九州大学大学院農学研究院
遺伝子資源開発研究センター

年　報　　　　　　第21号

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Number 21

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九州大学大学院　農学研究院
遺伝子資源開発研究センター

年報　　第21号

平成29年

九州大学大学院　農学研究院附属遺伝子資源開発研究センター

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I.　センターの概要

1.　目的

本センターは、遺伝子資源の収集、保存、開発から評価、利用に至る研究教育を遂行する。特に、高度な技法で遺伝情報の解析を行い、遺伝子レベルでの農業遺伝子資源に関する応用展開研究と戦略的プロジェクト研究の推進並びに遺伝子資源のDNA・細胞レポジトリー機能の充実を図る。

2.　沿革

昭和62年5月	本学附属家蚕遺伝子実験施設を振替え、附属遺伝子資源研究センターが10年の時限施設として設置され、教授、助教授、助手各2名が配置された。
平成元年4月	教授、助教授各1が追加配置された。本学大学院農学研究科に設置された独立専攻遺伝子資源工学専攻の協力講座として、昆虫遺伝子資源学、遺伝子開発管理学の2講座に参加した。
平成3年4月	遺伝子資源工学専攻に微生物遺伝子工学講座が新設され、これに参加した。
平成9年4月	附属遺伝子資源研究センターは時限により廃止され、新たに、教授3名、助教授3名、助手2名の振替えによって附属遺伝子資源開発研究センターが設置された。
平成12年4月	大学院重点化に伴い、大学院　農学研究院附属遺伝子資源開発研究センターに改組された。また、大学院教育として生物資源環境科学府　遺伝子資源工学専攻　遺伝子資源開発学講座（昆虫遺伝子資源学分野、植物遺伝子資源学分野、微生物遺伝子工学分野）に改組された。
平成22年4月	農学研究院・学府組織改組に伴い、昆虫遺伝子資源学分野、植物遺伝子資源学分野は、生命機能科学部門　システム生物学講座に、微生物遺伝子工学分野は分子微生物学・バイオマス資源科学講座に所属し、教育に参画した。

3. 組織・教職員

センター長　熊丸敏博

家蚕遺伝子開発分野

職名	氏名	職名	氏名
教授	伴野　豊	技術職員	西川　和弘
助教	山本　幸治	技術職員	田村　圭
学術研究員	藤井　告	技術職員	山本　和典
学術研究員	福森　善寿	技術補佐員	江口　誠一

植物遺伝子開発分野

職名	氏名	職名	氏名
教授	熊丸　敏博	テクニカルスタッフ	藤田　喜久男
准教授	久保　貴彦	テクニカルスタッフ	原田　良子
特任助教	福田　真子	テクニカルスタッフ	有隅　久美子
特任助教	松坂　弘明	テクニカルスタッフ	須山　奈緒美
研究支援推進員	仲島　久美子	技術補佐員	村田　亜紀
		技術補佐員	米田　未和子

微生物遺伝子開発分野

職名	氏名	職名	氏名
教授	土居　克実	技術補佐員	守川　佳乃子
助教	藤野　泰寛		

4. 研究と事業内容

家蚕遺伝子開発分野

・カイコ遺伝子資源の収集、開発、評価、保存、活用並びに遺伝子機能の発現機構の解明
・文部科学省ナショナルバイオリソースプロジェクトNBRP（カイコ）の中核機関として我国のバイオリソース事業への貢献

植物遺伝子開発分野

・イネ種子貯蔵タンパク質の生合成･集積を制御する遺伝的機構の解明
・ナショナルバイオリソースプロジェクト(NBRP)におけるイネ突然変異系統の整備
・在来イネ遺伝子資源の保存と特性評価に関する研究

