described based on the structure, there are many unclear points for ToMV replication. We are going to obtain the structural information of the full length replication protein.

Fig. 1. ToMV-inoculated nontransgenic (left) and transgenic tomato expressing the *Tm-1* gene (right).

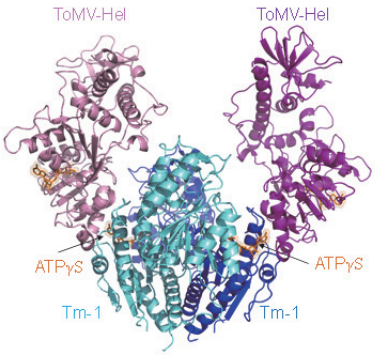


Fig. 2. Crystal structure of the ToMV-Hel and Tm-1 (431) complex. Tm-1 (431) molecules are shown in blue and cyan, and ToMV-Hel molecules shown in violet and light pink.

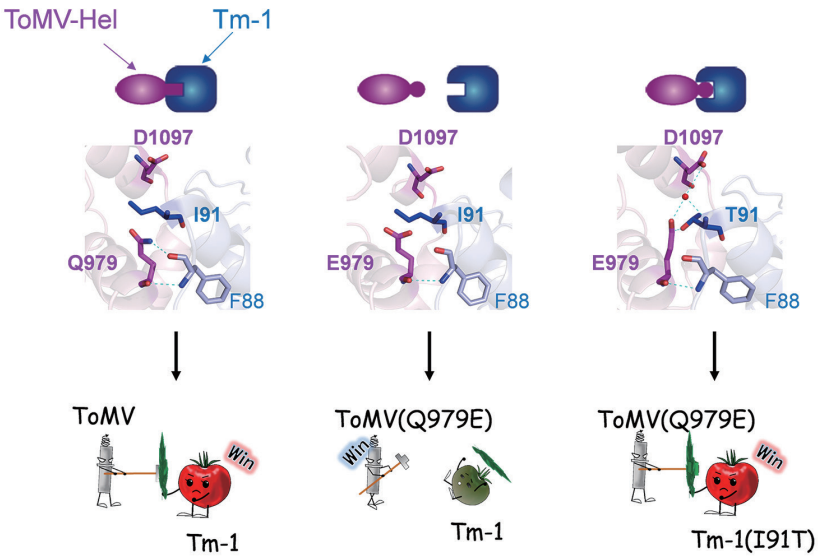


Fig. 3. The arms race between ToMV-Hel and Tm-1. Left:Tm-1 binds ToMV-Hel and thereby inhibits RNA replication. I91 of Tm-1 makes hydrophobic interaction with Q979 and D1097 of ToMV-Hel. Center:When Q979 is replaced by E, Tm-1 cannot bind ToMV-Hel and viral replication is allowed. Right:A naturally occurring amino acid change (I91 to T) in Tm-1 renders the ability to bind ToMV-Hel with the Q979E substitution.

Collaborators

Yuichiro Kezuka, Takamasa Nonaka (Iwate Medical University), Tsuyoshi Inoue, Hiroyoshi Matsumura (Osaka University)

References

1. Ishibashi K, Kezuka Y, Kobayashi C, Kato M, Inoue T, Nonaka T, Ishikawa M, Matsumura H, Katoh E (2014) Structural basis for the recognition-evasion arms race between *Tomato mosaic virus* and the resistance gene *Tm-1* ***Proceedings of the National Academy of Sciences of the United States of America*** 111 (33):E3486-E3495
2. Kato M, Kezuka Y, Kobayashi C, Ishibashi K, Nonaka T, Ishikawa M, Katoh E (2013) Crystallization and preliminary X-ray crystallographic analysis of the inhibitory domain of the tomato mosaic virus resistance protein Tm-1 ***Acta Crystallographica Section F*** 69 (12):1411-1414
3. Nishikiori M, Sugiyama S, Xiang H, Niiyama M, Ishibashi K, Inoue T, Ishikawa M, Matsumura H, Katoh E (2012) Crystal structure of the superfamily 1 helicase from *Tomato mosaic virus* ***Journal of Virology*** 86 (14):7565-7576

Intellectual Contribution

Isolation and genome analysis of biocontrol *Pseudomonas* strains

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[1]Plant-Microbe Interactions Research Unit, [2]Agrogenomics Research Center

We have isolated three strains of pseudomonads, namely, Cab57, Os17 and St29 that suppress plant diseases in the rhizosphere that could facilitate effective and ecological applications of biocontrol agents. The strain Cab57 was identified as *Pseudomonas protegens*, whereas strains Os17 and St29 were found to be the closest to *P. protegens* although they showed different 16S rRNA pattern. Comparative genome analysis also revealed the biocontrol factors which are important for the plant protection efficacy of the strain Os17.

Keywords:plant protection, biocontrol strains, comparative genome analysis

Background

Pseudomonas protegens and some other root-colonizing pseudomonads classified into the *Pseudomonas fluorescens* group are effective biocontrol strains, which suppress plant diseases in the rhizosphere. Screening for plant beneficial pseudomonads has led to advances in biocontrol research particularly in effective and ecological application of biocontrol agents. In this study, we aimed to screen biocontrol pseudomonad strains relative to *P. protegens* in the fields in Japan. We also identified biocontrol factors which are important for the plant protection efficacy through the genomic comparison and characterization of each strain.

Results and Discussion

1. Approximately 2,800 fluorescent pseudomonads were obtained from plant roots. Based on PCR analysis, a total of 48 isolates were selected as candidates of 2,4-diacetylphloroglucinol-producing (*phlD*$^+$) strains,. Among them, Cab57 was identified as *P. protegens* based on 16S rRNA gene analysis (with 100% identity) and whole-genome analysis. The genome is organized into a single circular chromosome with 6.8 Mbp (Table 1).
2. Strains Os17 and St29, which organized into a single circular chromosome with 6.9 Mbp and 6.8 Mbp, respectively, were found to belong to the same species. Although these strains were relatively closest to *P. protegens*, they were also found to be different based on 16S rRNA gene and whole-genome analyses (Table 1).
3. The genes associated with Gac/Rsm signal transduction pathway are fully conserved in these strains as reported in *P. protegens*. Strain Cab57 was found to exhibit typical Gac/Rsm activities and antibiotic production, which were enhanced by knocking-out the *retS* gene, a sensor kinase acting as an antagonist of GacS (Fig. 1A).
4. Strains Cab57 and Os17 showed prominent plant protection efficacy at the same level of strain *P. protegens* CHA0, whereas strain St29 was less effective (Table 1).
5. Comparative genome analysis revealed that the complete rhizoxin analog biosynthesis gene cluster (ca. 79 kb) found in the Os17 genome was absent in the St29 genome. In an *rzxB* mutant, which lacks the polyketide synthase essential for the production of rhizoxin analogs, the growth inhibition activity against fungal and oomycete pathogens (Fig. 1B) and the plant protection efficacy were attenuated as compared with those of wild-type Os17. These findings suggest that rhizoxin analogs are important biocontrol factors of this strain.

Future prospects

1. Strains Cab57 and Os17 are effective for the application as biocontrol agents.
2. These strains are also useful as antibiotic producers.

Table 1. General genomic features and comparison of plant protection efficacy of *Pseudomonas protegens* CHA0, *P. protegens* Cab57, *Pseudomonas* sp. Os17, and *Pseudomonas* sp. St29.

	CHA0*	Cab57	Os17	St29
Genome size（bp）	6,867,980	6,827,892	6,885,464	6,833,117
Coding sequence number	6,115	6,186	6,195	6,217
G+C content（%）	63.4	63.3	63.5	63.3
Gac/Rsm homologs	conserved	conserved	conserved	conserved
Plant protection efficacy	++	++	++	+

* Jousset *et al.*, ***Genome Announcement*** 2014 2(2): e00322-14.

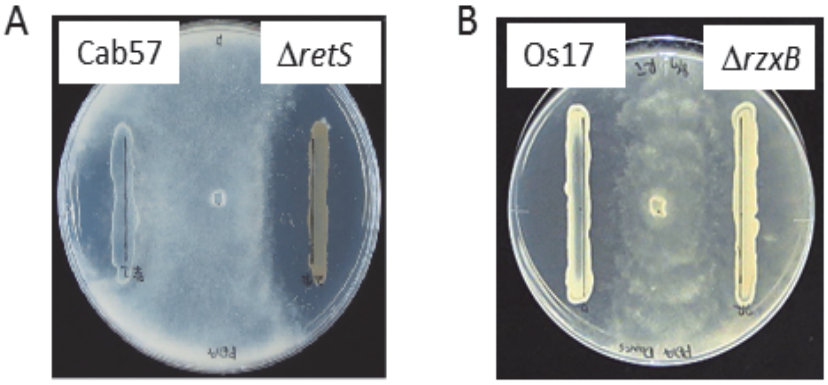

Fig. 1. Antibiotic activities of *Pseudomonas* strains toward *Pythium ultimum*. (A) Antibiotic activity of *P. protegens* Cab57 wild type was enhanced by knocking-out the *retS* gene. (B) Antibiotic activity of *Pseudomonas* sp. Os17 wild type was attenuated by knocking-out the *rzxB* gene.

Collaborators

Kosumi Yamada (University of Tsukuba), Nobutaka Someya (National Agriculture and Food Research Organization)

References

1. Takeuchi K, Noda N, Someya N (2014) Complete genome sequence of the biocontrol strain *Pseudomonas protegens* Cab57 discovered in Japan reveals strain-specific diversity of this species ***PLoS ONE*** 9 (4):e93683
2. Takeuchi K, Noda N, Katayose Y, Mukai Y, Numa H, Yamada K, Someya N (2015) Rhizoxin analogs contribute to the biocontrol activity of newly isolated *Pseudomonas* strain ***Molecular Plant-Microbe Interactions*** 28 (3):333-342
3. Patent application #JP-2015-118395 (Japan)

Development of multi-disease resistant rice with increased yield by optimizing gene expression level of *WRKY45*

Hiroshi Takatsuji, Shingo Goto, Fuyuko Shimoda-Sasakura, Mai Suetsugu, Michael Gomez Selvaraj, Nagao Hayashi, Muneo Yamazaki, Manabu Ishitani, Masaki Shimono, Shoji Sugano, Akane Matsushita, Takanari Tanabata

Disease Resistant Crops Research Unit

Driving the gene for the rice transcription factor WRKY45 with a strong promoter rendered the rice plants extremely resistant to multiple diseases at the cost of significant reduction in yield. We have succeeded in developing rice plants that are resistant to multiple diseases while retaining good yield.

Keywords:rice, blast disease, chemical defense inducer, WRKY45, *OsUbi7* promoter

Background

Yield of rice decreases due to various infectious diseases such as blast disease caused by fungal pathogen and leaf-blight disease caused by bacterial pathogen. WRKY45 is a rice transcription factor that plays a central role in the functioning of chemical defense inducers, such as probenazole and benzothiadiazole. Overexpression of its gene in rice rendered rice plants extremely resistant to multiple diseases including rice blast and leaf blight (multi-disease resistance), which should lead to drastically reduced necessity of pesticides. However, driving *WRKY45* gene under the control of strong promoter (P_{ZmUbi}) severely reduced the yield. Here, we attempted to optimize *WRKY45*-expressing rice by driving *WRKY45* cDNA with several rice-derived promoters of various strengths to develop practical multi-disease rice.

Results and Discussion

1. We fused 2-kb upstream sequences of 16 rice genes, which have different constitutive expression levels, upstream of *WRKY45* cDNA and obtained a large number of transformant lines (T_0). Of these, we selected homozygous lines that showed resistance to both rice blast and leaf blight diseases.
2. Evaluation of the growth and yield of the homozygous lines in confined greenhouse revealed that *OsUbi7*-promoter-driven *WRKY45* expression rice lines (P_{OsUbi7}) showed the best balance in terms of multi-disease resistance and rice yield.
3. Field trials in confined fields in Japan and overseas (Korea and Columbia) showed that P_{OsUbi7} lines showed growth and yield very similar to those of control untransformed Nipponbare rice (Fig. 1). A nursery upland experiment revealed that P_{OsUbi7} plants have strong resistance to rice blast.
4. P_{OsUbi7} lines were resistant to all 4 races of blast fungus tested and 6 races of leaf blight pathogens (*Xoo*) tested including 3 races isolated in foreign countries, indicating that the disease resistance of P_{OsUbi7} lines is race-nonspecific (Fig. 2).
5. Exposure to low temperature (8℃ , 7 d) followed by room temperature in nursery killed most of P_{ZmUbi} plants, while Nipponbare plants survived (Fig. 3). Treatment with 250 mM NaCl for 3 d also killed only the the P_{ZmUbi} plants. Defense genes such as *PR* genes were induced under both the stress conditions in the P_{ZmUbi} plants but not in Nipponbare suggesting a relationship between the gene expression and the stress hypersensitivity of the P_{ZmUbi} plants. Unlike the P_{ZmUbi} plants, P_{OsUbi7} plants survived the stress conditions similar to Nipponbare (Fig. 3). These results indicate that the use of the *OsUbi7* promoter for driving *WRKY45* saved the stress hypersensitivity resulting from *WRKY45* overexpression.

Future prospects

1. We are now selecting the best P_{OsUbi7} lines with a genetic background of forage rice towards the development of practical multi-disease resistant forage rice.
2. We are also developing a *WRKY45* expression line in which *WRKY45* is driven by a pathogen-inducible promoter, and can be cultivated without using pesticides.
3. Foreign companies and organizations are interested in our technology.

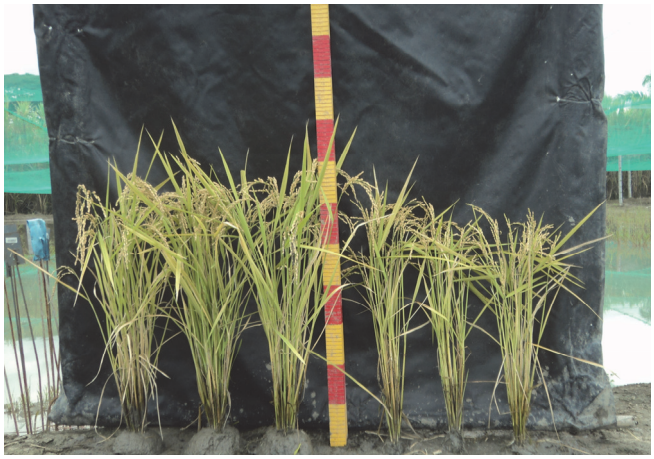

Fig. 1. Field trial in a confined field in Colombia.
P_{ZmUbi} plants (right) show poorer growth and less yield but P_{OsUbi7} plants (left) showed comparable agronomical traits comparable with Nipponbare.

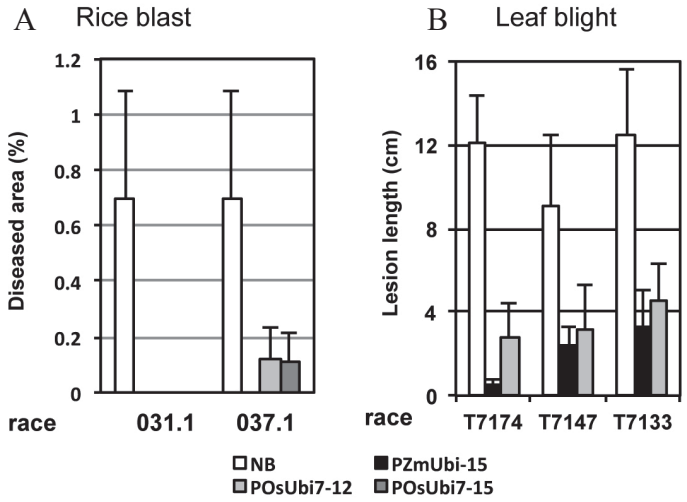

Fig. 2. Disease resistance of *WRKY45* expression lines.
P_{ZmUbi}, P_{OsUbi7}, and Nipponbare plants were tested for disease resistance.
(A) Blast resistance against 2 races of fungal pathogen.
(B) Leaf blight resistance against 3 races of bacterial pathogen isolated in Japan.

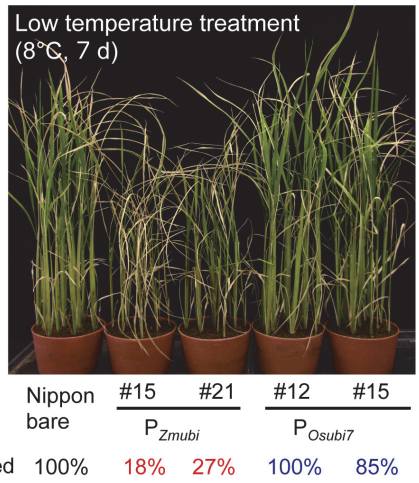

Fig. 3. Low-temperature sensitivity of *WRKY45* expression lines. Most of P_{ZmUbi} plants exposed to low temperature (8℃) for 7d, and transferred to room temperature died,whereas the P_{OsUbi7} and Nipponbare plants survived.

Reference

1. Goto S, Sasakura-Shimoda F, Suetsugu M, Selvaraj M.G, Hayashi N, Yamazaki M, Ishitani M, Shimono M, Sugano S, Matsushita A, Tanabata T, Takatsuji H (2014) Development of disease-resistant rice by optimized expression of *WRKY45* ***Plant Biotechnology Journal*** DOI:10.1111/pbi.12303
2. International publication #WO2012/121093

Intellectual Contribution

Identification and characterization of a novel carrier protein involved in ant chemical communication

Toshimasa Yamazaki[1], Wataru Tsuchiya[1], Zui Fujimoto[1], Mitsuhiro Miyazawa[2], Jun Ishibashi[2]

[1]Biomolecular Research Unit, [2]Insect Mimetics Research Unit

A new carrier protein involved in ant chemical communication was identified and characterized. The ligand-binding pocket of this protein was composed of a flexible β-structure that allowed it to bind and deliver a wide range of hydrophobic semiochemicals. The defined molecular mechanism of ligand recognition may help us to develop new tools for pest ant management.

Keywords:worker ant, semiochemical, carrier protein, structure-based drug design

Background

Ants are eusocial insects that distribute tasks among individuals belonging to different castes. To fulfill caste-specific tasks, ants have developed a sophisticated system of chemical communication using sensory organs (sensilla) that detect molecules carrying task-specific information (semiochemicals). The antennae are the major chemosensory organs in ants. Once the semiochemicals have entered the sensillum through pores, they reach the aqueous sensillum lymph, which contains carrier proteins that bind the hydrophobic molecules and deliver them to various chemoreceptors residing in the membrane of the neuron's dendrites (Fig. 1). Worker ants are responsible for various tasks that are required for colony maintenance. In their chemical communication, α-helical carrier proteins, odorant-binding proteins (OBPs) and chemosensory proteins (CSPs), which accumulate in the sensillum lymph in the antennae, play roles in transporting semiochemicals to chemoreceptors. However, the number of these carrier proteins is not sufficient to bind the large number of semiochemicals that are recognized by ants. It is therefore hypothesized that there are undefined carrier proteins involved in chemical communication in worker ants and some of them must possess a ligand-binding pocket that interacts with a variety of semiochemicals. Screening for worker-antenna-specific genes in the Japanese carpenter ant, *Camponotus japonicas*, enabled us to identify a novel carrier protein that is capable of delivering various hydrophobic semiochemicals to chemosensory receptor neurons.

Results and Discussion

1. We identified a worker-antenna-specific gene that is involved in chemical communication of the Japanese carpenter ant, *Camponotus japonicas*. We named the protein encoded by this gene CjapNPC2 due to its high similarity to the Niemann-Pick type C2 (NPC2) protein which is an essential carrier protein for intracellular cholesterol transport in vertebrates including human. CjapNPC2 was exclusively expressed in the antennae and was specifically accumulated in the lymph-filled cavities of the basiconic sensilla.
2. Ligand binding studies revealed that CjapNPC2 is able to bind various hydrophobic molecules including long-chain fatty acids, alcohols and acetates at neutral pH but not at an acidic pH, suggesting the pH-dependent ligand binding and dissociation that are characteristic features of the carrier protein. In addition, some of the tested ligands can provide electrophysiological signals in the antenna of worker ants. It is noteworthy that CjapNPC2 is not able to bind cholesterol while the vertebrate NPC2 does not bind to long-chain fatty acids (Fig. 2).
3. The crystal structures of the apo and oleic acid-bound CjapNPC2 unveiled the molecular mechanism of the ligand recognition which distinct from those by the α-helical carrier proteins of OBP and CSP (Fig. 3). CjapNPC2 adopts a β-sandwich structure with a large hydrophobic cavity for binding of the ligand in a U-shaped conformation (Fig. 3A). Intrinsic flexibility of the ligand-binding cavity of CjapNPC2, particularly at the entrance regions, may contribute to its moderate selectivity and thus facilitate entry and binding of a wide range of potential semiochemicals.