微生物遺伝子開発分野

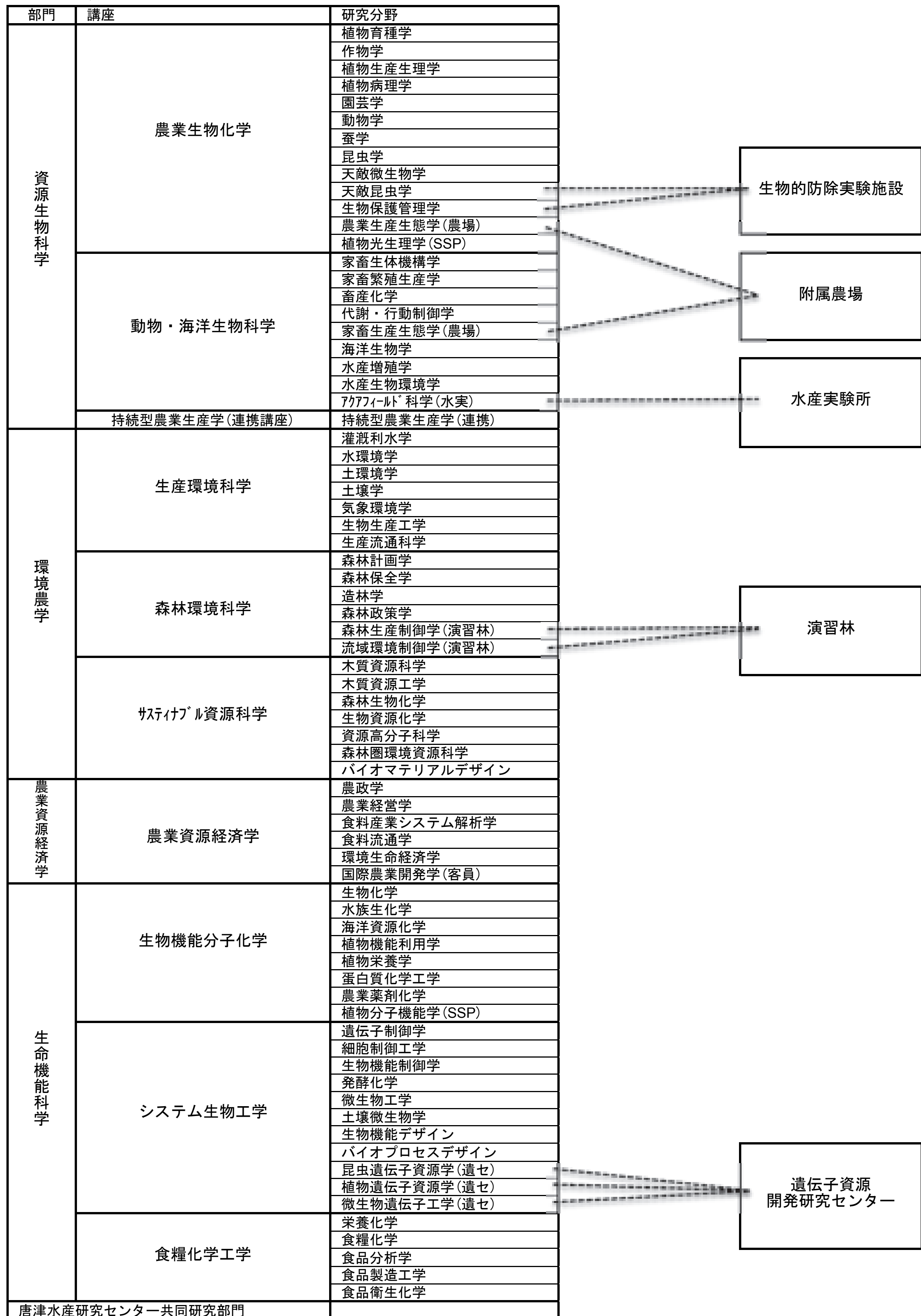
・微生物遺伝子資源の探索と評価、保存と利用開発、並びに有用遺伝子の高度機能化と応用展開に関する研究

各分野とも、所定の許可を得た本学部内外の学生や研究者等に対し、研究の場や遺伝子資源材料を提供し、さらに研究指導と教育を行っている。

5. 遺伝子資源開発研究センター運営委員会委員　（平成30年3月31日現在）

委員長	熊丸　敏博	(遺伝子資源開発研究センター)
教授	伴野　豊	(遺伝子資源開発研究センター)
教授	土居　克実	(遺伝子資源開発研究センター)
准教授	田代　康介	(生命機能科学部門)
准教授	片倉　喜範	(生命機能科学部門)
教授	吉村　淳	(資源生物科学部門)
准教授	小名　俊博	(環境農学部門)
教授	南石　晃明	(農業資源経済学部門)
教授	望月　俊宏	(附属農場)
教授	大賀　祥治	(附属演習林)
教授	青木　智佐	(附属生物的防除研究施設)

6. 組織図



部門	講座	研究分野
資源生物科学	農業生物化学	植物育種学
		作物学
		植物生産生理学
		植物病理学
		園芸学
		動物学
		蚕学
		昆虫学
		天敵微生物学
		天敵昆虫学
		生物保護管理学
		農業生産生態学(農場)
		植物光生理学(SSP)
	動物・海洋生物科学	家畜生体機構学
		家畜繁殖生産学
		畜産化学
		代謝・行動制御学
		家畜生産生態学(農場)
		海洋生物学
		水産増殖学
		水産生物環境学
		ｱｸｱﾌｨｰﾙﾄﾞ科学(水実)
	持続型農業生産学(連携講座)	持続型農業生産学(連携)
環境農学	生産環境科学	灌漑利水学
		水環境学
		土環境学
		土壌学
		気象環境学
		生物生産工学
		生産流通科学
	森林環境科学	森林計画学
		森林保全学
		造林学
		森林政策学
		森林生産制御学(演習林)
		流域環境制御学(演習林)
	ｻｽﾃｨﾅﾌﾞﾙ資源科学	木質資源科学
		木質資源工学
		森林生物化学
		生物資源化学
		資源高分子科学
		森林圏環境資源科学
		バイオマテリアルデザイン
農業資源経済学	農業資源経済学	農政学
		農業経営学
		食料産業システム解析学
		食料流通学
		環境生命経済学
		国際農業開発学(客員)
生命機能科学	生物機能分子化学	生物化学
		水族生化学
		海洋資源化学
		植物機能利用学
		植物栄養学
		蛋白質化学工学
		農業薬剤化学
		植物分子機能学(SSP)
	システム生物工学	遺伝子制御学
		細胞制御工学
		生物機能制御学
		発酵化学
		微生物工学
		土壌微生物学
		生物機能デザイン
		バイオプロセスデザイン
		昆虫遺伝子資源学(遺セ)
		植物遺伝子資源学(遺セ)
		微生物遺伝子工学(遺セ)
	食糧化学工学	栄養化学
		食糧化学
		食品分析学
		食品製造工学
		食品衛生化学
唐津水産研究センター共同研究部門		