Future prospects

1. As the function of CjapNPC2 is quite different from that known in vertebrates, this protein is an attractive target for development of new tools for pest ant management.
2. The defined molecular mechanism of ligand recognition by CjapNPC2 should open the door to the structure-based design of safe ant insecticides that disrupt chemical communication.

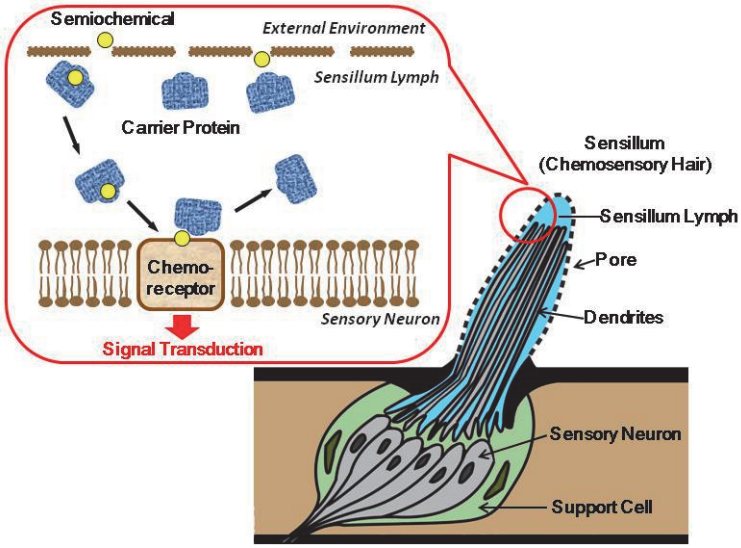

Fig. 1. Schematic representation of the general structure of an ant olfactory hair. The ant chemosensory signal transduction pathway is initiated by transport of a semiochemical molecule in the sensillum lymph as a complex with its carrier protein to the proper chemoreceptor.

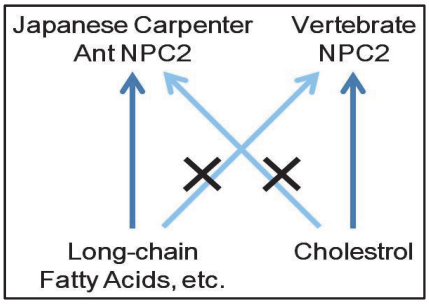

Fig. 2. Ligand selectivity of Niemann-Pick type c2 proteins (NPC2). CjapNPC2 from the Japanese carpenter ant can bind and deliver various potential semiochemicals including long-chain fatty acids, alcohols and acetates but not cholesterol. In contrast, vertebrate NPC2, does not bind long-chain fatty acids.

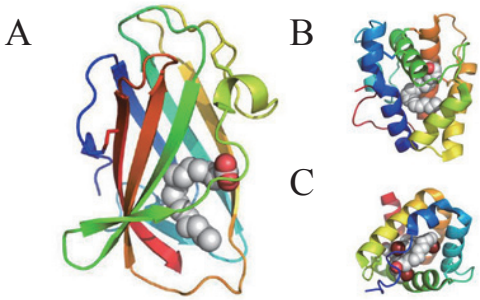

Fig. 3. Structures of semiochemical carrier proteins in insects. (A) Niemann-Pick type C2 protein from the Japanese carpenter ant (CjapNPC2) in complex with oleic acid. (B) Odrant-binding protein. (C) Chemosensory protein. Bound molecules are shown as space-filling models.

Collaborators

Yuko Ishida (Toyama Prefectural University), Takeshi Fujii, Yukio Ishikawa (University of Tokyo), Shigeru Matsuyama (University of Tsukuba)

Reference

1. Ishida Y, Tsuchiya W, Fujii T, Fujimoto Z, Miyazawa M, Ishibashi J, Matsuyama S, Ishikawa Y, Yamazaki T (2014) Niemann–Pick type C2 protein mediating chemical communication in the worker ant ***Proceedings of the National Academy of Sciences of the United States of America*** 111 (10):3847-3852

Intellectual Contribution

Synergistic defensive function of raphides and protease

Kotaro Konno[1], Takashi A. Inoue[1], Masatoshi Nakamura[2]

[1]Insect-Plant Interaction Research Unit, [2]Genetic Resource Conservation Research Unit

We clarified that raphides, calcium oxalate needle crystals, exert strong insecticidic or growth-inhibiting activities against insects and function as a defense of plants against herbivorous insects by synergistically intensifying the defense activity of cysteine protease that coexist with raphides in plant tissues.

Keywords:raphide, calcium oxalate, needle crystal, cysteine protease, plant defense, plant-insect interaction

Background

Raphides, tiny needle-shaped calcium oxalate crystals, are present in large amount in tissues of many plant species including kiwifruit, pineapple, taro, yam, and grape. Although raphides may play defensive roles against herbivores, there are no direct experimental evidence showing their modes of function based on purified raphides. Since raphides frequently coincide in the same plant tissue with other defensive substances such as protease in the case of kiwifruit and pineapple, we hypothesized that raphides make holes in plant tissues and/or cell membranes and facilitate other defense substances to reach their targets, thereby intensifying the defense activity of defense substances, which can be called the needle effect. Therefore, we performed clear bioassays feeding the larvae of the Eri silkmoth (*Samia ricini*) with leaves from the host castor oil plant (*Ricinus communis*) painted with the raphides purified from kiwifruits (*Actinidia deliciosa*) in the presence or absence of cysteine protease (or other defense proteins) that often coincide with raphides in plant tissues.

Results and Discussion

1. We successfully purified raphides by homogenizing kiwifruit tissues in heavy liquid (dense CsCl solution with a specific gravity of 1.8), centrifugation of the homogenate, and collecting the precipitate with specific gravity of more than 2. The collected raphides were very sharp with a length of 0.1mm (Fig. 1).
2. A much stronger defense activity was observed in the presence of both raphides and cysteine protease than either raphides or cysteine protease only (Fig. 2, Table 1). When neonate larvae of the Eri silkmoth were fed with leaves of castor oil plant painted with either 41.7μg/cm^2 of raphides (Fig. 2B) or 0.22mg/cm^2 of cysteine protease only (Fig. 2C), the mortality rate was very low (0%) and the larvae grew nearly as well as when they were fed with unpainted castor oil plant leaves (Fig. 2A). Even when the larvae were fed with leaves painted twice the concentration of either raphides or cysteine protease, defensive activities remained weak. In contrast, when larvae were fed with leaves painted with both raphides and cysteine protease together, extremely strong defense activity was observed with larval mortality of 69% without any trace of growth, the larvae died within two hours and the body turned black and soft (Fig. 2D). These results indicated that raphides and cysteine protease exert synergistic defensive function against insect herbivores. Our results also clarified that the toxicity of cysteine protease was intensified to 16-32 times in the presence of a small amount of raphides (Table 1).
3. The needle-like shape is an essential factor in the synergistic defense effect of raphides; amorphous calcium oxalate crystal and cysteine protease did not show any synergistic effect.
4. Raphides also showed synergistic defense activities with chitinase as well as protease suggesting that raphides may function as a general intensifier of various defense substances.

Future prospects

1. Synergism between raphides and other defensive substances will facilitate a deeper understanding of the defense mechanisms of many cultivated plants that contain raphides such as kiwifruit, pineapple, taro, yam, and grape.
2. Raphides can possibly be used as an intensifier of agrochemicals and defense proteins, and the synergistic function may help in developing insect resistant crop varieties.

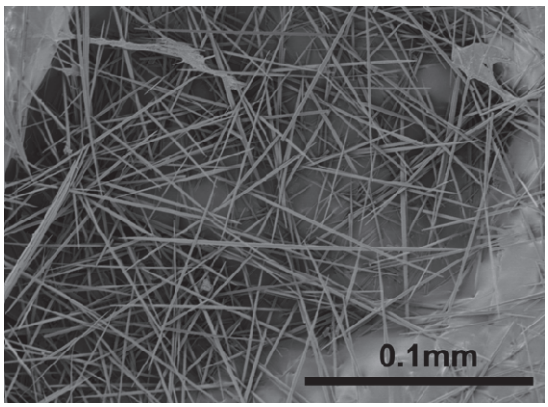

Fig. 1. Raphides, needle-shape calcium oxalate crystals that were purified from kiwifruit have very sharp shape measuring ca. 0.1 mm in length.

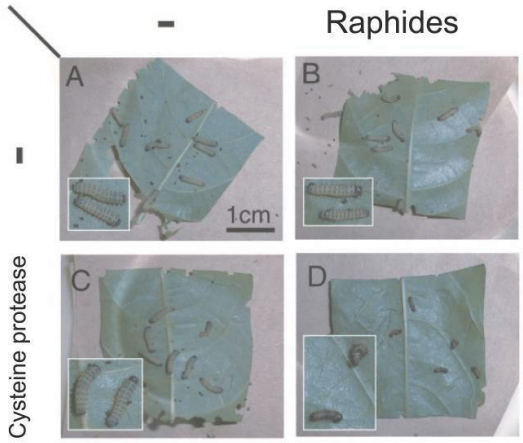

Fig. 2. Synergistic defensive function of raphides and cysteine protease against insects. Leaves painted with raphides alone, cysteine protease alone, or those painted with both are fed to the larvae of Eri silkmoth and defensive activities were compared among treatments. (A) Control (unpainted leaf), (B) Leaf painted with raphides. (C) Leaf painted with cysteine protease, (D) Leaf painted with both raphides and cysteine protease. Strong defensive activities (growth inhibition and insecticidic activity) were observed only when the leaf was painted with both raphides and cysteine protease (D).

Table 1. Numerical relationship between raphides and cysteine protease in terms of synergism defensive effects based on mortalities of Eri silkworm in day 1 (%) are indicated in the table (n=16-17, — ; not tested).

Cysteine protease (mg/cm^2) \ Raphides ($\mu g/cm^2$)	0	5.2	10.4	41.7	83.3
0	0.0	0.0	0.0	0.0	0.0
0.014	0.0	0.0	0.0	0.0	—
0.028	0.0	0.0	0.0	0.0	—
0.056	0.0	31.3	23.5	23.1	—
0.11	0.0	31.3	87.5	37.5	—
0.22	0.0	81.3	87.5	68.8	—
0.44	23.1	—	—	—	—
0.89	0.0	—	—	—	—

Reference

1. Konno K, Inoue T.A, Nakamura M (2014) Synergistic defensive function of raphides and protease through the needle effect ***PLoS ONE*** 9 (3):e91341

Intellectual Contribution

Cloning of the brown planthopper resistance gene *BPH26* from indica rice cultivar induces sucking inhibition

Yasumori Tamura[1], Makoto Hattori[1], Akira Takahashi[2], Jianzhong Wu[3], Naoki Sentoku[4]

[1]Insect-Plant Interaction Research Unit, [2]Disease Resistant Crops Research Unit, [3]Advanced Genomics Laboratory, [4]Functional Plant Research Unit

Brown planthopper (BPH) is a serious pest of rice. A BPH-resistance gene, *BPH26*, was cloned from an indica rice cultivar. It was revealed that although the stylet of BPH could reach the phloem, it could not suck the phloem sap from the rice plants carrying *BPH26* and subsequently died from starvation.

Keywords:*indica* rice cultivar, brown planthopper, *Nilaparvata lugens* Stål, BPH resistance gene, sucking inhibition, *BPH26*, NBS-LRR

Background

The brown planthopper (BPH), *Nilaparvata lugens* Stål, is an important pest of rice (Fig. 1), which causes serious damage to rice cultivation by sucking the phloem sap until the plant dies. Since BPH strains showing resistance to insecticides such as imidacloprid have emerged, the issue of BPH damage to rice cultivation has become more serious. Although it has been reported that *BPH26* could confer resistance to BPH which has recently migrated to Japan because of the coexistence of *BPH25*, the *BPH26* has not been cloned yet. Development and utilization of BPH-resistant rice varieties will promote environment-friendly and low-cost agricultural practices involving limited use of pesticides.

Results and Discussion

1. The chromosomal location and nucleotide sequences, and functions of *BPH26* were analyzed, and DNA markers of *BPH26* were developed for marker-assisted breeding.
2. The *BPH26* was identified using transgenic rice lines with the gene. It was revealed that BPH could not suck the phloem sap from rice varieties carrying *BPH26* and died from starvation, although the stylet could reach the phloem (Fig. 2). When BPH was released on two varieties, one with *BPH26* and the other without *BPH26*, only the variety with *BPH26* survived the insect infestation (Fig. 3 and Fig. 4).
3. The BPH26 protein closely resembles rice NBS-LRR proteins, which are involved in signal perception and transduction during infection by pathogens. This suggests that BPH26 protein is a receptor involved in the signal perception and transduction activated during BPH attack.

Future prospects

1. The *BPH26* gene in coexistence of *BPH25* conferred resistance to the BPH biotype that neither *BPH26* nor *BPH25* was effective against.
2. DNA markers of *BPH25* for marker-assisted breeding are currently under development. A broad-spectrum BPH-resistant variety against some BPH biotypes can be developed by using two DNA markers for the two genes (*BPH25* and *BPH26*).

Fig. 1. A brown planthopper sucking rice phloem sap. The rice plant dies because of the loss of nutrient content in the phloem sap.

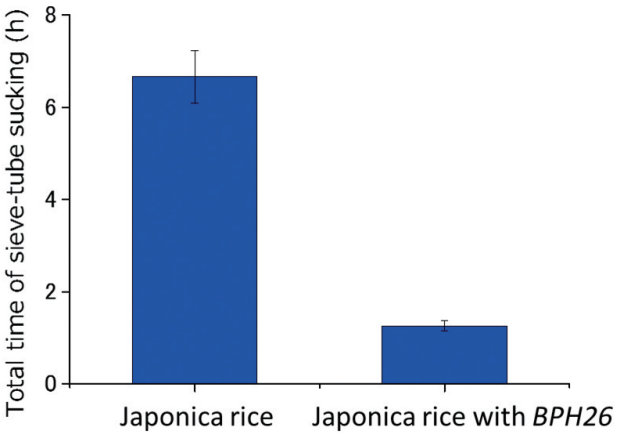

Fig. 2. Total duration of phloem ingestion in the two rice lines during a 10-hour measurement period.

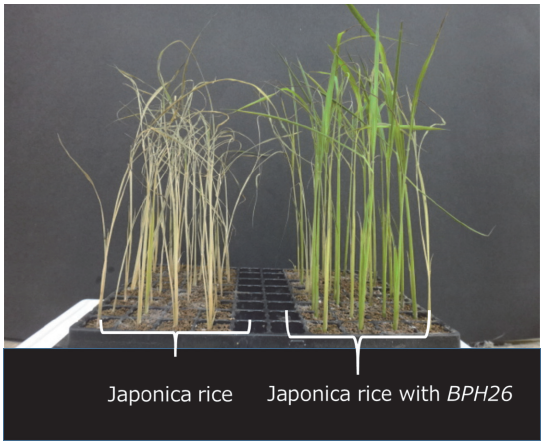

Fig. 3. Rice seedlings after one week of BPH release. Although the susceptible *japonica* rice variety (left) was almost wilted, the rice variety carrying *BPH26* (right) grew vigorously.

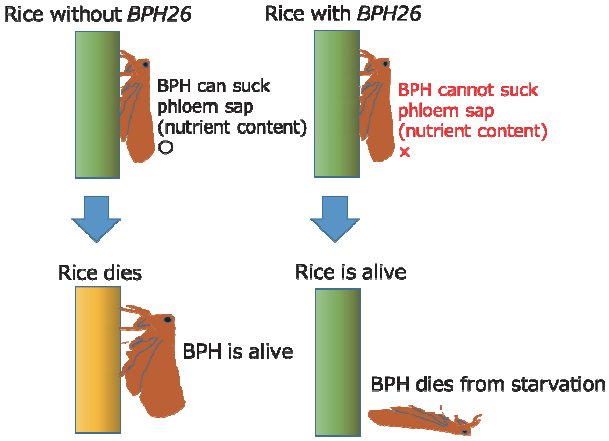

Fig. 4. Diagram showing *BPH26* induced resistance. BPH cannot suck the phloem sap and subsequently dies from starvation.

Collaborators

Hideshi Yasui (Kyushu University), Hirofumi Yoshioka, Miki Yoshioka (Nagoya University)

Reference

1. Tamura Y, Hattori M, Yoshioka H, Yoshioka M, Takahashi A, Wu J, Sentoku N, Yasui H (2014) Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. *indica* cultivar ADR52 ***Scientific Reports*** 4:5872

Start of experimental rearing of transgenic silkworms as 'Type 1 Use' in an isolated zone

Natuo Kômoto[1], Kenji Yukuhiro[1], Tetsuya Iizuka[1], Eiji Okada[1], Mai Tsuda[2], Yutaka Tabei[3], Shuichiro Tomita[1], Makoto Kiuchi[4], Hideki Sezutsu[1]

[1]Transgenic Silkworm Research Unit, [2]Soybean Applied Genomics Research Unit, [3]Research Promotion Section for Genetically Modified Organisms, [4]Genetic Resources Conservation Research Unit

In order to achieve the introduction of transgenic silkworms into sericultural farms, we have started the experimental rearing of transgenic silkworms as a first case in compliance with the 'Type 1 Use' of transgenic animals in Japan, and conducted studies in management approaches and monitoring.

Keywords:transgenic silkworms, Type 1 Use, biodiversity risk assessment, high function silk

Background

We are developing transgenic silkworms which produce silk with high value in order to establish new industries. However, introduction of genetically modified silkworm in sericultural farms requires ministerial approval after application with a biological diversity risk assessment report (Fig. 1). The silkworm, *Bombyx mori*, is an extremely domesticated animal, which is unable to survive or reproduce in nature. Appropriate assessment of the impact of possible crossing with a wild relative, *B. mandarina*, is expected. We reviewed the possibility of incidental crossing that may have occurred around sericultural farms. We have also started experimental rearing of transgenic silkworm producing green-fluorescent silk, with ministerial approval for the usage in an isolated zone in compliance with the 'Type 1 Use' of transgenic animals in Japan, in order to evaluate the impact on biodiversity.

Results and Discussion

1. The mitochondrial *COI* haplotypes of 3,633 *B. mandarina* moths collected in 37 areas in Japan from Hokkaido to Kumamoto are clearly different from those of 147 strains of *B. mori*, showing no signs of crossing between the two species.
2. The fifth-instar larvae of *B. mori* (800 in total) reared outdoor were completely captured by insects and birds and no cocoons were found.
3. There is no statistically-significant difference in walking distance of fifth-instar larvae between transgenic silkworms producing green-fluorescent silk and non-transgenic silkworms (Fig. 2A). The female moths of the transgenic strain laid eggs in smaller areas.
4. There is no statistically-significant difference in the impact of sericultural wastes on the germination and growth of broccoli and soil bacteria (Fig. 2B).
5. For the investigation of impact of transgenic silkworms on biodiversity in the condition of sericultural farms, we have started experimental rearing of silkworms producing green-fluorescent silk as 'Type 1 Use' in an isolated zone (Fig. 3). Monitoring around the zone yielded no hybrids between the transgenic silkworm and wild silkworm, *B. mandarina*.

Future prospects

1. The procedures presented in this study have made it possible to evaluate the impact of transgenic silkworm rearing on biological diversity from the perspective of behavioral characteristics and hazardous substance production. In addition, the data obtained by the experimental rearing in the isolated zone will encourage the approval of transgenic silkworm rearing in sericultural farms.

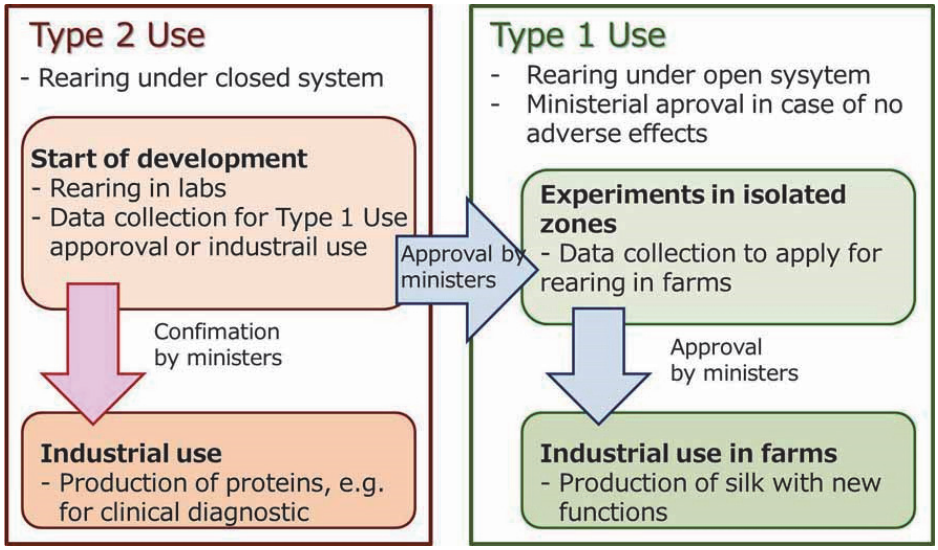

Fig. 1. Development of transgenic silkworms.

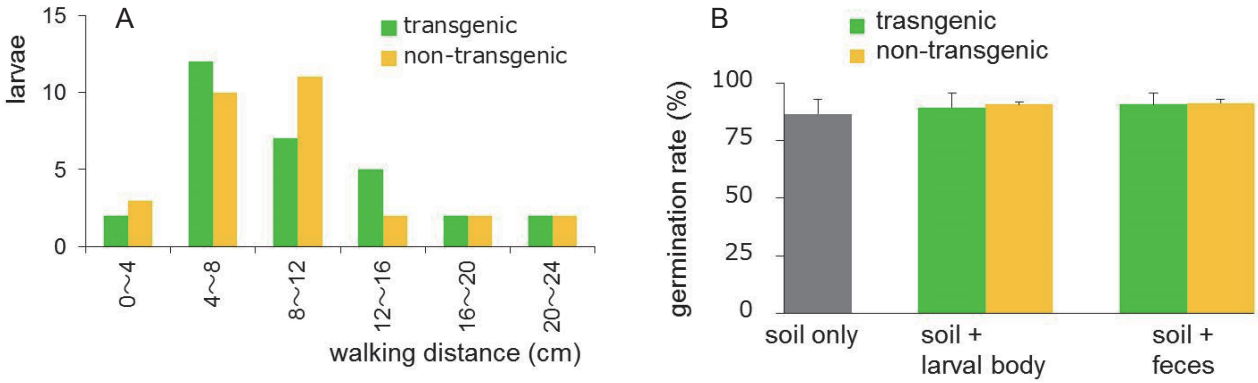

Fig. 2. Comparison of transgenic and non-transgenic silkworms. (A) At walking distance after 16 hours, there was no statistically significant difference between transgenic and non-transgenic silkworm larvae (thirty larvae each, 2nd day of 5th instar). (B) The effect on germination rate was analysed in broccoli seeds sown in soil mixed with larval body or feces of transgenic and non-transgenic silkworms. Five tests with 30 seeds were conducted and a statistically significant difference was not observed.

Fig. 3. Experimental rearing of transgenic silkworms which produce green-fluorescent silk for 'Type 1 Use' in the isolated zone. Left: Feeding silkworms with mulberry leaves. Right: Rearing house in the isolated zone.

Reference

1. Yukuhiro K, Iwata K, Kômoto N, Tomita S, Itoh M, Kiuchi M (2012) Nucleotide sequences of mitochondrial cytochrome C oxidase subunit I (COI) gene show clear differences between the domesticated silkmoth *Bombyx mori* and the wild mulberry silkmoth *Bombyx mandarina* from Japan ***Journal of Insect Biotechnology and Sericology*** 81 (1):29-35

Advances in transgenic silkworm screening and gene knock-in

Takuya Tsubota[1], Keiro Uchino[1], Takao K. Suzuki[1], Hiromitsu Tanaka[2], Takumi Kayukawa[3], Takaaki Daimon[3], Tetsuro Shinoda[3], Hideki Sezutsu[1]

[1]Transgenic Silkworm Research Unit, [2]Insect-Microbe Research Unit, [3]Insect Growth Regulation Research Unit

We identified a novel strong and ubiquitous promoter in the silkworm. Using this promoter, screening of the transgenic silkworm in the embryonic stage has become very efficient. We also developed a novel technique to integrate exogenous gene into the targeted chromosome via genome editing. Efficient selection of knock-in individuals is now possible by using these techniques.

Keywords:promoter, genome editing, knock-in, gene functional analysis, useful material production

Background

Silkworm is frequently used for the production of useful materials according to its high ability to generate recombinant proteins. However, the efficiency to produce transgenic silkworm is not high and a novel technique to select transgenic silkworm easily at an early developmental stage has been required. In addition, the amount of recombinant protein expressed using transgenic silkworm is not high enough. Gene knock-in is a promising technique to increase protein expression level but this technique has not been available in the silkworm. Thus, we attempted to improve the screening technique and develop novel knock-in technology.

Results and Discussion

1. The *hsp90* gene was found to be expressed in a strong and ubiquitous pattern in all developmental stages (embryo, larva, pupa and adult) of silkworm. In addition, the promoter fragment responsible for such an expression pattern was isolated.
2. Transgenic silkworm expressing the *Green Fluorescent Protein* (*GFP*) gene using *hsp90* promoter showed strong and ubiquitous GFP expression in various developmental stages (Fig. 1). In particular, GFP expression could be detected for at least five days during the embryonic stage suggesting that *hsp90* promoter can be utilized for the easy screening of transgenic silkworm embryo.
3. Novel knock-in technique was developed using this promoter. Although homologous recombination-mediated knock-in was very difficult in the silkworm, utilization of programmable nucleases and MMEJ repair mechanism succeeded in the efficient integration of *hsp90* promoter and GFP into the silkworm urate granule formation gene (Fig. 2).
4. The exogenous gene was inserted in a very precise manner suggesting that this gene knock-in technique is a very versatile tool.

Future prospects

1. Screening of the transgenic silkworm has become very easy using the *hsp90* promoter. This promoter also enables the detailed analysis of each gene function to facilitate the study for efficient production of useful materials or the development of novel insecticides.
2. Integration of exogenous genes into the highly-active endogenous genomic locus will also be possible using the novel knock-in technique to facilitate the production of large amount of useful materials. In addition, this technique will also contribute to the development of novel high-performance silk.

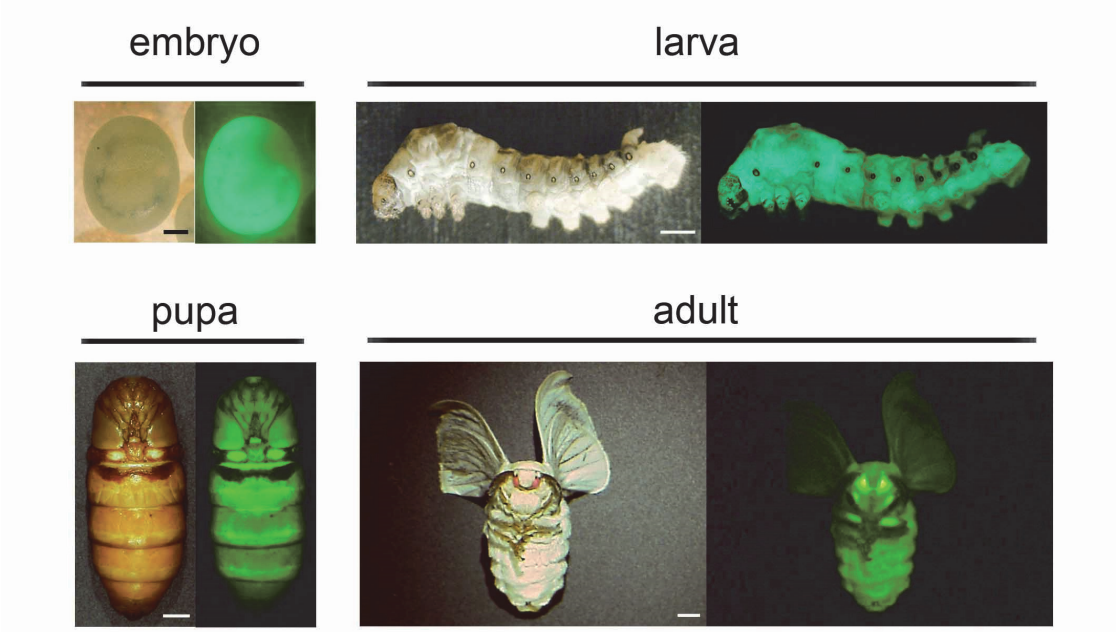

Fig. 1. Induction of *GFP* expression by *hsp90* promoter in the embryo, larva, pupa and adult of transgenic silkworm. The left panel of each figure indicates the bright-field image and the right shows GFP-fluorescence. *GFP* is expressed in green-colored region. *hsp90* promoter is ubiquitously active in all of the developmental stages. Scale bar is 0.3mm in the embryo, 1mm in the larva and 3mm in the pupa and adult. Partially modified from ***G3*:*Genes, Genomes, Genetics*** doi:10.1534/g3.114.011643.

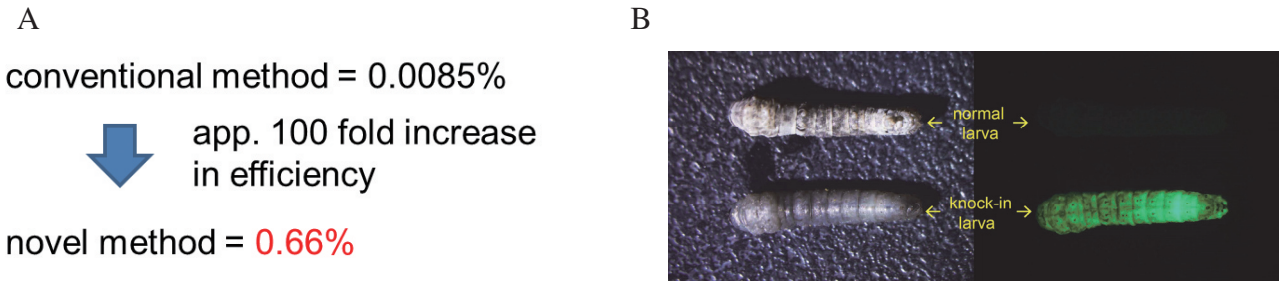

Fig. 2. Development of a novel knock-in technology. (A) Comparison of the knock-in efficiency between the conventional and novel method. Around 100 fold increase was achieved using the novel technique. (B) Images of the knock-in silkworm. The left panel indicates the bright-field image and the right panel shows GFP-fluorescence. The upper and lower individuals depict the normal and knock-in larva, respectively. In the knock-in larva the oily skin phenotype can be observed according to the disruption of urate granule formation gene. In addition, the larva shows ubiquitous green fluorescence due to the insertion of *GFP* gene. Partially modified from ***Nature Communications*** doi:10.1038/ncomms6560.

Collaborators

Shota Nakade, Yuto Sakane, Satoshi Kume, Naoaki Sakamoto, Masanobu Obara, Takashi Yamamoto, Tetsushi Sakuma, Ken-ichi T. Suzuki (Hiroshima University)

References

1. Tsubota T, Uchino K, Suzuki T.K, Tanaka H, Kayukawa T, Shinoda T, Sezutsu H (2014) Identification of a novel strong and ubiquitous promoter/enhancer in the silkworm *Bombyx mori* ***G3*:*Genes, Genomes, Genetics*** 4 (7):1347-1357
2. Nakade S, Tsubota T, Sakane Y, Kume S, Sakamoto N, Obara M, Daimon T, Sezutsu H, Yamamoto T, Sakuma T, Suzuki K.T (2014) Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9 ***Nature Communications*** 5:5560
3. International publication #WO2015/022971

“Tough Silk” produced by transgenic silkworm expressing spider dragline silk protein

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[1]Silk Materials Research Unit, [2]Transgenic silkworm Research Unit

A transgenic silkworm which produces new silk material combining the tensile strength and elasticity of spider dragline silk has been successfully generated. This new spider-type silk, “Tough Silk” is 1.5 times tougher as compared to normal silk. A processing method similar to normal silk has also been successfully applied in production of textile using “Tough Silk”.

Keywords:garden spider, transgenic silkworm, dragline, toughness, silk

Background

Developing a transgenic silkworm which expresses the modified spider dragline protein in cocoon silk fibroin could facilitate in improving the tensile strength and elasticity of silk known for high quality natural fiber. Since spider dragline has excellent tensile properties, we cloned the cDNA of a dragline protein gene from a garden spider (*Araneus ventricosus*). In 2007, we succeeded in generating a transgenic silkworm using an experimental strain silkworm as host. However, because of the limitations presented by the poor physical properties of the silk produced, reeling of raw silk from the cocoons and weaving with the silk fibers could not be performed. Therefore for practical applications of transgenic silk, it is necessary to use a silkworm strain that can produce smooth, uniform silk such as the C515 in commercial strain and to introduce the dragline cDNA of *Araneus ventricosus* into that strain.

Results and Discussion

1. In order to generate transgenic silkworm, it is important to perform micro-injection of DNAs before cell division of diapause-broken eggs. However, it was quite difficult to break diapause of eggs artificially for micro-injection. We improved the traditional acid-treatment by adjusting the C515 strain, and finally succeeded in generating a transgenic silkworm expressing the spider dragline protein (Fig. 1).
2. The cocoons from the transgenic silkworm could be pulled into raw silk and mass production could be performed as shown in Fig. 2. Using densitometry, we calculated the amount of spider dragline protein against the total fibroin and that was about 1 % w/w. From the tensile test results, the toughness (breaking energy of a fiber) improved by 53.2 % as compared with native raw silk (Fig. 3).
3. The transgenic raw silk could be degummed in a usual industrial process. After degumming, the glossy and elasticity of the transgenic silk were the same as normal silk. A vest woven using the spider-dragline-protein containing silk demonstrates the utility of transgenic silk (Fig 4). This “Tough Silk” is 1.5 times tougher than normal silk and could provide a new strong and tough fiber for textile industry.
4. Although there are studies showing that spider dragline protein could be produced using bacteria, development of a tough fiber for industrial purposes has never been reported so far. Our “Tough Silk” produced by the transgenic silkworm is the first application of fibers which contains spider dragline proteins.

Fig. 1. Comparison of the dry cocoon shell weight of the experimental and “Tough Silk” strains.

Fig. 2. A processing method similar to normal silk has been successfully applied in the production of textile using “Tough Silk”.

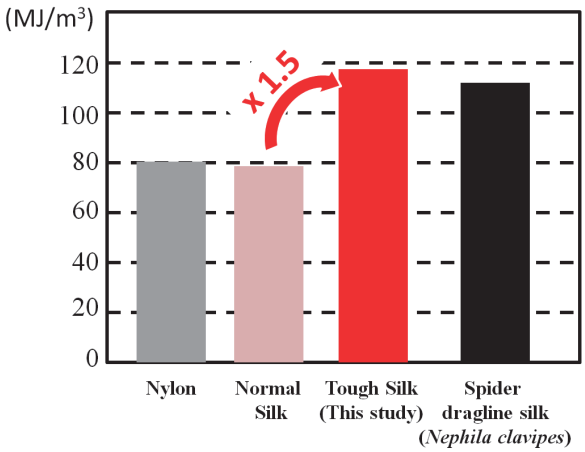

Fig. 3. Comparison of toughness with other fiber materials shows that “Tough Silk” requires 1.5 times more energy to break than normal silk.

Fig. 4. A vest made of “Tough Silk”.

Reference

1. Kuwana Y, Sezutsu H, Nakajima K, Tamada Y, Kojima K (2014) High-toughness silk produced by a transgenic silkworm expressing spider (*Araneus ventricosus*) dragline silk protein ***PLoS ONE*** 9 (8):e105325

Intellectual Contribution

Decoding the draft genome sequence of desiccation tolerant African midge

Takahiro Kikawada, Richard Cornette, Rie Hatanaka, Shingo Kikuta, Sachiko Shimura

Insect Mimetics Research Unit

We completed the draft genome sequence of an African midge, *Polypedilum vanderplanki*, which has a capability to survive under extreme desiccation tolerance, or the so-called anhydrobiosis. The draft sequence shows that *P. vanderplanki*-specific gene clusters and desiccation-inducible gene expression systems contribute to anhydrobiosis.

Keywords:anhydrobiosis, genome, horizontal gene transfer, adaptive evolution, gene duplications

Background

Our collaborative research with scientists from Japan, Russia and the USA successfully deciphered the draft genome sequence of an African midge, *Polypedilum vanderplanki*, possessing the capability to survive extreme desiccation. The draft sequence reveals that *P. vanderplanki*-specific gene clusters and desiccation-inducible gene expression systems contribute to anhydrobiosis. The genes connected with anhydrobiosis will be applied to develop new technologies such as long-term storage of cells, embryos and blood in a dry state at room temperature.

Results and Discussion

1. NIAS organized the international collaborative research team comprising of Kazan Federal University (Russia), Okinawa Institute of Science and Technology Graduate University (Japan), National Institute for Basic Biology (Japan), Kanazawa University (Japan), Lomonosov Moscow State University (Russia), Scientific Research Institute of Physico-Chemical Medicine (Russia), Russian Academy of Science and Vanderbilt University (USA). The team deciphered the draft genome sequence of the anhydrobiotic midge, *P. vanderplanki* and identified approximately 17,000 protein-coding loci.
2. Comparative genome analysis of *P. vanderplanki* and a congeneric desiccation-sensitive midge *P. nubifer* (Fig. 1) led to the identification of *P. vanderplanki*-specific genomic regions where these gene sets are located as 'anhydrobiosis-related gene island' (ARId). Moreover, this analysis provides evidence on the existence of desiccation-specific gene expression system in *P. vanderplanki* (Fig. 2).
3. ARIds consist mainly of multicopy genes for protective proteins, such as antioxidants, enzymes for repair of damaged proteins, and LEA proteins acting as molecular shields.
4. The *LEA* genes were horizontally acquired from soil bacteria in the habitat of *P. vanderplanki*.
5. We revealed the evolutionary process that led to the ability to acquire anhydrobiosis in *P. vanderplanki* diverged from an ancestral species about 25 million years ago (Fig. 3).

Future prospects

1. The key sets of genes connected with anhydrobiosis will be applied to develop new technologies for long-term storage of cells, embryos and blood in a dry state at room temperature.
2. Elucidation of the molecular mechanisms underlying dehydration-specific gene expression system will be useful in designing a methodology for induction of resistance to desiccation in different animal tissues and cells.

Fig. 1. Comparative genome analysis unveils the essential gene sets involved in anhydrobiosis.

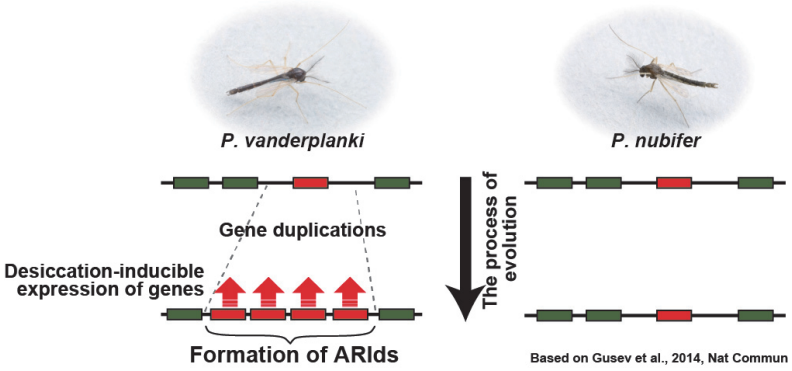

Fig. 2. The unique gene variation between *P. vanderplanki* and *P. nubifer* representing earlier species of midge. The large red blocks indicate gene clusters associated with tolerance to extreme conditions known as ARIds.

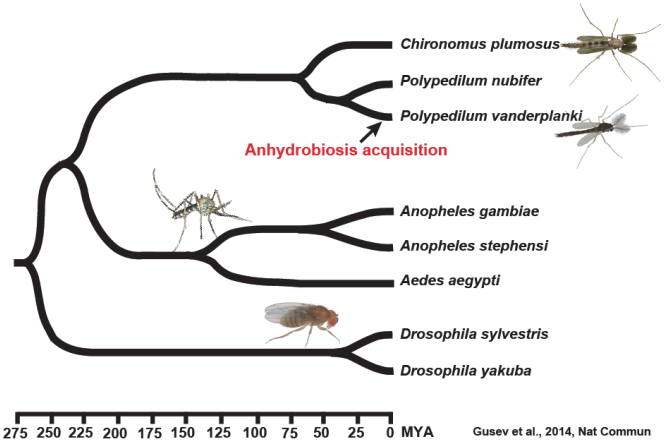

Fig. 3. The genes responsible for making *Polypedilum vanderplanki* resilient are unique genetic mutations that have been found only in this particular species. indicate gene clusters associated with tolerance to extreme conditions known as ARIds.

Reference

1. Gusev O, Suetsugu Y, Cornette R, Kawashima T, Logacheva M.D, Kondrashov A.S, Penin A.A, Hatanaka R, Kikuta S, Shimura S, Kanamori H, Katayose Y, Matsumoto T, Shagimardanova E, Alexeev D, Govorun V, Wisecaver J, Mikheyev A, Koyanagi R, Fujie M, Nishiyama T, Shigenobu S, Shibata T.F, Golygina V, Hasebe M, Okuda T, Satoh N, Kikawada T (2014) Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge ***Nature Communications*** 5:4784

List of Publications

1-1 Conservation of genetic resources for food and agriculture and intensification of their use

Original Papers

1. Aoki T, Vaughan M.M, McCormick S.P, Busman M, Ward T.J, Kelly A, O'Donnell K, Johnston P, Geiser D.M (2015) *Fusarium dactylidis* sp. nov., a novel nivalenol toxin-producing species sister to *F. pseudograminearum* isolated from orchard grass (*Dactylis glomerata*) in Oregon and New Zealand ***Mycologia*** 107(2):409-418
2. Chankaew S, Isemura T, Isobe S, Kaga A, Tomooka N, Somta P, Hirakawa H, Shirasawa K, Vaughan D.A, Srinives P (2014) Detection of genome donor species of neglected tetraploid crop *Vigna reflexo-pilosa* (Créole Bean), and genetic structure of diploid species based on newly developed EST-SSR markers from azuki bean (*Vigna angularis*) ***PLoS ONE*** 9(8):e104990
3. Freeman S, Otero Colina G, Rodríguez-Alvarado G, Fernández-Pavía S, Maymon M, Ploetz R.C, Aoki T, O'Donnell K (2014) First report of mango malformation disease caused by *Fusarium pseudocircinatum* in Mexico ***Plant Disease*** 98(11):1583
4. Fukuoka S, *Saka* N, Mizukami Y, Koga H, Yamanouchi U, Yoshioka Y, Hayashi N, Ebana K, Mizobuchi R, Yano M (2015) Gene pyramiding enhances durable blast disease resistance in rice ***Scientific Reports*** 5:7773
5. Ikejima S, Tsuneyama I, Ohnuma A, Ninagi O, Hara W (2014) Identification of the attached chromosomes in balanced lethal silkworm, *Bombyx mori* ***Sanshi-Konchu Biotec*** 83(3):283-288 (in Japanese with English summary)
6. Inoue T, Yuo T, Ohta T, Hitomi E, Ichitani K, Kawase M, Taketa S, Fukunaga K (2015) Multiple origins of the phenol reaction negative phenotype in foxtail millet, *Setaria italica* (L.) P. Beauv., were caused by independent loss-of-function mutations of the *polyphenol oxidase* (*Si7PPO*) gene during domestication ***Molecular Genetics and Genomics*** DOI: 10.1007/s00438-015-1022-x
7. Ishihara T, Hayano-Saito Y, Oide S, Ebana K, La N.T, Hayashi K, Ashizawa T, Suzuki F, Koizumi S (2014) Quantitative trait locus analysis of resistance to panicle blast in the rice cultivar Miyazakimochi ***Rice*** 7:2
8. Kasajima S, Satoh T, Itoh H, Yoshida H, Suzuki T, Morishita T, Shimizu A (2014) Comparison of dry matter partitioning between the semi dwarf cultivar and the original cultivar in Tartary buckwheat ***Fagopyrum*** 31:11-14
9. Kosegawa E, Kobayashi H, Misawa T (2014) Development of an acid-resistant egg sheet using shellac ***Sanshi-Konchu Biotec*** 83(3):273-276 (in Japanese with English summary)
10. Kurose D, Kanegae Y, Misawa T, Ebihara Y, Tanaka C, Watanabe T, Uematsu S, Tsushima S, Sato T (2015) Yellow spot of white lace flower caused by *Pleospora herbarum* in Japan ***Journal of General Plant Pathology*** 81(2):169-172
11. Matsuda F, Nakabayashi R, Yang Z, Okazaki Y, Yonemaru J, Ebana K, Yano M, Saito K (2015) Metabolome-genome-wide association study dissects genetic architecture for generating natural variation in rice secondary metabolism ***The Plant Journal*** 81(1):13-23
12. Matsumoto T, Yamamoto S, Fukui K, Rafique T, Engelmann F, Niino T (2015) Cryopreservation of persimmon shoot tips from dormant buds using the D cryo-plate technique ***The Horticulture Journal*** 84(2):106-110
13. Matsumoto T, Yoshimatsu K, Kawahara N, Yamamoto S, Niino T (2014) Development of *in vitro* propagation by node culture and cryopreservation by V-Cryo-plate method for *Perilla frutescens* ***Advances in Horticultural Science*** 28(2):79-83
14. Misawa T, Satou M, Yasuoka S, Matsushita Y, Uzuhashi S, Sato T, Yamauchi N, Shirakawa T (2014) Downy mildew Chinese chive caused by *Peronospora destructor* (Berkeley) Caspary ex

Berkeley ***Annual Report of the Society of Plant Protection of North Japan*** 65:68-71 (in Japanese with English summary)
15. Yamashiro M, Waki T, Nakayama K, Aoki T (2015) Stem and root rot of *Gentiana triflora* Pall. caused by a species of the *Fusarium solani* species complex ***Japanese Journal of Phytopathology*** 81(1):43-47 (in Japanese with English summary)
16. Motohashi T, Umemuro H, Takyu T, Sato Y, Kondo K (2014) Mutation breeding of *Glebionis coronalia* by exposing gamma irradiation ***Chromosome Botany*** 9(2):55-56
17. Nagai T (2014) A defective bacteriophage produced by *Bacillus subtilis* MAFF 118147 and a mutant producing no normal particles of the defective bacteriophage ***Food Science and Technology Research*** 20(6):1229-1234
18. Naito M, Harumi T, Kuwana T (2015) Long-term culture of chicken primordial germ cells isolated from embryonic blood and production of germline chimaeric chickens ***Animal Reproduction Science*** 153: 50-61
19. Nakano M, Yamada T, Masuda Y, Sato Y, Kobayashi H, Ueda H, Morita R, Nishimura M, Kitamura K, Kusaba M (2014) A green-cotyledon/stay-green mutant exemplifies the ancient whole-genome duplications in soybean ***Plant and Cell Physiology*** 55(10):1763-1771
20. Netsu O, Hiraguri A, Uehara-Ichiki T, Komatsu K, Sasaya T (2015) Functional comparison of RNA silencing suppressor between the p5 protein of rice grassy stunt virus and the p3 protein of rice stripe virus ***Virus Research*** 203:10-19
21. Niino T, Wunna, Watanabe K, Nohara N, Rafique T, Yamamoto S, Fukui K, Arizaga M.V, Castillo Martinez C.R, Matsumoto T, Engelmann F (2014) Cryopreservation of mat rush lateral buds by air dehydration using aluminum cryo-plate ***Plant Biotechnology*** 31(3):281-287
22. Onogi A, Ideta O, Inoshita Y, Ebana K, Yoshioka T, Yamasaki M, Iwata H (2015) Exploring the areas of applicability of whole-genome prediction methods for Asian rice (*Oryza sativa* L.) ***Theoretical and Applied Genetics*** 128(1):41-53
23. O'Donnell K, Sink S, Libeskind-Hadas R, Hulcr J, Kasson M.T, Ploetz R.C, Konkol J.L, Ploetz J.N, Carrillo D, Campbell A, Duncan R.E, Liyanage P.N.H, Eskalen A, Na F, Geiser D.M, Bateman C, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé A.A, Rooney A.P (2014) Discordant phylogenies suggest repeated host shifts in the *Fusarium* - *Euwallacea* ambrosia beetle mutualism ***Fungal Genetics and Biology*** DOI: 10.1016/j.fgb.2014.10.014
24. Park Y-J, Nishikawa T, Matsushima K, Minami M, Nemoto K (2014) A rapid and reliable PCR-restriction fragment length polymorphism (RFLP) marker for the identification of *Amaranthus cruentus* species ***Breeding Science*** 64(4):422-426
25. Park Y-J, Nishikawa T, Matsushima K, Minami M, Tomooka N, Nemoto K (2014) Molecular characterization and genetic diversity of the *starch branching enzyme* (*SBE*) gene from *Amaranthus*: the evolutionary origin of grain amaranths ***Molecular Breeding*** 34(4):1975-1985
26. Rafique T, Yamamoto S, Fukui K, Mahmood Z, Niino T (2015) Cryopreservation of sugarcane using the V cryo-plate technique ***CryoLetters*** 36(1):51-59
27. Sato T, Aoki M, Aoki T, Kubota M, Yaguchi T, Uzuhashi S, Tomioka K (2014) Fungi isolated from spoiled bean sprouts in Japan ***Japan Agricultural Research Quarterly*** 48(3):317-329
28. Sawada H, Miyoshi T, Ide Y (2014) Novel MLSA group (Psa5) of *Pseudomonas syringae* pv. *actinidiae* causing bacterial canker of kiwifruit (*Actinidia chinensis*) in Japan ***Japanese Journal of Phytopathology*** 80(3):171-184 (in Japanese with English summary)
29. Sawada H, Azegami K (2014) First report of root mat (hairy root) of tomato (*Lycopersicon esculentum*) caused by *Rhizobium radiobacter* harboring cucumopine Ri plasmid in Japan ***Japanese Journal of Phytopathology*** 80(2):98-114 (in Japanese with English summary)
30. Scandiani M.M, Luque A.G, Razori M.V, Casalini L.C, Aoki T, O'Donnell K, Cervigni G.D.L, Spampinato C.P (2015) Metabolic profiles of soybean roots during early stages of *Fusarium tucumaniae* infection ***Journal of Experimental Botany*** 66(1):391-402

31. Sehrawat N, Bhat K.V, Kaga A, Tomooka N, Yadav M, Jaiwal P.K (2014) Development of new gene-specific markers associated with salt tolerance for mungbean (*Vigna radiata* L. Wilczek) ***Spanish Journal of Agricultural Research*** 12(3):732-741
32. Takahashi Y, Akiba M, Iizumi T, Tomooka N (2015) Collection and conservation of wild leguminous crop relatives on Iki and Hirado Islands, Nagasaki prefecture, Japan, 2013 ***Annual report on Exploration and Introduction of Plant Genetic Resources*** 30:1-27
33. Takahashi Y, Naito K, Ogiso-Tanaka E, Inoue J, Hirashima S, Tomooka N (2015) Collection and field survey of wild vigna genetic resources in the Yaeyama archipelago, Okinawa prefecture, Japan, 8th to 14th July, 2013 ***Annual report on Exploration and Introduction of Plant Genetic Resources*** 30:29-51
34. Takahashi Y, Peou U, Lay Heng S, Channa T, Makara O, Tomooka N (2015) Collection and conservation of leguminous crops and their wild relatives in Cambodia, 2013 ***Annual report on Exploration and Introduction of Plant Genetic Resources*** 30:109-143
35. Takemoto-Kuno Y, Mitsueda H, Suzuki K, Hirabayashi H, Ideta O, Aoki N, Umemoto T, Ishii T, Ando I, Kato H, Nemoto H, Imbe T, Takeuchi Y (2015) *qAC2*, a novel QTL that interacts with *Wx* and controls the low amylose content in rice (*Oryza sativa* L.) ***Theoretical and Applied Genetics*** 128(4):563-573
36. Tateishi K, Kasahara Y, Watanabe K, Hosokawa N, Doi H, Nakajima K, Adachi H, Nomoto A (2015) A new cell line from the fat body of *Spodoptera litura* (Lepidoptera, Noctuidae) and detection of lysozyme activity release upon immune stimulation ***In Vitro Cellular & Developmental Biology - Animal*** 51(1):15-18
37. Tomooka N, Naito K, Kaga A, Sakai H, Isemura T, Ogiso-Tanaka E, Iseki K, Takahashi Y (2014) Evolution, domestication and neo-domestication of the genus *Vigna* ***Plant Genetic Resources: Characterization and Utilization*** 12(S1):S168-S171
38. Wang L, Kikuchi S, Muto C, Naito K, Isemura T, Ishimoto M, Cheng X, Kaga A, Tomooka N (2015) Reciprocal translocation identified in *Vigna angularis* dominates the wild population in East Japan ***Journal of Plant Research*** (Online First)
39. Yamamoto S, Wunna, Rafique T, Arizaga M.V, Fukui K, Gutierrez E.J.C, Castillo Martinez C.R, Watanabe K, Niino T (2015) The aluminum cryo-plate increases efficiency of cryopreservation protocols for potato shoot tips ***American Journal of Potato Research*** 92(2):250-257

Reviews and Monographs

1. Nagai T (2014) Natto ***Handbook of Indigenous Foods Involving Alkaline Fermentation*** 2(1.1.1):8-18
2. Nagai T (2014) Antioxidative activity ***Handbook of Indigenous Foods Involving Alkaline Fermentation*** 5(3.4.1):342-346
3. Nagai T (2014) Probiotic activity ***Handbook of Indigenous Foods Involving Alkaline Fermentation*** 5(3.4.2):346-351
4. Nagai T (2014) Immunomodulating activity ***Handbook of Indigenous Foods Involving Alkaline Fermentation*** 5(3.4.3):351-355
5. Nagai T (2014) Fibrinolytic activity ***Handbook of Indigenous Foods Involving Alkaline Fermentation*** 5(3.4.4):355-359
6. Nagai T (2014) Poly-γ-glutamic acid ***Handbook of Indigenous Foods Involving Alkaline Fermentation*** 8(2):498-508
7. Naito K, Monden Y, Yasuda K, Saito H, Okumoto Y (2014) *mPing*: The bursting transposon ***Breeding Science*** 64(2):109-114
8. Naito M (2015) Embryo manipulation in chickens ***The Journal of Poultry Science*** 52(1):7-14
9. Sawada H (2014) Studies on the classification and evolution of phytopathogenic bacteria of the genera *Agrobacterium* and *Pseudomonas* ***Journal of General Plant Pathology*** 80(6):519-522

10. Tomooka N, Isemura T, Naito K, Kaga A, Vaughan D (2014) *Vigna* species ***Broadening the Genetic Base of Grain Legumes*** (9):175-208

1-2-1 Enhancing the potential of the genome sequence and resources from agriculturally important organisms

Original Papers

1. Alam M.M, Nakamura H, Ichikawa H, Kobayashi K, Yaeno T, Yamaoka N, Nishiguchi M (2015) Overexpression of *OsHAP2E* for a CCAAT-binding factor confers resistance to *Cucumber mosaic virus* and *Rice necrosis mosaic virus* ***Journal of General Plant Pathology*** 81(1):32-41
2. Alam M.M, Nakamura H, Ichikawa H, Miyao A, Hirochika H, Kobayashi K, Yamaoka N, Nishiguchi M (2014) Response of an *aspartic protease* gene *OsAP77* to fungal, bacterial and viral infections in rice ***Rice*** 7:9
3. Alam M.M, Tanaka T, Nakamura H, Ichikawa H. Kobayashi K, Yaeno T, Yamaoka N, Shimomoto K, Takayama K, Nishina H, Nishiguchi M (2015) Overexpression of a rice *heme activator protein* gene (*OsHAP2E*) confers resistance to pathogens, salinity and drought, and increases photosynthesis and tiller number ***Plant Biotechnology Journal*** 13(1):85-96
4. Endo M, Kumagai M, Motoyama R, Sasaki-Yamagata H, Mori-Hosokawa S, Hamada M, Kanamori H, Nagamura Y, Katayose Y, Itoh T, Toki S (2015) Whole-genome analysis of herbicide-tolerant mutant rice generated by *Agrobacterium*-mediated gene targeting ***Plant and Cell Physiology*** 56(1):116-125
5. Endo M, Mikami M, Toki S (2015) Multigene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice ***Plant and Cell Physiology*** 56(1):41-47
6. Habu Y, Ando T, Ito S, Nagaki K, Kishimoto N, Taguchi-Shiobara F, Numa H, Yamaguchi K, Shigenobu S, Murata M, Meshi T, Yano M (2015) Epigenomic modification in rice controls meiotic recombination and segregation distortion ***Molecular Breeding*** 35(4):103
7. Hayashi G, Shibato J, Imanaka T, Cho K, Kubo A, Kikuchi S, Satoh K, Kimura S, Ozawa S, Fukutani S, Endo S, Ichikawa K, Agrawal G.K, Shioda S, Fukumoto M, Rakwal R (2014) Unraveling low-level gamma radiation–responsive changes in expression of early and late genes in leaves of rice seedlings at litate village, Fukushima ***Journal of Heredity*** 105(5):723-738
8. Hayashi M, Shiro S, Kanamori H, Mori-Hosokawa S, Sasaki-Yamagata H, Sayama T, Nishioka M, Takahashi M, Ishimoto M, Katayose Y, Kaga A, Harada K, Kouchi H, Saeki Y, Umehara Y (2014) A thaumatin-like protein, Rj4, controls nodule symbiotic specificity in soybean ***Plant and Cell Physiology*** 55(9):1679-1689
9. Ishikawa G, Nakamura K, Ito H, Saito M, Sato M, Jinno H, Yoshimura Y, Nishimura T, Maejima H, Uehara Y, Kobayashi F, Nakamura T (2014) Association mapping and validation of QTLs for flour yield in the soft winter wheat variety Kitahonami ***PLoS ONE*** 9(10):e111337
10. Iwakami S, Endo M, Saika H, Okuno J, Nakamura N, Yokoyama M, Watanabe H, Toki S, Uchino A, Inamura T (2014) Cytochrome P450 CYP81A12 and CYP81A21 are associated with resistance to two acetolactate synthase inhibitors in *Echinochloa phyllopogon* ***Plant Physiology*** 165(2):618-629
11. Khodayari H, Saeidi H, Akhavan A, Pourkheirandish M, Komatsuda T (2014) Microsatellite analysis of genetic diversity of wild barley (*Hordeum vulgare* subsp. *spontaneum*) using different sampling methods in Iran ***The Iranian Journal of Botany*** 20(1):41-50
12. Kobayashi T, Yamamoto K, Suetsugu Y, Kuwazaki S, Hattori M, Jairin J, Sanada-Morimura S, Matsumura M (2014) Genetic mapping of the rice resistance-breaking gene of the brown planthopper *Nilaparvata lugens* ***Proceedings of the Royal Society B: Biological Sciences*** 281(1787):20140726

13. Liang C, Hirose T, Okamura M, Tanimoto R, Miyao A, Hirochika H, Terao T, Li T, Ohsugi R, Aoki N (2014) Phenotypic analyses of rice *lse2* and *lse3* mutants that exhibit hyperaccumulation of starch in the leaf blades ***Rice*** 7:32
14. Mizuno H, Yazawa T, Kasuga S, Sawada Y, Ogata J, Ando T, Kanamori H, Yonemaru J, Wu J, Yokota Hirai M, Matsumoto T, Kawahigashi H (2014) Expression level of a *flavonoid 3'-hydroxylase* gene determines pathogen-induced color variation in sorghum ***BMC Research Notes*** 7:761
15. Nishizawa-Yokoi A, Endo M, Ohtsuki N, Saika H, Toki S (2015) Precision genome editing in plants via gene targeting and *piggyBac*-mediated marker excision ***The Plant Journal*** 81(1):160-168
16. Nuruzzaman M, Kanno T, Amada R, Habu Y, Kasajima I, Ishikawa T, Kawai-Yamada M, Uchimiya H (2014) Does the upstream region possessing MULE-like sequence in rice upregulate *PsbS1* gene expression? ***PLoS ONE*** 9(9):e102742
17. Oono Y, Yazawa T, Kawahara Y, Kanamori H, Kobayashi F, Sasaki H, Mori S, Wu J, Handa H, Itoh T, Matsumoto T (2014) Genome-wide transcriptome analysis reveals that cadmium stress signaling controls the expression of genes in drought stress signal pathways in rice ***PLoS ONE*** 9(5):e96946
18. Ordonio R.L, Ito Y, Hatakeyama A, Ohmae-Shinohara K, Kasuga S, Tokunaga T, Mizuno H, Kitano H, Matsuoka M, Sazuka T (2014) Gibberellin deficiency pleiotropically induces culm bending in sorghum: an insight into sorghum semi-dwarf breeding ***Scientific Reports*** 4:5287
19. Satoh K, Saji S, Ito S, Shimizu H, Saji H, Kikuchi S (2014) Gene response in rice plants treated with continuous fog influenced by pH, was similar to that treated with biotic stress ***Rice*** 7:10
20. Shiono K, Yamauchi T, Yamazaki S, Mohanty B, Malik A.I, Nagamura Y, Nishizawa N.K, Tsutsumi N, Colmer T.D, Nakazono M (2014) Microarray analysis of laser-microdissected tissues indicates the biosynthesis of suberin in the outer part of roots during formation of a barrier to radial oxygen loss in rice (*Oryza sativa*) ***Journal of Experimental Botany*** 65(17):4795-4806
21. Song X.J, Kuroha T, Ayano M, Furuta T, Nagai K, Komeda N, Segami S, Miura K, Ogawa D, Kamura T, Suzuki T, Higashiyama T, Yamasaki M, Mori H, Inukai Y, Wu J, Kitano H, Sakakibara H, Jacobsen S.E, Ashikari M (2015) Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice ***Proceedings of the National Academy of Sciences of the United States of America*** 112(1):76-81
22. Takahashi S, Kojo K.H, Kutsuna N, Endo M, Toki S, Isoda H, Hasezawa S (2015) Differential responses to high- and low-dose ultraviolet-B stress in tobacco Bright Yellow-2 cells ***Frontiers in Plant Science*** 6:254
23. The International Wheat Genome Sequencing Consortium (IWGSC) (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome ***Science*** 345(6194):1251788
24. Tsukazaki H, Yaguchi S, Sato S, Hirakawa H, Katayose Y, Kanamori H, Kurita K, Itoh T, Kumagai M, Mizuno S, Hamada M, Fukuoka H, Yamashita K, McCallum J.A, Shigyo M, Wako T (2015) Development of transcriptome shotgun assembly-derived markers in bunching onion (*Allium fistulosum*) ***Molecular Breeding*** 35(1):55
25. Ui H, Sameri M, Pourkheirandish M, Chang M-C, Shimada H, Stein N, Komatsuda T, Handa H (2015) High-resolution genetic mapping and physical map construction for the fertility restorer *Rfm1* locus in barley ***Theoretical and Applied Genetics*** 128(2):283-290
26. Van Bockhaven J, Spíchal L, Novák O, Strnad M, Asano T, Kikuchi S, Höfte M, De Vleesschauwer D (2015) Silicon induces resistance to the brown spot fungus *Cochliobolus miyabeanus* by preventing the pathogen from hijacking the rice ethylene pathway ***New Phytologist*** 206(2):761-773
27. Van Bockhaven J, Steppe K, Bauweraerts I, Kikuchi S, Asano T, Höfte M, De Vleesschauwer D (2015) Primary metabolism plays a central role in moulding silicon-inducible brown spot resistance in rice ***Molecular Plant Pathology*** DOI: 10.1111/mpp.12236

28. Wakasa Y, Oono Y, Yazawa T, Hayashi S, Ozawa K, Handa H, Matsumoto T, Takaiwa F (2014) RNA sequencing-mediated transcriptome analysis of rice plants in endoplasmic reticulum stress conditions ***BMC Plant Biology*** 14:101
29. Wang N, Ning S, Wu J, Tagiri A, Komatsuda T (2015) An epiallele at *cly1* affects the expression of floret closing (cleistogamy) in barley ***Genetics*** 199(1):95-104
30. Yoda S, Yamaguchi J, Mita K, Yamamoto K, Banno Y, Ando T, Daimon T, Fujiwara H (2014) The transcription factor Apontic-like controls diverse colouration pattern in caterpillars ***Nature Communications*** 5:4936

Reviews and Monograph

1. Kikuchi S (2014) Genome-wide view of the expression profiles of NAC-domain genes in response to infection by rice viruses ***Omics Technologies and Crop Improvement*** (5):127-152
2. Komatsuda T (2014) The genetics of inflorescence architecture in *Hordeum* ***Journal of Systematics and Evolution*** 52(6):779-782
3. Saika H, Nishizawa-Yokoi A, Toki S (2014) The non-homologous end-joining pathway is involved in stable transformation in rice ***Frontiers in Plant Science*** 5:560
4. Shimatani Z, Nishizawa-Yokoi A, Endo M, Toki S, Terada R (2015) Positive-negative-selection-mediated gene targeting in rice ***Frontiers in Plant Science*** 5:748

1-2-2 Bioinformatics approach for advancement of agrobiological research

Original Papers

1. Gusev O, Suetsugu Y, Cornette R, Kawashima T, Logacheva M.D, Kondrashov A.S, Penin A.A, Hatanaka R, Kikuta S, Shimura S, Kanamori H, Katayose Y, Matsumoto T, Shagimardanova E, Alexeev D, Govorun V, Wisecaver J, Mikheyev A, Koyanagi R, Fujie M, Nishiyama T, Shigenobu S, Shibata T.F, Golygina V, Hasebe M, Okuda T, Satoh N, Kikawada T (2014) Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge ***Nature Communications*** 5:4784
2. International *Glossina* Genome Initiative (2014) Genome sequence of the tsetse fly (*Glossina morsitans*): Vector of African trypanosomiasis ***Science*** 344(6182):380-386
3. Kobayashi T, Yamamoto K, Suetsugu Y, Kuwazaki S, Hattori M, Jairin J, Sanada-Morimura S, Matsumura M (2014) Genetic mapping of the rice resistance-breaking gene of the brown planthopper *Nilaparvata lugens* ***Proceedings of the Royal Society B: Biological Sciences*** 281(1787):20140726
4. Nojima Y, Ito K, Ono H, Nakazato T, Bono H, Yokoyama T, Sato R, Suetsugu Y, Nakamura Y, Yamamoto K, Satoh J, Tabunoki H, Fugo H (2015) Superoxide dismutases, SOD1 and SOD2, play a distinct role in the fat body during pupation in silkworm *Bombyx mori* ***PLoS ONE*** 10(2):e0116007
5. Oono Y, Yazawa T, Kawahara Y, Kanamori H, Kobayashi F, Sasaki H, Mori S, Wu J, Handa H, Itoh T, Matsumoto T (2014) Genome-wide transcriptome analysis reveals that cadmium stress signaling controls the expression of genes in drought stress signal pathways in rice ***PLoS ONE*** 9(5):e96946
6. The International Wheat Genome Sequencing Consortium (IWGSC) (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome ***Science*** 345(6194):1251788
7. Tsukazaki H, Yaguchi S, Sato S, Hirakawa H, Katayose Y, Kanamori H, Kurita K, Itoh T, Kumagai M, Mizuno S, Hamada M, Fukuoka H, Yamashita K, McCallum J.A, Shigyo M, Wako T (2015) Development of transcriptome shotgun assembly-derived markers in bunching onion (*Allium fistulosum*) ***Molecular Breeding*** 35(1):55
8. Xue J, Zhou X, Zhang C-X, Yu L-L, Fan H-W, Wang Z, Xu H-J, Xi Y, Zhu Z-R, Zhou W-W, Pan P-L, Li B-L, Colbourne J.K, Noda H, Suetsugu Y, Kobayashi T, Zheng Y, Liu S, Zhang R, Liu Y,

Luo Y-D, Fang D-M, Chen Y, Zhan D-L, Lv X-D, Cai Y, Wang Z-B, Huang H-J, Cheng R-L, Zhang X-C, Lou Y-H, Yu B, Zhuo J-C, Ye Y-X, Zhang W-Q, Shen Z-C, Yang H-M, Wang J, Wang J, Bao Y-Y, Cheng J-A (2014) Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation ***Genome Biology*** 15:521

9. Yasukochi Y, Ohno M, Shibata F, Jouraku A, Nakano R, Ishikawa Y, Sahara K (2014) A FISH-based chromosome map for the European corn borers yields insights into ancient chromosomal fusions in the silkworm ***bioRχiv*** DOI: http://dx.doi.org/10.1101/013284
10. Yoda S, Yamaguchi J, Mita K, Yamamoto K, Banno Y, Ando T, Daimon T, Fujiwara H (2014) The transcription factor Apontic-like controls diverse colouration pattern in caterpillars ***Nature Communications*** 5:4936

1-2-3 Genomics approach for advancement of research in crop improvement

Original Papers

1. Arai-Sanoh Y, Takai T, Yoshinaga S, Nakano H, Kojima M, Sakakibara H, Kondo M, Uga Y (2014) Deep rooting conferred by *DEEPER ROOTING 1* enhances rice yield in paddy fields ***Scientific Reports*** 4:5563
2. Fujii K, Kato S, Sayama T, Tanaka Y, Nakazaki T, Ishimoto M, Shiraiwa T (2015) Stability verification of the effects of stem determination and earliness of flowering on green stem disorder of soybean against genetic background and environment ***Plant Production Science*** 18(2):166-179
3. Fukuda A, Sugimoto K, Ando T, Yamamoto T, Yano M (2015) Chromosomal locations of a gene underlying heat-accelerated brown spot formation and its suppressor genes in rice ***Molecular Genetics and Genomics*** 290(3):1085-1094
4. Fukuoka S, Saka N, Mizukami Y, Koga H, Yamanouchi U, Yoshioka Y, Hayashi N, Ebana K, Mizobuchi R, Yano M (2015) Gene pyramiding enhances durable blast disease resistance in rice ***Scientific Reports*** 5:7773
5. Fukuoka S, Yamamoto S, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N, Yasuda N, Fujita Y, Nguyen T.T.T, Koizumi S, Sugimoto K, Matsumoto T, Yano M (2014) Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast ***Scientific Reports*** 4:4550
6. Funatsuki H, Suzuki M, Hirose A, Inaba H, Yamada T, Hajika M, Komatsu K, Katayama T, Sayama T, Ishimoto M, Fujino K (2014) Molecular basis of a shattering resistance boosting global dissemination of soybean ***Proceedings of the National Academy of Sciences of the United States of America*** 111(50):17797-17802
7. Hayashi M, Shiro S, Kanamori H, Mori-Hosokawa S, Sasaki-Yamagata H, Sayama T, Nishioka M, Takahashi M, Ishimoto M, Katayose Y, Kaga A, Harada K, Kouchi H, Saeki Y, Umehara Y (2014) A thaumatin-like protein, Rj4, controls nodule symbiotic specificity in soybean ***Plant and Cell Physiology*** 55(9):1679-1689
8. Hirata K, Masuda R, Tsubokura Y, Yasui T, Yamada T, Takahashi K, Nagaya T, Sayama T, Ishimoto M, Hajika M (2014) Identification of quantitative trait loci associated with boiled seed hardness in soybean ***Breeding Science*** 64(4):362-370
9. Inoue Y, Kobae Y, Omoto E, Tanaka A, Banba M, Takai S, Tamura Y, Hirose A, Komatsu K, Otagaki S, Matsumoto S, Taniguchi M, Masuta C, Ishimoto M, Hata S (2014) The soybean mycorrhiza-inducible phosphate transporter gene, *GmPT7*, also shows localized expression at the tips of vein endings of senescent leaves ***Plant and Cell Physiology*** 55(12):2102-2111
10. Iwata H, Ebana K, Uga Y, Hayashi T (2015) Genomic prediction of biological shape: Elliptic Fourier analysis and kernel partial least squares (PLS) regression applied to grain shape prediction in rice (*Oryza sativa* L.) ***PLoS ONE*** 10(3):e0120610

11. Kato S, Fujii K, Yumoto S, Ishimto M, Shiraiwa T, Sayama T, Kikuchi A, Nishio T (2015) Seed yield and its components of indeterminate and determinate lines in recombinant inbred lines of soybean ***Breeding Science*** 65(2):154-160
12. Kato S, Sayama T, Fujii K, Yumoto S, Kono Y, Hwang T-Y, Kikuchi A, Takada Y, Tanaka Y, Shiraiwa T, Ishimoto M (2014) A major and stable QTL associated with seed weight in soybean across multiple environments and genetic backgrounds ***Theoretical and Applied Genetics*** 127(6):1365-1374
13. Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S, Uga Y (2015) QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of *DEEPER ROOTING 1* ***Rice*** 8:16
14. Maruyama N, Fujiwara K, Yokoyama K, Cabanos C, Hasegawa H, Takagi K, Nishizawa K, Uki Y, Kawarabayashi T, Shouji M, Ishimoto M, Terakawa T (2014) Stable accumulation of seed storage proteins containing vaccine peptides in transgenic soybean seeds ***Journal of Bioscience and Bioengineering*** 118(4):441-447
15. Matsubara K, Yamamoto E, Mizobuchi R, Yonemaru J, Yamamoto T, Kato H, Yano M (2015) Hybrid breakdown caused by epistasis-based recessive incompatibility in a cross of rice (*Oryza sativa* L.) ***Journal of Heredity*** 106(1):113-122
16. Matsuda F, Nakabayashi R, Yang Z, Okazaki Y, Yonemaru J, Ebana K, Yano M, Saito K (2015) Metabolome-genome-wide association study dissects genetic architecture for generating natural variation in rice secondary metabolism ***The Plant Journal*** 81(1):13-23
17. Miyamoto T, Ochiai K, Nonoue Y, Matsubara K, Yano M, Matoh T (2015) Expression level of the sodium transporter gene *OsHKT2;1* determines sodium accumulation of rice cultivars under potassium-deficient conditions ***Soil Science & Plant Nutrition*** DOI: 10.1080/00380768.2015.1005539
18. Mizobuchi R, Sato H, Fukuoka S, Tsushima S, Yano M (2015) Fine mapping of *RBG2*, a quantitative trait locus for resistance to *Burkholderia glumae*, on rice chromosome 1 ***Molecular Breeding*** 35(1):15
19. Mizobuchi R, Sato H, Fukuoka S, Yamamoto S, Kawasaki-Tanaka A, Fukuta Y (2014) Mapping of a QTL for field resistance to blast (*Pyricularia oryzae* Cavara) in Ingngoppor-tinawon, a rice (*Oryza sativa* L.) landrace from the Philippines ***Japan Agricultural Research Quarterly*** 48(4):425-431
20. Oki N, Komatsu K, Takahashi M, Takahashi M, Kono Y, Ishimoto M (2015) Field assessment of resistance QTL to common cutworm (*Spodoptera litura* Fabricius) in soybean ***Crop Science*** 55(2):624-630
21. Ookawa T, Inoue K, Matsuoka M, Ebitani T, Takarada T, Yamamoto T, Ueda T, Yokoyama T, Sugiyama C, Nakaba S, Funada R, Kato H, Kanekatsu M, Toyota K, Motobayashi T, Vazirzanjani M, Tojo S, Hirasawa T (2014) Increased lodging resistance in long-culm, low-lignin *gh2* rice for improved feed and bioenergy production ***Scientific Reports*** 4:6567
22. Sakazono S, Nagata T, Matsuo R, Kajihara S, Watanabe M, Ishimoto M, Shimamura S, Harada K, Takahashi R, Mochizuki T (2014) Variation in root development response to flooding among 92 soybean lines during early growth stages ***Plant Production Science*** 17(3):228-236
23. Sasaki K, Takeuchi Y, Miura K, Yamaguchi T, Ando T, Ebitani T, Higashitani A, Yamaya T, Yano M, Sato T (2015) Fine mapping of a major quantitative trait locus, *qLG-9*, that controls seed longevity in rice (*Oryza sativa* L.) ***Theoretical and Applied Genetics*** 128(4):769-778
24. Sato H, Matsumoto K, Ota C, Yamakawa T, Kihara J, Mizobuchi R (2015) Confirming a major QTL and finding additional loci responsible for field resistance to brown spot(*Bipolaris oryzae*) in rice ***Breeding Science*** 65(2):170-175
25. Shiono K, Ando M, Nishiuchi S, Takahashi H, Watanabe K, Nakamura M, Matsuo Y, Yasuno N, Yamanouchi U, Fujimoto M, Takanashi H, Ranathunge K, Franke R, Shitan N, Nishizawa N.K, Takamure I, Yano M, Tsutsumi N, Schreiber L, Yazaki K, Nakazono M, Kato K (2014)

RCN1/OsABCG5, an ATP-binding cassette (ABC) transporter, is required for hypodermal suberization of roots in rice (*Oryza sativa*) ***The Plant Journal*** 80(1):40-51
26. Takahashi R, Ishimaru Y, Shimo H, Bashir K, Senoura T, Sugimoto K, Ono K, Suzui N, Kawachi N, Ishii S, Yin Y-G, Fujimaki S, Yano M, Nishizawa N.K, Nakanishi H (2014) From laboratory to field: *OsNRAMP5*-knockdown rice is a promising candidate for Cd phytoremediation in paddy fields ***PLoS ONE*** 9(6):e98816
27. Takai T, Ikka T, Kondo K, Nonoue Y, Ono N, Arai-Sanoh Y, Yoshinaga S, Nakano H, Yano M, Kondo M, Yamamoto T (2014) Genetic mechanisms underlying yield potential in the rice high-yielding cultivar Takanari, based on reciprocal chromosome segment substitution lines ***BMC Plant Biology*** 14:295
28. Tanaka Y, Kumagai E, Tazoe Y, Adachi S, Homma K (2014) Leaf photosynthesis and its genetic improvement from the perspective of energy flow and CO_2 diffusion ***Plant Production Science*** 17(2):111-123
29. Uga Y, Kitomi Y, Yamamoto E, Kanno N, Kawai S, Mizubayashi T, Fukuoka S (2015) A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of *DEEPER ROOTING 1* ***Rice*** 8:8
30. Yamada T, Shimada S, Hajika M, Hirata K, Takahashi K, Nagaya T, Hamaguchi H, Maekawa T, Sayama T, Hayashi T, Ishimoto M, Tanaka J (2014) Major QTLs associated with green stem disorder insensitivity of soybean (*Glycine max* (L.) Merr.) ***Breeding Science*** 64(4):331-338
31. Yamaguchi N, Kurosaki H, Ishimoto M, Kawasaki M, Senda M, Miyoshi T (2015) Early-maturing and chilling-tolerant soybean lines derived from crosses between Japanese and Polish cultivars ***Plant Production Science*** 18(2):234-239
32. Yamaguchi N, Sayama T, Sasama H, Yamazaki H, Miyoshi T, Tanaka Y, Ishimoto M (2014) Mapping of quantitative trait loci associated with terminal raceme length in soybean ***Crop Science*** 54(6):2461-2468
33. Yamaguchi N, Sayama T, Yamazaki H, Miyoshi T, Ishimoto M, Funatsuki H (2014) Quantitative trait loci associated with lodging tolerance in soybean cultivar 'Toyoharuka' ***Breeding Science*** 64(4):300-308
34. Yamamoto E, Iwata H, Tanabata T, Mizobuchi R, Yonemaru J, Yamamoto T, Yano M (2014) Effect of advanced intercrossing on genome structure and on the power to detect linked quantitative trait loci in a multi-parent population: a simulation study in rice ***BMC Genetics*** 15:50
35. Yano K, Ookawa T, Aya K, Ochiai Y, Hirasawa T, Ebitani T, Takarada T, Yano M, Yamamoto T, Fukuoka S, Wu J, Ando T, Ordonio R.L, Hirano K, Matsuoka M (2015) Isolation of a novel lodging resistance QTL gene involved in strigolactone signaling and its pyramiding with a QTL gene involved in another mechanism ***Molecular Plant*** 8(2):303-314
36. Yonemaru J, Mizobuchi R, Kato H, Yamamoto T, Yamamoto E, Matasubara K, Hirabayashi H, Takeuchi Y, Tsunematsu H, Ishii T, Ohta H, Maeda H, Ebana K, Yano M (2014) Genomic regions involved in yield potential detected by genome-wide association analysis in Japanese high-yielding rice cultivars ***BMC Genomics*** 15:346

Reviews

1. Ahmadi N, Audebert A, Bennett M.J, Bishopp A, de Oliveira A.C, Courtois B, Diedhiou A, Diévart A, Gantet P, Ghesquière A, Guiderdoni E, Henry A, Inukai Y, Kochian L, Laplaze L, Lucas M, Luu D.T, Manneh B, Mo X, Muthurajan R, Périn C, Price A, Robin S, Sentenac H, Sine B, Uga Y, Véry A.A, Wissuwa M, Wu P, Xu J (2014) The roots of future rice harvests ***Rice*** 7:29
2. Matsubara K, Hori K, Ogiso-Tanaka E, Yano M (2014) Cloning of quantitative trait genes from rice reveals conservation and divergence of photoperiod flowering pathways in Arabidopsis and rice ***Frontiers in Plant Science*** 5:193
3. Uga Y, Kitomi Y, Ishikawa S, Yano M (2015) Genetic improvement for root growth angle to enhance crop production ***Breeding Science*** 65(2):111-119

1-2-4 Genomics approach for advancement of research in livestock production

Original Papers

1. Harumi T, Kobayashi E, Naito M (2015) Allele-specific polymerase chain reaction typing and sequencing of mitochondrial D-loop region in broiler chickens in Japan ***Animal Science Journal*** DOI: 10.1111/asj.12369
2. Hirawatari K, Hanzawa N, Kuwahara M, Aoyama H, Miura I, Wakana S, Gotoh H (2015) Polygenic expression of teratozoospermia and normal fertility in B10.MOL-TEN1 mouse strain ***Congenital Anomalies*** 55(2):92-98
3. Komatsu M, Sato Y, Negami T, Terada T, Sasaki O, Yasuda J, Arakawa A, Yoshida C, Takahashi H, Malau-Aduli A.E.O, Suzuki K, Shimizu K (2015) Overdominance effect of the bovine ghrelin receptor (*GHSR1a*)-*DelR242* locus on growth in Japanese Shorthorn weaner bulls: heterozygote advantage in bull selection and molecular mechanisms ***G3: Genes, Genomes, Genetics*** 5(2):271-279
4. Kumagai Y, Sekimoto M, Okamoto M, Kurita R, Kojima M, Degawa M (2014) Involvement of hepatic IL-1 in the strain-dependent sex differences in serum total cholesterol levels in rats ***Biological & Pharmaceutical Bulletin*** 37(4):654-658
5. Nakamura S, Maehara T, Watanabe S, Ishihara M, Sato M (2015) Liver lobe and strain difference in gene expression after hydrodynamics-based gene delivery in mice ***Animal Biotechnology*** 26(1):51-57
6. Sakurai T, Watanabe S, Kamiyoshi A, Sato M, Shindo T (2014) A single blastocyst assay optimized for detecting CRISPR/Cas9 system-induced indel mutations in mice ***BMC Biotechnology*** 14:69
7. Satou K, Suto J (2015) Effect of the Y chromosome on testis weight in mice ***Journal of Veterinary Medical Science*** DOI: doi.org/10.1292/jvms.14-0423
8. Sato M, Inada E, Saitoh I, Matsumoto Y, Ohtsuka M, Miura H, Nakamura S, Sakurai T, Watanabe S (2015) A combination of targeted toxin technology and the *piggyBac*-mediated gene transfer system enables efficient isolation of stable transfectants in nonhuman mammalian cells ***Biotechnology Journal*** 10(1):143-153
9. Suto J (2015) Genetic analysis of litter size in mice ***Journal of Veterinary Medical Science*** 77(3):353-358
10. Suto J (2015) Genetic analysis of low survival rate of pups in RR/Sgn inbred mice ***Journal of Veterinary Medical Science*** DOI: doi.org/10.1292/jvms.14-0361
11. Suto J, Satou K (2014) Effect of the Y chromosome on plasma high-density lipoprotein-cholesterol levels in Y-chromosome-consomic mouse strains ***BMC Research Notes*** 7:393
12. Suto J, Satou K (2015) Further characterization of diabetes mellitus and body weight loss in males of the congenic mouse strain DDD.Cg-A^{y} ***Journal of Veterinary Medical Science*** 77(2):203-210
13. Taniguchi M, Arakawa A, Motoyama M, Nakajima I, Nii M, Mikawa S (2015) Genomic structural analysis of porcine fatty acid desaturase cluster on chromosome 2 ***Animal Science Journal*** 86(4):369-377

1-2-5 Structural and functional analysis of biomolecules related to agriculture

Original Papers

1. Bagautdinov B, Matsuura Y, Yamamoto H, Sawano M, Ogasahara K, Takehira M, Kunishima N, Katoh E, Yutani K (2015) Thermodynamic analysis of unusually thermostable CutA1 protein from human brain and its protease susceptibility ***The Journal of Biochemistry*** 157(3):169-176
2. Ishibashi K, Kezuka Y, Kobayashi C, Kato M, Inoue T, Nonaka T, Ishikawa M, Matsumura H, Katoh E (2014) Structural basis for the recognition-evasion arms race between *Tomato mosaic virus* and the

resistance gene *Tm-1* ***Proceedings of the National Academy of Sciences of the United States of America*** 111(33):E3486-E3495

3. Matsuzawa J, Aikawa H, Umeda T, Ashikawa Y, Suzuki-Minakuchi C, Kawano Y, Fujimoto Z, Okada K, Yamane H, Nojiri H (2014) Crystallization and preliminary X-ray diffraction analyses of the redox-controlled complex of terminal oxygenase and ferredoxin components in the Rieske nonhaem iron oxygenase carbazole 1,9a-dioxygenase ***Acta Crystallographica Section F*** 70(10):1406-1409
4. Muramatsu M, Suzuki R, Yamazaki T, Miyao M (2015) Comparison of plant-type phospho*enol*pyruvate carboxylases from rice: Identification of two plant-specific regulatory regions of the allosteric enzyme ***Plant and Cell Physiology*** 56(3):468-480
5. Ohishi K, Suzuki R, Maeda T, Tsuda M, Abe E, Yoshida T, Endo Y, Okamura M, Nagamine T, Yamamoto H, Ueda M, Maruyama T (2014) Recent host range expansion of canine distemper virus and variation in its receptor, the signaling lymphocyte activation molecule, in carnivores ***Journal of Wildlife Diseases*** 50(3):596-606
6. Suzuki R, Suzuki N, Fujimoto Z, Momma M, Kimura K, Kitamura S, Kimura A, Funane K (2015) Molecular engineering of cycloisomaltooligosaccharide glucanotransferase from *Bacillus circulans* T-3040: structural determinants for the reaction product size and reactivity ***Biochemical Journal*** 467(2):259-270

Monographs

1. Fujimoto Z (2014) Basic procedure of X-ray crystallography for analysis of lectin–sugar interactions ***Methods in Molecular Biology*** 1200(39):481-490
2. Fujimoto Z, Tateno H, Hirabayashi J (2014) Lectin structures: Classification based on the 3-D structures ***Methods in Molecular Biology*** 1200(46):579-606
3. Kajiwara H (2015) Gene analysis using mass spectrometric cleaved amplified polymorphic sequence (MS-CAPS) with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) ***Methods in Molecular Biology*** 1245(16):205-214

2-1-1 Elucidation of the mechanisms involved in biomass production, growth, differentiation, and environmental response of agricultural crops

Original Papers

1. Alam M.M, Nakamura H, Ichikawa H, Kobayashi K, Yaeno T, Yamaoka N, Nishiguchi M (2015) Overexpression of *OsHAP2E* for a CCAAT-binding factor confers resistance to *Cucumber mosaic virus* and *Rice necrosis mosaic virus* ***Journal of General Plant Pathology*** 81(1):32-41
2. Alam M.M, Nakamura H, Ichikawa H, Miyao A, Hirochika H, Kobayashi K, Yamaoka N, Nishiguchi M (2014) Response of an *aspartic protease* gene *OsAP77* to fungal, bacterial and viral infections in rice ***Rice*** 7:9
3. Alam M.M, Tanaka T, Nakamura H, Ichikawa H. Kobayashi K, Yaeno T, Yamaoka N, Shimomoto K, Takayama K, Nishina H, Nishiguchi M (2015) Overexpression of a rice *heme activator protein* gene (*OsHAP2E*) confers resistance to pathogens, salinity and drought, and increases photosynthesis and tiller number ***Plant Biotechnology Journal*** 13(1):85-96
4. Baba-Kasai A, Hara N, Takano M (2014) Tissue-specific and light-dependent regulation of phytochrome gene expression in rice ***Plant, Cell and Environment*** 37(12):2654-2666
5. Fukayama H, Fujiwara N, Hatanaka T, Misoo S, Miyao M (2014) Nocturnal phosphorylation of phospho*enol*pyruvate carboxylase in the leaves of hygrophytic C_3 monocots ***Bioscience, Biotechnology and Biochemistry*** 78(4):609-613

6. Fukayama H, Masumoto C, Taniguchi Y, Baba-Kasai A, Katoh Y, Ohkawa H, Miyao M (2015) Characterization and expression analyses of two plastidic enolase genes in rice ***Bioscience, Biotechnology and Biochemistry*** 79(3):402-409
7. Ibl V, Kapusi E, Arcalis E, Kawagoe Y, Stoger E (2014) Fusion, rupture, and degeneration: the fate of *in vivo*-labelled PSVs in developing barley endosperm ***Journal of Experimental Botany*** 65(12):3249-3261
8. Ishikawa M, Ishikawa M, Toyomasu T, Aoki T, Price W.S (2015) Ice nucleation activity in various tissues of *Rhododendron* flower buds: their relevance to extraorgan freezing ***Frontiers in Plant Science*** 6:149
9. Ishikawa M, Oda A, Fukami R, Kuriyama A (2015) Factors contributing to deep supercooling capability and cold survival in dwarf bamboo (*Sasa senanensis*) leaf blades ***Frontiers in Plant Science*** 5:791
10. Ishikawa M (2014) Ice nucleation activity in plant tissues ***Cryobiology and Cryotechnology*** 60(2):79-88 (in Japanese with English summary)
11. Kishimoto T, Yamazaki H, Saruwatari A, Murakawa H, Sekozawa Y, Kuchitsu K, Price W.S, Ishikawa M (2014) High ice nucleation activity located in blueberry stem bark is linked to primary freeze initiation and adaptive freezing behaviour of the bark ***AoB PLANTS*** 6:plu044
12. Kurotani K, Hayashi K, Hatanaka S, Toda Y, Ogawa D, Ichikawa H, Ishimaru Y, Tashita R, Suzuki T, Ueda M, Hattori T, Takeda S (2015) Elevated levels of CYP94 family gene expression alleviate the jasmonate response and enhance salt tolerance in rice ***Plant and Cell Physiology*** 56(4):779-789
13. Matsuzaki J, Kawahara Y, Izawa T (2015) Punctual transcriptional regulation by the rice circadian clock under fluctuating field conditions ***The Plant Cell*** 27(3):633-648
14. Miyazawa S, Hayashi K, Nakamura H, Hasegawa T, Miyao M (2014) Elevated CO_2 decreases the photorespiratory NH_3 production but does not decrease the NH_3 compensation point in rice leaves ***Plant and Cell Physiology*** 55(9):1582-1591
15. Muramatsu M, Suzuki R, Yamazaki T, Miyao M (2015) Comparison of plant-type phospho*enol*pyruvate carboxylases from rice: Identification of two plant-specific regulatory regions of the allosteric enzyme ***Plant and Cell Physiology*** 56(3):468-480
16. Okubo T, Liu D, Tsurumaru H, Ikeda S, Asakawa S, Tokida T, Tago K, Hayatsu M, Aoki N, Ishimaru K, Ujiie K, Usui Y, Nakamura H, Sakai H, Hayashi K, Hasegawa T, Minamisawa K (2015) Elevated atmospheric CO_2 levels affect community structure of rice root-associated bacteria ***Frontiers in Microbiology*** 6:136
17. Shiraya T, Mori T, Maruyama T, Sasaki M, Takamatsu T, Oikawa K, Itoh K, Kaneko K, Ichikawa H, Mitsui T (2015) Golgi/plastid-type manganese superoxide dismutase involved in heat-stress tolerance during grain filling of rice ***Plant Biotechnology Journal*** DOI: 10.1111/pbi.12314
18. Suzuki H, Okamoto A, Kojima A, Nishimura T, Takano M, Kagawa T, Kadota A, Kanegae T, Koshiba T (2014) Blue-light regulation of *ZmPHOT1* and *ZmPHOT2* gene expression and the possible involvement of Zmphot1 in phototropism in maize coleoptiles ***Planta*** 240(2):251-261
19. Tanabe S, Yokotani N, Nagata T, Fujisawa Y, Jiang C-J, Abe K, Ichikawa H, Mitsuda N, Ohme-Takagi M, Nishizawa Y, Minami E (2014) Spatial regulation of defense-related genes revealed by expression analysis using dissected tissues of rice leaves inoculated with *Magnaporthe oryzae* ***Journal of Plant Physiology & Pathology*** 2(4):1000135
20. Ujiie K, Yamamoto T, Yano M, Ishimaru K (2015) Genetic factors determining varietal differences in characters affecting yield between two rice (*Oryza sativa* L.) varieties, Koshihikari and IR64 ***Genetic Resources and Crop Evolution*** (Online First)
21. Xie X, Kagawa T, Takano M (2014) The phytochrome B/phytochrome C heterodimer is necessary for phytochrome C-mediated responses in rice seedlings ***PLoS ONE*** 9(5):e97264
22. Yokotani N, Tsuchida-Mayama T, Ichikawa H, Mitsuda N, Ohme-Takagi M, Kaku H, Minami E, Nishizawa Y (2014) OsNAC111, a blast disease-responsive transcription factor in rice, positively

regulates the expression of defense-related genes ***Molecular Plant-Microbe Interactions*** 27(10):1027-1034

Review and Monographs

1. Izawa T (2015) Deciphering and prediction of plant dynamics under field conditions ***Current Opinion in Plant Biology*** 24:87-92
2. Osugi A, Izawa T (2014) Critical gates in day-length recognition to control the photoperiodic flowering ***Advances in Botanical Research*** 72(4):103-130
3. Sakai T, Uehara Y, Nagashima A (2014) Function of ABCBs in light signaling ***Signaling and Communication in Plants*** 22:301-311

2-1-2 Elucidation of the regulatory mechanisms involved in insect growth, development and differentiation

Original Papers

1. Enya S, Ameku T, Igarashi F, Iga M, Kataoka H, Shinoda T, Niwa R (2014) A Halloween gene *noppera-bo* encodes a glutathione *S*-transferase essential for ecdysteroid biosynthesis via regulating the behaviour of cholesterol in *Drosophila* ***Scientific Reports*** 4:6586
2. Kamimura M, Matsumoto H, Kiuchi M, Ito Y, Fujiwara H, Shinoda T (2014) Development of a cell-based assay for ecdysteroid quantification using an early ecdysteroid-inducible gene promoter ***Applied Entomology and Zoology*** 49(3):443-452
3. Lozano J, Kayukawa T, Shinoda T, Belles X (2014) A role for Taiman in insect metamorphosis ***PLoS Genetics*** 10(10):e1004769
4. Matsumoto H, Ueno C, Nakamura Y, Kinjoh T, Ito Y, Shimura S, Noda H, Imanishi S, Mita K, Fujiwara H, Hiruma K, Shinoda T, Kamimura M (2015) Identification of two juvenile hormone inducible transcription factors from the silkworm, *Bombyx mori* ***Journal of Insect Physiology*** doi:10.1016/j.jinsphys.2015.02.011
5. Mizoguchi A, Kamimura M, Kiuchi M, Kataoka H (2015) Positive feedback regulation of prothoracicotropic hormone secretion by ecdysteroid - A mechanism that determines the timing of metamorphosis ***Insect Biochemistry and Molecular Biology*** 58:39-45
6. Nagamine K, Kayukawa T, Hoshizaki S, Matsuo T, Shinoda T, Ishikawa Y (2014) Cloning, phylogeny, and expression analysis of the Broad-Complex gene in the longicorn beetle *Psacothea hilaris* ***SpringerPlus*** 3:539
7. Nakade S, Tsubota T, Sakane Y, Kume S, Sakamoto N, Obara M, Daimon T, Sezutsu H, Yamamoto T, Sakuma T, Suzuki K.T (2014) Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9 ***Nature Communications*** 5:5560
8. Namiki S, Daimon T, Iwatsuki C, Shimada T, Kanzaki R (2014) Antennal lobe organization and pheromone usage in bombycid moths ***Biology Letters*** 10(4):20140096
9. Sekiné K , Furusawa T, Hatakeyama M (2015) The *boule* gene is essential for spermatogenesis of haploid insect male ***Developmental Biology*** 399(1):154-163
10. Sugahara R, Jouraku A, Nakakura T, Kusakabe T, Yamamoto T, Shinohara Y, Miyoshi H, Shiotsuki T (2015) Two adenine nucleotide translocase paralogues involved in cell proliferation and spermatogenesis in the silkworm *Bombyx mori* ***PLoS ONE*** 10(3):e0119429
11. Sugahara R, Mon H, Lee J.M, Shiotsuki T, Kusakabe T (2014) Differential contribution of the Fanconi anemia-related proteins to repair of several types of DNA damage in cultured silkworm cells ***FEBS Letters*** 588(21):3959-3963
12. Toyota K, Miyakawa H, Hiruta C, Furuta K, Ogino Y, Shinoda T, Tatarazako N, Miyagawa S, Shaw J.R, Iguchi T (2015) Methyl farnesoate synthesis is necessary for the environmental sex

determination in the water flea *Daphnia pulex* ***Journal of Insect Physiology*** (In Press):Corrected Proof

13. Tsubota T, Uchino K, Suzuki T.K, Tanaka H, Kayukawa T, Shinoda T, Sezutsu H (2014) Identification of a novel strong and ubiquitous promoter/enhancer in the silkworm *Bombyx mori* ***G3: Genes, Genomes, Genetics*** 4(7):1347-1357
14. Uehara T, Yamaguchi T, Kotaki T, Shimoda M (2014) Evaluation of phototactic behavior by two-dimensional open field test in the brown-winged green bug, *Plautia stali* (Scott) (Hemiptera: Pentatomidae) ***Japanese Journal of Applied Entomology and Zoology*** 58(1):36-38 (in Japanese with English summary)
15. Yamamoto K, Higashiura A, Hossain T, Yamada N, Shiotsuki T, Nakagawa A (2015) Structural characterization of the catalytic site of a *Nilaparvata lugens* delta-class glutathione transferase ***Archives of Biochemistry and Biophysics*** 566:36-42
16. Yoda S, Yamaguchi J, Mita K, Yamamoto K, Banno Y, Ando T, Daimon T, Fujiwara H (2014) The transcription factor Apontic-like controls diverse colouration pattern in caterpillars ***Nature Communications*** 5:4936

Review and Monograph

1. Shimmi O, Matsuda S, Hatakeyama M (2014) Insights into the molecular mechanisms underlying diversified wing venation among insects ***Proceedings of the Royal Society B: Biological Sciences*** 281(1789):20140264
2. Daimon T (2015) Highly efficient targeted gene disruption in the silkworm, *Bombyx mori*, using genome editing tools ***Targeted Genome Editing Using Site-Specific Nucleases*** II(5):81-96

2-1-3 Elucidation of the molecular mechanisms involved in the development and differentiation of germ cell and stem cell of livestock

Original Papers

1. Appeltant R, Somfai T, Nakai M, Bodó S, Maes D, Kikuchi K, Van Soom A (2015) Interactions between oocytes and cumulus cells during in vitro maturation of porcine cumulus-oocyte complexes in a chemically defined medium: effect of denuded oocytes on cumulus expansion and oocyte maturation ***Theriogenology*** 83(4):567-576
2. Hosoe M, Yoshida N, Hashiyada Y, Teramoto H, Takahashi T, Niimura S (2014) Sericin accelerates the production of hyaluronan and decreases the incidence of polyspermy fertilization in bovine oocytes during *in vitro* maturation ***Journal of Reproduction and Development*** 60(4):268-273
3. Kaneko H, Kikuchi K, Tanihara F, Noguchi J, Nakai M, Ito J, Kashiwazaki N (2014) Normal reproductive development of pigs produced using sperm retrieved from immature testicular tissue cryopreserved and grafted into nude mice ***Theriogenology*** 82(2):325-331
4. Katoh Y, Takebayashi K, Kikuchi A, Iki A, Kikuchi K, Tamba M, Kawashima A, Matsuda M, Okamura N (2014) Porcine sperm capacitation involves tyrosine phosphorylation and activation of aldose reductase ***Reproduction*** 148(4):389-401
5. Naito M, Harumi T, Kuwana T (2015) Long-term culture of chicken primordial germ cells isolated from embryonic blood and production of germline chimaeric chickens ***Animal Reproduction Science*** 153: 50-61
6. Naito M, Harumi T, Kuwana T (2015) Expression of GFP gene in gonads of chicken embryos by transfecting primordial germ cells *in vitro* or *in vivo* using the piggyBac transposon vector system ***The Journal of Poultry Science*** DOI: http://doi.org/10.2141/jpsa.0140197

7. Nakai M, Ozawa M, Maedomari N, Noguchi J, Kaneko H, Ito J, Onishi A, Kashiwazaki N, Kikuchi K (2014) Delay in cleavage of porcine embryos after intracytoplasmic sperm injection (ICSI) shows poorer embryonic development ***Journal of Reproduction and Development*** 60(3):256-259
8. Nguyen B.X, Kikuchi K, Uoc N.T, Dang-Nguyen T.Q, Linh N.V, Men N.T, Nguyen T.T, Nagai T (2015) Production of Ban miniature pig embryos by *in vitro* fertilization: A comparative study with Landrace ***Animal Science Journal*** 86(5):487-493
9. Shinagawa T, Huynh L.M, Takagi T, Tsukamoto D, Tomaru C, Kwak H-G, Dohmae N, Noguchi J, Ishii S (2015) Disruption of *Th2a* and *Th2b* genes causes defects in spermatogenesis ***Development*** 142:1287-1292
10. Somfai T, Yoshioka K, Tanihara F, Kaneko H, Noguchi J, Kashiwazaki N, Nagai T, Kikuchi K (2014) Generation of live piglets from cryopreserved oocytes for the first time using a defined system for *in vitro* embryo production ***PLoS ONE*** 9(5):e97731

Review

1. Naito M (2015) Embryo manipulation in chickens ***The Journal of Poultry Science*** 52(1):7-14

2-1-4 Elucidation of the mechanisms involved in the control of the behavior and reproduction of livestock

Original Papers

1. Hosoe M, Yoshida N, Hashiyada Y, Teramoto H, Takahashi T, Niimura S (2014) Sericin accelerates the production of hyaluronan and decreases the incidence of polyspermy fertilization in bovine oocytes during *in vitro* maturation ***Journal of Reproduction and Development*** 60(4):268-273
2. Ito S, Iwashita Y, Hagiwara S, Yamamoto N, Sakumoto R, Tani M, Okamoto C, Yayou K (2014) Comparison of the maintenance behaviours Japanese Brown and Japanese Black cows during the summer ***Animal Behaviour and Management*** 50(4):162-168 (in Japanese with English summary)
3. Kasuya E, Sutoh M (2014) Possible roles of central serotonin in physiological responses to hot environment in cattle ***Proceedings of Japanese Society for Animal Nutrition and Metabolism*** 58(2):37-43 (in Japanese with English summary)
4. Kumagai A, Yoshioka S, Sakumoto R, Okuda K (2014) Auto-amplification system for prostaglandin F2α in bovine corpus luteum ***Molecular Reproduction and Development*** 81(7):646-654
5. Matsuda F, Nakatsukasa K, Suetomi Y, Naniwa Y, Ito D, Inoue N, Wakabayashi Y, Okamura H, Maeda K, Uenoyama Y, Tsukamura H, Ohkura S (2015) The luteinising hormone surge-generating system is functional in male goats as in females: Involvement of kisspeptin neurones in the medial preoptic area ***Journal of Neuroendocrinology*** 27(1):57–65
6. Misu R, Oishi S, Yamada A, Yamamura T, Matsuda F, Yamamoto K, Noguchi T, Ohno H, Okamura H, Ohkura S, Fujii N (2014) Development of novel neurokinin 3 receptor (NK3R) selective agonists with resistance to proteolytic degradation ***Journal of Medicinal Chemistry*** 57(20):8646-8651
7. Misu R, Yamamoto K, Yamada A, Noguchi T, Ohno H, Yamamura T, Okamura H, Matsuda F, Ohkura S, Oishi S, Fujii N (2015) Structure-activity relationship study on senktide for development of novel potent neurokinin-3 receptor selective agonists ***MedChemComm*** 6:469-476
8. Ohara H, Mogi K, Ichimaru T, Ohkura S, Takeuchi Y, Mori Y, Okamura H (2014) Effects of exposure to male goat hair extracts on luteinizing hormone secretion and neuronal activation in seasonally anestrous ewes ***Journal of Veterinary Medical Science*** 76(10):1329-1337
9. Ohta T, Koshi K, Ushizawa K, Hosoe M, Takahashi T, Yamaguchi T, Kizaki K, Hashizume K (2014) Expression profiles of perforin, granzyme B and granulysin genes during the estrous cycle and gestation in the bovine endometrium ***Animal Science Journal*** 85(7):763-769

10. Sakumoto R, Hayashi K, Hosoe M, Iga K, Kizaki K, Okuda K (2015) Gene expression profiles in the bovine corpus luteum (CL) during the estrous cycle and pregnancy: possible roles of chemokines in regulating CL function during pregnancy ***Journal of Reproduction and Development*** 61(1):42-48
11. Watanabe K, Ishida M, Ito S, Kasuya E, Sutoh M, Yayou K (2014) The relationship between awake-sleep states and automatic nervous balance in cattle ***Animal Behaviour and Management*** 50(3):119-126 (in Japanese with English summary)
12. Yamamura T, Wakabayashi Y, Ohkura S, Navarro V.M, Okamura H (2015) Effects of intravenous administration of neurokinin receptor subtype-selective agonists on gonadotropin-releasing hormone pulse generator activity and luteinizing hormone secretion in goats ***Journal of Reproduction and Development*** 61(1):20-29
13. Yamamura T, Wakabayashi Y, Sakamoto K, Matsui H, Kusaka M, Tanaka T, Ohkura S, Okamura H (2014) The effects of chronic subcutaneous administration of an investigational kisspeptin analog, TAK-683, on gonadotropin-releasing hormone pulse generator activity in goats ***Neuroendocrinology*** 100(2-3):250-264
14. Yayou K, Ito S, Yamamoto N (2015) Relationships between postnatal plasma oxytocin concentrations and social behaviors in cattle ***Animal Science Journal*** DOI: 10.1111/asj.12363

Monograph

1. Okamura H, Mori Y (2014) Multiple-unit activity recording of the gonadotropin-releasing hormone pulse generator ***Neurophysiology of Neuroendocrine Neurons*** 2(14):323-346

2-2-1 Elucidation of the mechanisms involved in plant pathogenic microbe infections and development of innovative technologies for their applications

Original Papers

1. Akimoto-Tomiyama C, Furutani A, Ochiai H (2014) Real time live imaging of phytopathogenic bacteria *Xanthomonas campestris* pv. campestris MAFF106712 in 'Plant Sweet Home' ***PLoS ONE*** 9(4):e94386
2. Fujimoto T, Mizukubo T, Abe H, Seo S (2015) Sclareol induces plant resistance to root-knot nematode partially through ethylene-dependent enhancement of lignin accumulation ***Molecular Plant-Microbe Interactions*** 28(4):398-407
3. Hasegawa M, Mitsuhara I, Seo S, Okada K, Yamane H, Iwai T, Ohashi Y (2014) Analysis on blast fungus-responsive characters of a flavonoid phytoalexin sakuranetin; Accumulation in infected rice leaves, antifungal activity and detoxification by fungus ***Molecules*** 19(8):11404-11418
4. Ishibashi K, Kezuka Y, Kobayashi C, Kato M, Inoue T, Nonaka T, Ishikawa M, Matsumura H, Katoh E (2014) Structural basis for the recognition-evasion arms race between *Tomato mosaic virus* and the resistance gene *Tm-1* ***Proceedings of the National Academy of Sciences of the United States of America*** 111(33):E3486-E3495
5. Kawamura-Nagaya K, Ishibashi K, Huang Y-P, Miyashita S, Ishikawa M (2014) Replication protein of tobacco mosaic virus cotranslationally binds the 5′ untranslated region of genomic RNA to enable viral replication ***Proceedings of the National Academy of Sciences of the United States of America*** 111(16):E1620-E1628
6. Komoda K, Ishibashi K, Kawamura-Nagaya K, Ishikawa M (2014) Possible involvement of eEF1A in *Tomato spotted wilt virus* RNA synthesis ***Virology*** 468-470:81-87
7. Miyashita S, Ishibashi K, Kishino H, Ishikawa M (2015) Viruses roll the dice: The stochastic behavior of viral genome molecules accelerates viral adaptation at the cell and tissue levels ***PLoS Biology*** 13(3):e1002094

8. Takeuchi K, Noda N, Katayose Y, Mukai Y, Numa H, Yamada K, Someya N (2015) Rhizoxin analogs contribute to the biocontrol activity of a newly isolated *Pseudomonas* strain ***Molecular Plant-Microbe Interactions*** 28(3):333-342
9. Takeuchi K, Noda N, Someya N (2014) Complete genome sequence of the biocontrol strain *Pseudomonas protegens* Cab57 discovered in Japan reveals strain-specific diversity of this species ***PLoS ONE*** 9(4):e93683
10. Ueda H, Mitsuhara I, Tabata J, Kugimiya S, Watanabe T, Suzuki K, Yoshida S, Kitamoto H (2015) Extracellular esterases of phylloplane yeast *Pseudozyma antarctica* induce defect on cuticle layer structure and water-holding ability of plant leaves ***Applied Microbiology and Biotechnology*** DOI: 10.1007/s00253-015-6523-3

Reviews

1. Chujo T, Ishibashi K, Miyashita S, Ishikawa M (2015) Functions of the 5′- and 3′-untranslated regions of tobamovirus RNA ***Virus Research*** DOI: 10.1016/j.virusres.2015.01.028
2. Ishibashi K, Ishikawa M (2014) Mechanisms of tomato mosaic virus RNA replication and its inhibition by the host resistance factor *Tm-1* ***Current Opinion in Virology*** 9:8-13

2-2-2 Elucidation of the mechanisms involved in crop response to microbial infection and development of crop strains with multiple pathogen resistance

Original Papers

1. Akagi A, Fukushima S, Okada K, Jiang C-J, Yoshida R, Nakayama A, Shimono M, Sugano S, Yamane H, Takatsuji H (2014) WRKY45-dependent priming of diterpenoid phytoalexin biosynthesis in rice and the role of cytokinin in triggering the reaction ***Plant Molecular Biology*** 86(1-2):171-183
2. Chujo T, Miyamoto K, Ogawa S, Masuda Y, Shimizu T, Kishi-Kaboshi M, Takahashi A, Nishizawa Y, Minami E, Nojiri H, Yamane H, Okada K (2014) Overexpression of phosphomimic mutated OsWRKY53 leads to enhanced blast resistance in rice ***PLoS ONE*** 9(6):e98737
3. Fukuoka S, Saka N, Mizukami Y, Koga H, Yamanouchi U, Yoshioka Y, Hayashi N, Ebana K, Mizobuchi R, Yano M (2015) Gene pyramiding enhances durable blast disease resistance in rice ***Scientific Reports*** 5:7773
4. Fukuta Y, Koga I, Ung T, Sathya K, Kawasaki-Tanaka A, Koide Y, Kobayashi N, Obara M, Yadana H, Hayashi N (2014) Pathogenicity of rice blast (*Pyricularia oryzae* Cavara) isolates from Cambodia ***Japan Agricultural Research Quarterly*** 48(2):155-166
5. Goto S, Sasakura-Shimoda F, Suetsugu M, Selvaraj M.G, Hayashi N, Yamazaki M, Ishitani M, Shimono M, Sugano S, Matsushita A, Tanabata T, Takatsuji H (2014) Development of disease-resistant rice by optimized expression of *WRKY45* ***Plant Biotechnology Journal*** DOI: 110.1111/pbi.12303
6. Kouzai Y, Mochizuki S, Nakajima K, Desaki Y, Hayafune M, Miyazaki H, Yokotani N, Ozawa K, Minami E, Kaku H, Shibuya N, Nishizawa Y (2014) Targeted gene disruption of *OsCERK1* reveals its indispensable role in chitin perception and involvement in the peptidoglycan response and immunity in rice ***Molecular Plant-Microbe Interactions*** 27(9):975-982
7. Lv B-S, Ma H-Y, Li X-W, Wei L-X, Lv H-Y, Yang H-Y, Jiang C-J, Liang Z-W (2015) Proline accumulation is not correlated with saline-alkaline stress tolerance in rice seedlings ***Agronomy Journal*** 107(1):51-60
8. Matsui H, Fujiwara M, Hamada S, Shimamoto K, Nomura Y, Nakagami H, Takahashi A, Hirochika H (2014) Plasma membrane localization is essential for OsPti1a-mediated negative regulation of immune signaling in rice ***Plant Physiology*** 166(1):327-336

9. Miyamoto K, Nishizawa Y, Minami E, Nojiri H, Yamane H, Okada K (2015) Overexpression of the bZIP transcription factor OsbZIP79 suppresses the production of diterpenoid phytoalexin in rice cells ***Journal of Plant Physiology*** 173:19–27
10. Miyata K, Kozaki T, Kouzai Y, Ozawa K, Ishii K, Asamizu E, Okabe Y, Umehara Y, Miyamoto A, Kobae Y, Akiyama K, Kaku H, Nishizawa Y, Shibuya N, Nakagawa T (2014) The bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice ***Plant and Cell Physiology*** 55(11):1864-1872
11. Tanabe S, Yokotani N, Nagata T, Fujisawa Y, Jiang C-J, Abe K, Ichikawa H, Mitsuda N, Ohme-Takagi M, Nishizawa Y, Minami E (2014) Spatial regulation of defense-related genes revealed by expression analysis using dissected tissues of rice leaves inoculated with *Magnaporthe oryzae* ***Journal of Plant Physiology & Pathology*** 2(4):1000135
12. Wei L-X, Lv B-S, Wang M-M, Ma H-Y, Yang H-Y, Liu X-L, Jiang C-J, Liang Z-W (2015) Priming effect of abscisic acid on alkaline stress tolerance in rice (*Oryza sativa* L.) seedlings ***Plant Physiology and Biochemistry*** 90:50-57
13. Yokotani N, Tsuchida-Mayama T, Ichikawa H, Mitsuda N, Ohme-Takagi M, Kaku H, Minami E, Nishizawa Y (2014) OsNAC111, a blast disease-responsive transcription factor in rice, positively regulates the expression of defense-related genes ***Molecular Plant-Microbe Interactions*** 27(10):1027-1034

Review and Monograph

1. Takatsuji H (2014) Development of disease-resistant rice using regulatory components of induced disease resistance ***Frontiers in Plant Science*** 5:630
2. Takatsuji H, Jiang C-J (2014) Plant hormone crosstalks under biotic stresses ***Phytohormones: A Window to Metabolism, Signaling and Biotechnological Applications*** :323-350

2-2-3 Elucidation of the mechanisms involved in plant and soil microbe symbioses

Original Papers

1. Hayashi M, Shiro S, Kanamori H, Mori-Hosokawa S, Sasaki-Yamagata H, Sayama T, Nishioka M, Takahashi M, Ishimoto M, Katayose Y, Kaga A, Harada K, Kouchi H, Saeki Y, Umehara Y (2014) A thaumatin-like protein, Rj4, controls nodule symbiotic specificity in soybean ***Plant and Cell Physiology*** 55(9):1679-1689
2. Miyata K, Kozaki T, Kouzai Y, Ozawa K, Ishii K, Asamizu E, Okabe Y, Umehara Y, Miyamoto A, Kobae Y, Akiyama K, Kaku H, Nishizawa Y, Shibuya N, Nakagawa T (2014) The bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice ***Plant and Cell Physiology*** 55(11):1864-1872
3. Ohkama-Ohtsu N, Ichida S, Yamaya H, Ohwada T, Itakura M, Hara Y, Mitsui H, Kaneko T, Tabata S, Tejima K, Saeki K, Omori H, Hayashi M, Maekawa T, Murooka Y, Tajima S, Simomura K, Nomura M, Uchiumi T, Suzuki A, Shimoda Y, Abe M, Minamisawa K, Arima Y, Yokoyama T (2015) Peribacteroid solution of soybean root nodules partly induces genomic loci for differentiation into bacteroids of free-living *Bradyrhizobium japonicum* cells ***Soil Science & Plant Nutrition*** DOI: 10.1080/00380768.2014.994470
4. Soyano T, Hirakawa H, Sato S, Hayashi M, Kawaguchi M (2014) NODULE INCEPTION creates a long-distance negative feedback loop involved in homeostatic regulation of nodule organ production ***Proceedings of the National Academy of Sciences of the United States of America*** 111(40):14607-14612
5. Soyano T, Shimoda Y, Hayashi M (2015) NODULE INCEPTION antagonistically regulates gene expression with nitrate in *Lotus japonicus* ***Plant and Cell Physiology*** 56(2):368-376

Review and Monographs

1. Binder A, Soyano T, Hayashi M, Parniske M, Radutoiu S (2014) Plant genes involved in symbiotic signal perception/signal transduction ***The Lotus japonicus Genome*** II(6):59-71
2. Fukai E, Małolepszy A, Sandal N, Hayashi M, Andersen S.U (2014) Forward and reverse genetics: The *LORE1* retrotransposon insertion mutants ***The Lotus japonicus Genome*** VI(20):221-227
3. Soyano T, Hayashi M (2014) Transcriptional networks leading to symbiotic nodule organogenesis ***Current Opinion in Plant Biology*** 20:146-154

2-2-4 Elucidation of the mechanisms involved in insect pest infestation and plant resistance to insects

Original Papers

1. Kobayashi T, Yamamoto K, Suetsugu Y, Kuwazaki S, Hattori M, Jairin J, Sanada-Morimura S, Matsumura M (2014) Genetic mapping of the rice resistance-breaking gene of the brown planthopper *Nilaparvata lugens* ***Proceedings of the Royal Society B: Biological Sciences*** 281(1787):20140726
2. Matsumoto Y, Suetsugu Y, Nakamura M, Hattori M (2014) Transcriptome analysis of the salivary glands of *Nephotettix cincticeps* (Uhler) ***Journal of Insect Physiology*** 71:170–176
3. Matsumoto Y, Wakakuwa M, Yukuhiro F, Arikawa K, Noda H (2014) Attraction to different wavelength light emitting diodes (LEDs), the compound eye structure, and *opsin* genes in *Nilaparvata lugens* ***Japanese Journal of Applied Entomology and Zoology*** 58(2):111-118 (in Japanese with English summary)
4. Tamura Y, Hattori M, Yoshioka H, Yoshioka M, Takahashi A, Wu J, Sentoku N, Yasui H (2014) Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryza sativa* L. ssp. *indica* cultivar ADR52 ***Scientific Reports*** 4:5872
5. Taniai K, Hirayama C, Mita K, Asaoka K (2014) Starvation-responsive glycine-rich protein gene in the silkworm *Bombyx mori* ***Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*** 184(7):827-834
6. Xue J, Zhou X, Zhang C-X, Yu L-L, Fan H-W, Wang Z, Xu H-J, Xi Y, Zhu Z-R, Zhou W-W, Pan P-L, Li B-L, Colbourne J.K, Noda H, Suetsugu Y, Kobayashi T, Zheng Y, Liu S, Zhang R, Liu Y, Luo Y-D, Fang D-M, Chen Y, Zhan D-L, Lv X-D, Cai Y, Wang Z-B, Huang H-J, Cheng R-L, Zhang X-C, Lou Y-H, Yu B, Zhuo J-C, Ye Y-X, Zhang W-Q, Shen Z-C, Yang H-M, Wang J, Wang J, Bao Y-Y, Cheng J-A (2014) Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation ***Genome Biology*** 15:521

Review

1. Kadono-Okuda K, Ito K, Murthy G.N, Sivaprasad V, Ponnuvel K.M (2014) Molecular mechanism of densovirus resistance in silkworm, *Bombyx mori* ***Sericologia: Revue des vers à soie*** 54(1):1-10

2-2-5 Elucidation of insect-insect, insect-plant and insect-microbe interactions and their applications

Original Papers

1. Abe N, Yamaguchi T, Nakano A, Shimoda M (2014) Daily distribution of locomotor activity in *Aphidius gifuensis* (Hymenoptera: Braconidae) analyzed using infrared monitoring system ***Japanese Journal of Applied Entomology and Zoology*** 58(4):329-331 (in Japanese with English summary)
2. Chiu E, Hijnen M, Bunker R.D, Boudes M, Rajendran C, Aizel K, Oliéric V, Schulze-Briese C, Mitsuhashi W, Young V, Ward V.K, Bergoin M, Metcalf P, Coulibaly F (2015) Structural basis for the enhancement of virulence by viral spindles and their in vivo crystallization ***Proceedings of the National Academy of Sciences of the United States of America*** 112(13):3973-3978
3. Fujiwara-Tsujii N, Yasui H, Arakaki N (2014) Chemical and physical cues synergistically affect mating behavior sequences of male *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae) ***Zoological Science*** 31(9):553-558
4. Kobayashi H, Fujii-Muramatsu R, Noda H, Takeishi K (2014) Construction of an expressible BAC library of the unculturable insect microorganism, stink bug *Plautia stali* symbiont, for the search of biologically active and useful symbiont products ***Biological & Pharmaceutical Bulletin*** 37(4):528-533
5. Kubo-Irie M, Shimoda M, Sato A, Shida K, Yamaguchi T, Mohri H, Takeda K, Irie M (2015) Effect of nanoparticles injected into larvae on spermatogenesis in the pupal testis of the sweet potato hornworm, *Agrius convolvuli* (L.) ***Fundamental Toxicological Sciences*** 2(1):1-8
6. Maeda T, Kishimoto H, Wright L.C, James D.G (2015) Mixture of synthetic herbivore-induced plant volatiles attracts more *Stethorus punctum picipes* (Casey) (Coleoptera: Coccinellidae) than a single volatile ***Journal of Insect Behavior*** 28(2):126-137
7. Matsumoto Y, Wakakuwa M, Yukuhiro F, Arikawa K, Noda H (2014) Attraction to different wavelength light emitting diodes (LEDs), the compound eye structure, and *opsin* genes in *Nilaparvata lugens* ***Japanese Journal of Applied Entomology and Zoology*** 58(2):111-118 (in Japanese with English summary)
8. Murakami R, Miyamoto K (2014) Pathogenicity of *Euproctis pseudoconspersa* nucleopolyhedrovirus to mulberry silkworm ***Sericologia: Revue des vers à soie*** 54(1):43-48
9. Sahin Polan M, Ito K, Miyamoto K, Yokoyama T, Ninagi O (2014) Linkage analysis of the resistance gene expressed dominantly against the Bt insecticide Cry1Ac in the silkworm *Bombyx mori* ***Journal of Insect Biotechnology and Sericology*** 83(2):33-39
10. Uehara T, Yamaguchi T, Kotaki T, Shimoda M (2014) Evaluation of phototactic behavior by two-dimensional open field test in the brown-winged green bug, *Plautia stali* (Scott) (Hemiptera: Pentatomidae) ***Japanese Journal of Applied Entomology and Zoology*** 58(1):36-38 (in Japanese with English summary)
11. Watanabe K, Yukuhiro F, Matsuura Y, Fukatsu T, Noda H (2014) Intrasperm vertical symbiont transmission ***Proceedings of the National Academy of Sciences of the United States of America*** 111(20):7433-7437
12. Xue J, Zhou X, Zhang C-X, Yu L-L, Fan H-W, Wang Z, Xu H-J, Xi Y, Zhu Z-R, Zhou W-W, Pan P-L, Li B-L, Colbourne J.K, Noda H, Suetsugu Y, Kobayashi T, Zheng Y, Liu S, Zhang R, Liu Y, Luo Y-D, Fang D-M, Chen Y, Zhan D-L, Lv X-D, Cai Y, Wang Z-B, Huang H-J, Cheng R-L, Zhang X-C, Lou Y-H, Yu B, Zhuo J-C, Ye Y-X, Zhang W-Q, Shen Z-C, Yang H-M, Wang J, Wang J, Bao Y-Y, Cheng J-A (2014) Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation ***Genome Biology*** 15:521

2-2-6 Elucidation of molecular mechanisms in animal immune systems

Original Papers

1. Bergman I.M, Okumura N, Uenishi H, Hammer S.E, Knoll A, Edfors I, Juul-Madsen H.R (2015) Wild boars from Sweden, Austria, the Czech Republic and Japan possess intact mannose-binding lectin 2 (MBL2) genes ***International Journal of Immunogenetics*** 42(3):204-207
2. Chae J.J, McIntosh Ambrose W, Espinoza F.A, Mulreany D.G, Ng S, Takezawa T, Trexler M.M, Schein O.D, Chuck R.S, Elisseeff J.H (2015) Regeneration of corneal epithelium utilizing a collagen vitrigel membrane in rabbit models for corneal stromal wound and limbal stem cell deficiency ***Acta Ophthalmologica*** 93(1):e57-e66
3. Escalona Z, Álvarez B, Uenishi H, Toki D, Yuste M, Revilla C, Gómez del Moral M, Alonso F, Ezquerra A, Domínguez J (2015) Molecular characterization of porcine Siglec-10 and analysis of its expression in blood and tissues ***Developmental & Comparative Immunology*** 48(1):116-123
4. Ishimaru M, Yusuke N, Tsukimoto M, Harada H, Takenouchi T, Kitani H, Kojima S (2014) Purinergic signaling via P2Y receptors up-mediates IL-6 production by liver macrophages/Kupffer cells ***The Journal of Toxicological Sciences*** 39(3):413-423
5. Kitani H, Sakuma C, Takenouchi T, Sato M, Yoshioka M, Yamanaka N (2014) Establishment of *c-myc*-immortalized Kupffer cell line from a C57BL/6 mouse strain ***Results in Immunology*** 4:68-74
6. Morozumi T, Iseki H, Toki D, Takagi M, Tsunemitsu H, Uenishi H (2014) Concise and broadly applicable method for determining the genomic sequences of North-American-type porcine reproductive and respiratory syndrome viruses in various clusters ***Journal of Veterinary Medical Science*** 76(9):1249-1255
7. Sakuma C, Sato M, Oshima T, Takenouchi T, Chiba J, Kitani H (2015) Anti-WASP intrabodies inhibit inflammatory responses induced by Toll-like receptors 3, 7, and 9, in macrophages ***Biochemical and Biophysical Research Communications*** 458(1):28-33
8. Sakuma C, Sato M, Takenouchi T, Kitani H (2015) Specific binding of the WASP N-terminal domain to Btk is critical for TLR2 signaling in macrophages ***Molecular Immunology*** 63(2):328-336
9. Sekiyama K, Waragai M, Akatsu H, Sugama S, Takenouchi T, Takamatsu Y, Fujita M, Sekigawa A, Rockenstein E, Inoue S, La Spada A.R, Masliah E, Hashimoto M (2014) Disease modifying effect of adiponectin in model of α-synucleinopathies ***Annals of Clinical and Translational Neurology*** 1(7):479-489
10. Shinkai H, Matsumoto T, Toki D, Okumura N, Terada K, Uenishi H (2015) Porcine NOD1 polymorphisms with impaired ligand recognition and their distribution in pig populations ***Molecular Immunology*** 63(2):305-311
11. Takenouchi T, Suzuki S, Shinkai H, Tsukimoto M, Sato M, Uenishi H, Kitani H (2014) Extracellular ATP does not induce P2X7 receptor-dependent responses in cultured renal- and liver-derived swine macrophages ***Results in Immunology*** 4:62-67
12. Toki Y, Takenouchi T, Harada H, Tanuma S, Kitani H, Kojima S, Tsukimoto M (2015) Extracellular ATP induces P2X7 receptor activation in mouse Kupffer cells, leading to release of IL-1β, HMGB1, and PGE2, decreased MHC class I expression and necrotic cell death ***Biochemical and Biophysical Research Communications*** 458(4):771-776
13. Yoshida J, Oshikata A, Yokoo S, Yamagami S, Takezawa T, Amano S (2014) Development and evaluation of porcine atelocollagen vitrigel membrane with a spherical curve and transplantable artificial corneal endothelial grafts ***Investigative Ophthalmology & Visual Science*** 55(8):4975-4981

Reviews and Monographs

1. Aoki S, Takezawa T, Miyazaki-Oshikata A, Ikeda S, Nagase K, Koba S, Inoue T, Uchihashi K, Nishijima-Matsunobu A, Kakihara N, Hirayama H, Narisawa Y, Toda S (2014) Collagen vitrigel membrane: a powerful tool for skin regeneration ***Inflammation and Regeneration*** 34(3):117-123

2. Takenouchi T, Sekiyama K, Tsukimoto M, Iwamaru Y, Fujita M, Sugama S, Kitani H, Hashimoto M (2015) Role of autophagy in P2X7 receptor-mediated maturation and unconventional secretion of IL-1β in microglia ***Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging*** 6(14):211-222
3. Takenouchi T, Tsukimoto M, Hashimoto M, Kitani H (2014) Inflammasome activation by danger signals: extracellular ATP and pH ***Inflammasome*** 1(1):76-80

3-1 Innovation of of technologies for development of genetically modified crops and intensification of their use

Original Papers

1. Fukuda K, Ishida W, Harada Y, Wakasa Y, Takagi H, Takaiwa F, Fukushima A (2015) Prevention of allergic conjunctivitis in mice by a rice-based edible vaccine containing modified Japanese cedar pollen allergens ***British Journal of Ophthalmology*** 99(5):705-709
2. Iizuka M, Wakasa Y, Tsuboi H, Asashima H, Hirota T, Kondo Y, Matsumoto I, Sumida T, Takaiwa F (2014) Prophylactic effect of the oral administration of transgenic rice seeds containing altered peptide ligands of type II collagen on rheumatoid arthritis ***Bioscience, Biotechnology and Biochemistry*** 78(10):1662-1668
3. Iizuka M, Wakasa Y, Tsuboi H, Asashima H, Hirota T, Kondo Y, Matsumoto I, Takaiwa F, Sumida T (2014) Suppression of collagen-induced arthritis by oral administration of transgenic rice seeds expressing altered peptide ligands of type II collagen ***Plant Biotechnology Journal*** 12(8):1143-1152
4. Miyata K, Kozaki T, Kouzai Y, Ozawa K, Ishii K, Asamizu E, Okabe Y, Umehara Y, Miyamoto A, Kobae Y, Akiyama K, Kaku H, Nishizawa Y, Shibuya N, Nakagawa T (2014) The bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice ***Plant and Cell Physiology*** 55(11):1864-1872
5. Nishimura T, Saeki M, Kaminuma O, Takaiwa F, Hiroi T (2014) Transgenic plants for allergen-specific immunotherapy ***World Journal of Immunology*** 4(3):141-148
6. Ogo Y, Takahashi H, Wang S, Takaiwa F (2014) Generation mechanism of novel, huge protein bodies containing wild type or hypoallergenic derivatives of birch pollen allergen Bet v 1 in rice endosperm ***Plant Molecular Biology*** 86(1-2):111-123
7. Ogo Y, Wakasa Y, Hirano K, Urisu A, Matsuda T, Takaiwa F (2014) Generation of transgenic rice with reduced content of major and novel high molecular weight allergens ***Rice*** 7:19
8. Wakasa Y, Oono Y, Yazawa T, Hayashi S, Ozawa K, Handa H, Matsumoto T, Takaiwa F (2014) RNA sequencing-mediated transcriptome analysis of rice plants in endoplasmic reticulum stress conditions ***BMC Plant Biology*** 14:101
9. Wakasa Y, Takagi H, Watanabe N, Kitamura N, Fujiwara Y, Ogo Y, Hayashi S, Yang L, Ohta M, Wai Wai Thet Tin, Sekikawa K, Takano M, Ozawa K, Hiroi T, Takaiwa F (2015) Concentrated protein body product derived from rice endosperm as an oral tolerogen for allergen-specific immunotherapy - A new mucosal vaccine formulation against Japanese cedar pollen allergy ***PLoS ONE*** 10(3):e0120209
10. Yang L, Kawakatsu T, Wakasa Y, Yoine M, Takaiwa F (2014) RNA silencing is induced by the expression of foreign recombinant products in transgenic rice ***Plant Science*** 225:138-146

Reviews and Monographs

1. Nanasato Y, Tabei Y (2015) Cucumber (*Cucumis sativus* L.) and Kabocha Squash (*Cucurbita moschata* Duch) ***Methods in Molecular Biology*** 1223(24):299-310
2. Takaiwa F (2014) Plant-based vaccines against pollen allergy ***Genetically Engineered Plants as a Source of Vaccines Against Wide Spread Diseases*** (12):243-264

3. Tsuda M, Ohsawa R, Tabei Y (2014) Possibilities of direct introgression from *Brassica napus* to *B. juncea* and indirect introgression from *B. napus* to related Brassicaceae through *B. juncea* ***Breeding Science*** 64(1):74-82

3-2 Development of novel technologies for efficient use of genetically modified silkworm

Original Papers

1. Asakura T, Isozaki M, Saotome T, Tatematsu K, Sezutsu H, Kuwabara N, Nakazawa Y (2014) Recombinant silk fibroin incorporated cell-adhesive sequences produced by transgenic silkworm as a possible candidate for use in vascular graft ***Journal of Materials Chemistry B*** 2(42):7375-7383
2. Fujiwara T, Kazawa T, Sakurai T, Fukushima R, Uchino K, Yamagata T, Namiki S, Haupt S.S, Kanzaki R (2014) Odorant concentration differentiator for intermittent olfactory signals ***The Journal of Neuroscience*** 34(50):16581-16593
3. Iizuka T, Nakajima K, Okada K (2015) Double cocoon mounting methods ***The Journal of Silk Science and Technology of Japan*** 23:37-42 (in Japanese with English summary)
4. Iizuka T, Inami Y, Misawa T, Hashimoto K, Nakamura K, Okada E (2015) New formaldehyde gas fumigation method using a the decomposition apparatus of a formaldehyde gas ***The Journal of Silk Science and Technology of Japan*** 23:51-55 (in Japanese with English summary)
5. Kimoto M, Tsubota T, Uchino K, Sezutsu H, Takiya S (2015) LIM-homeodomain transcription factor Awh is a key component activating all three fibroin genes, *fibH*, *fibL* and *fhx*, in the silk gland of the silkworm, *Bombyx mori* ***Insect Biochemistry and Molecular Biology*** 56:29-35
6. Kiya T, Morishita K, Uchino K, Iwami M, Sezutsu H (2014) Establishment of tools for neurogenetic analysis of sexual behavior in the silkmoth, *Bombyx mori* ***PLoS ONE*** 9(11):e113156
7. Komoto N, Tsuda M, Okada E, Iizuka T, Kuwabara N, Sezutsu H, Tabei Y (2014) Development of methods for risk assessment of transgenic silkworms rearing on biodiversity ***Sanshi-Konchu Biotec*** 83(2):171-179 (in Japanese with English summary)
8. Kuwana Y, Sezutsu H, Nakajima K, Tamada Y, Kojima K (2014) High-toughness silk produced by a transgenic silkworm expressing spider (*Araneus ventricosus*) dragline silk protein ***PLoS ONE*** 9(8):e105325
9. Matsumoto Y, Ishii M, Ishii K, Miyaguchi W, Horie R, Inagaki Y, Hamamoto H, Tatematsu K, Uchino K, Tamura T, Sezutsu H, Sekimizu K (2014) Transgenic silkworms expressing human insulin receptors for evaluation of therapeutically active insulin receptor agonists ***Biochemical and Biophysical Research Communications*** 455(3-4):159-164
10. Mochida Y, Takemura Y, Matsumoto M, Ohnuma A, Uchino K, Iizuka T, Kômoto N, Sezutsu H, Kiuchi M (2014) Cryopreservation of germplasm of transgenic silkworms ***Sanshi-Konchu Biotec*** 83(2):163-170 (in Japanese with English summary)
11. Nakade S, Tsubota T, Sakane Y, Kume S, Sakamoto N, Obara M, Daimon T, Sezutsu H, Yamamoto T, Sakuma T, Suzuki K.T (2014) Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9 ***Nature Communications*** 5:5560
12. Nomura T, Suganuma M, Higa Y, Kataoka Y, Funaguma S, Okazaki H, Suzuki T, Kobayashi I, Sezutsu H, Fujiyama K (2015) Improvement of glycosylation structure by suppression of β-*N*-acetylglucosaminidases in silkworm ***Journal of Bioscience and Bioengineering*** 119(2):131-136
13. Suzuki T.K, Tomita S, Sezutsu H (2014) Gradual and contingent evolutionary emergence of leaf mimicry in butterfly wing patterns ***BMC Evolutionary Biology*** 14:229

14. Tsubota T, Uchino K, Suzuki T.K, Tanaka H, Kayukawa T, Shinoda T, Sezutsu H (2014) Identification of a novel strong and ubiquitous promoter/enhancer in the silkworm *Bombyx mori* ***G3: Genes, Genomes, Genetics*** 4(7):1347-1357

Monograph

1. Sezutsu H, Tamura T (2014) Silkworm transgenesis and applications ***Transgenic Insects: Techniques and Applications*** 2(9):138-151

3-3 Development of novel technologies for efficient use of genetically modified animals

Original Papers

1. Miwa Y, Yazaki S, Iwamoto M, Suzuki S, Iwasaki K, Haneda M, Yamamoto K, Maruyama S, Onishi A, Kobayashi T (2015) Functional difference between membrane-bound and soluble human thrombomodulin ***Transplantation*** 99(4):702-709
2. Nakai M, Ozawa M, Maedomari N, Noguchi J, Kaneko H, Ito J, Onishi A, Kashiwazaki N, Kikuchi K (2014) Delay in cleavage of porcine embryos after intracytoplasmic sperm injection (ICSI) shows poorer embryonic development ***Journal of Reproduction and Development*** 60(3):256-259
3. Suzuki S, Suzuki M, Nakai M, Sembon S, Fuchimoto D, Onishi A (2014) Transcriptional and histological analyses of the thymic developmental process in the fetal pig ***Experimental Animals*** 63(2):215-225

3-4 Development of novel technologies using biomaterials based on silk proteins

Original Papers

1. Hashimoto T, Taniguchi Y, Kameda T, Tamada Y, Kurosu H (2015) Changes in the properties and protein structure of silk fibroin molecules in autoclaved fabrics ***Polymer Degradation and Stability*** 112:20-26
2. Hosoe M, Yoshida N, Hashiyada Y, Teramoto H, Takahashi T, Niimura S (2014) Sericin accelerates the production of hyaluronan and decreases the incidence of polyspermy fertilization in bovine oocytes during *in vitro* maturation ***Journal of Reproduction and Development*** 60(4):268-273
3. Ito T, Kameda T, Tsuji Y, Tonooka N (2015) Suppressing gelation and promoting skin absorption of silk fibroin aqueous solution ***The Journal of Silk Science and Technology of Japan*** 23:57-65 (in Japanese with English summary)
4. Kambe Y, Sutherland T.D, Kameda T (2014) Recombinant production and film properties of full-length hornet silk proteins ***Acta Biomaterialia*** 10(8):3590-3598
5. Kameda T (2015) Influence of pH, temperature, and concentration on stabilization of aqueous hornet silk solution and fabrication of salt-free materials ***Biopolymers*** 103(1):41-52
6. Kuwana Y, Sezutsu H, Nakajima K, Tamada Y, Kojima K (2014) High-toughness silk produced by a transgenic silkworm expressing spider (*Araneus ventricosus*) dragline silk protein ***PLoS ONE*** 9(8):e105325
7. Sajwan S, Sidorov R, Stašková T, Žaloudíková A, Takasu Y, Kodrík D, Zurovec M (2015) Targeted mutagenesis and functional analysis of adipokinetic hormone-encoding gene in *Drosophila* ***Insect Biochemistry and Molecular Biology*** 61:79-86
8. Sutherland T.D, Sriskantha A, Church J.S, Strive T, Trueman H.E, Kameda T (2014) Stabilization of viruses by encapsulation in silk proteins ***ACS Applied Materials & Interfaces*** 6(20):18189-18196

9. Teramoto H, Kojima K (2014) Production of *Bombyx mori* silk fibroin incorporated with unnatural amino acids ***Biomacromolecules*** 15(7):2682-2690
10. Teramoto H, Kojima K (2013) Residue-specific incorporation of phenylalanine analogues into protein biosynthesis in silkworm cultured cells ***Journal of Insect Biotechnology and Sericology*** 82(3):61-69
11. Teramoto H, Kojima K (2015) Incorporation of methionine analogues into *Bombyx mori* silk fibroin for click modifications ***Macromolecular Bioscience*** 15(5):719-727
12. Teramoto H, Nakajima K, Kojima K (2015) Characterization of *Bombyx mori* silk fiber incorporating an unnatural amino acid (4-chlorophenylalanine) ***The Journal of Silk Science and Technology of Japan*** 23:27-35 (in Japanese with English summary)

3-5 Elucidation of insect-specific biological functions and development of novel technologies for their applications

Original Papers

1. Gusev O, Suetsugu Y, Cornette R, Kawashima T, Logacheva M.D, Kondrashov A.S, Penin A.A, Hatanaka R, Kikuta S, Shimura S, Kanamori H, Katayose Y, Matsumoto T, Shagimardanova E, Alexeev D, Govorun V, Wisecaver J, Mikheyev A, Koyanagi R, Fujie M, Nishiyama T, Shigenobu S, Shibata T.F, Golygina V, Hasebe M, Okuda T, Satoh N, Kikawada T (2014) Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge ***Nature Communications*** 5:4784
2. Hatanaka R, Furuki T, Shimizu T, Takezawa D, Kikawada T, Sakurai M, Sugawara Y (2014) Biochemical and structural characterization of an endoplasmic reticulum-localized late embryogenesis abundant (LEA) protein from the liverwort *Marchantia polymorpha* ***Biochemical and Biophysical Research Communications*** 454(4):588-593
3. Hatanaka R, Gusev O, Cornette R, Shimura S, Kikuta S, Okada J, Okuda T, Kikawada T (2015) Diversity of the expression profiles of late embryogenesis abundant (LEA) protein encoding genes in the anhydrobiotic midge *Polypedilum vanderplanki* ***Planta*** DOI: 10.1007/s00425-015-2284-6
4. Kato Y (2015) Tunable translational control using site-specific unnatural amino acid incorporation in *Escherichia coli* ***PeerJ*** 3:e904
5. Kobayashi H, Fujii-Muramatsu R, Noda H, Takeishi K (2014) Construction of an expressible BAC library of the unculturable insect microorganism, stink bug *Plautia stali* symbiont, for the search of biologically active and useful symbiont products ***Biological & Pharmaceutical Bulletin*** 37(4):528-533
6. Tsubota T, Uchino K, Suzuki T.K, Tanaka H, Kayukawa T, Shinoda T, Sezutsu H (2014) Identification of a novel strong and ubiquitous promoter/enhancer in the silkworm *Bombyx mori* ***G3: Genes, Genomes, Genetics*** 4(7):1347-1357
7. Yamakawa K, Furuki T, Furuta T, Hatanaka R, Kikawada T, Niwa T, Taguchi H, Furusawa H, Okahata Y, Sakurai M (2013) Experimental study on the mechanism underlying the anti-aggregation function of a group3LEA peptide ***Cryobiology and Cryotechnology*** 59(2):95-99 (in Japanese with English summary)

Review

1. Indo H.P, Yen H-C, Nakanishi I, Matsumoto K, Tamura M, Nagano Y, Matsui H, Gusev O, Cornette R, Okuda T, Minamiyama Y, Ichikawa H, Suenaga S, Oki M, Sato T, Ozawa T, St. Clair D.K, Majima H.J (2015) A mitochondrial superoxide theory for oxidative stress diseases and aging ***Journal of Clinical Biochemistry and Nutrition*** 56(1):1-7

Research Highlights for FY2014

No. 4

Date of Issue: September 2015
Published by the National Institute of Agrobiological Sciences
Kannondai, Tsukuba, Ibaraki, 305-8602 Japan
TEL: +81-29-838-7406
FAX: +81-29-838-7408
URL: http://www.nias.affrc.go.jp/index_e.html

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