II. 研究成果

1. 研究業績・出版物リスト

【家蚕遺伝子開発分野】

A. 原著論文

1) Fujii T, Banno Y.
Unique phenotype of the metamorphosis-defective mutant Ishigameyoh (gap): Establishment of a PCR-based marker for efficacious mutant maintenance in *Bombyx mori*.
Journal of Insect Biotechnology and Sericology, **86**(3), 95-103 (2017) doi:10.11416/jibs.86.3_095

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Gene, **629**, 92-100 (2017) doi: 10.1016/j.gene.2017.07.071 doi: 10.1016/j.gene.2017.07.071

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5) Fukumori H, Lee J, Fujii T, Kajiura Z, Banno Y.
Long-term preservation of eri and ailanthus silkworms using frozen gonads.
Cryobiology, **77**, 71-74 (2017) doi: 10.1016/j.cryobiol.2017.05.003

6) Yamashita M, Xu J, Morokuma D, Hirata K, Hino M, Mon H, Takahashi M, Hamdan SM, Sakashita K, Iiyama K, Banno Y, Kusakabe T, Lee JM.
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Molecular Biotechnology, **59**(6), 221-233 (2017) doi: 10.1007/s12033-017-0008-9

7) Hirowatari A, Chen Z, Mita K, Yamamoto K,
Enzymatic characterization of two epsilon-class glutathione S-transferases of *Spodoptera*

litura,
Archives of Insect Biochemistry & Physiology, e21443 (2017)

8) Cheng T*, Wu J*, Wu Y*, Chilukuri R*, Huang L*, Yamamoto K*, Feng L*, Li W* et al., Arunkumar KP, Kishino H, Goldsmith MR, Feng Q, Xia Q, Mita K (*equal contribution),
Wide distribution of a major East Asian noctuid pest explained by genomic adaptation to polyphagy and insecticides,
Nature Ecology & Evolution, **1** (11) 1745-1756 (2017)

9) Yamamoto K, Higashiura A, Suzuki M, Aritake K, Urade Y, Nakagawa A,
Molecular structure of a prostaglandin D synthase requiring glutathione from the brown planthopper, *Nilaparvata lugens*,
Biochemical and Biophysical Research Communications, **492**, 166-171 (2017)

10) Yamamoto K, Ozakiya Y, Uno T,
Localization of an Aldo-Keto Reductase (AKR2E4) in the Silkworm *Bombyx mori* (Lepidoptera: Bombycidae),
Journal of insect science, **17** (5), 1-3 (2017)

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1) 山本幸治,
特集「昆虫の農薬代謝分子機構」にあたって,
蚕糸昆虫バイオテック, **86**(3), 141-142 (2017)

2) 山本幸治,
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3) 行弘研司, 河本夏雄, 冨田秀一郎, 瀬筒秀樹, 志村幸子, 伴野 豊,
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4) 吉永啓恵, 長崎紀代美, 江田将子, 福森寿善, 伴野 豊,
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5) 福森寿善, 伴野 豊,
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日本薬学会第138年会, 2018年03月, 金沢市.

7) 田中龍一郎, 西久保南苗, 細木 美来, 坂崎 文俊, 伴野 豊, 秋澤 俊史,
血中尿酸低下作用物質の探索（26）—o06 系統油蚕の5齢期における血中尿酸の代謝と排泄（II）—,
日本薬学会第138年会, 2018年03月, 金沢市.

8) Haque MR, Hirowatari A, Furuya S, Yamamoto K,
Identification and characterization of the 5, 10- Methylene- tetrahydrofolate dehydrogenase from silkworm (*Bombyx mori*),
2017 AFELiSA, 2017年11月8日, 九州大学.

【植物遺伝子開発分野】

A. 原著論文

1) Nagamoto J., A. Sawazaki, M. Miyago, B. Shirouchi, M. Sakata, Li-T. Tong, T. Kumamaru, M. Sato.
Rapid semi-quatification of triacylglycerol, phosphatidylcholines and free fatty acids in rice bran of one grain,
Journal of Integrative Agriculture **17**, Online published (2018)

2) Yamatani H., K. Kohzuma, M. Nakano, T. Takami, Y. Kato, Y. Hayashi, Y. Monden, Y. Okumoto, T. Abe, T. Kumamaru, A. Tanaka, W. Sakamoto, Kusaba.
Impairment of Lhca4, a subunit of LHCI, causes high accumulation of chlorophyll and the stay-green phenotype in rice.
Journal of Experimental Botany, **69** (5), 1027–1035, (2018)

3) Chou H. L., L. Tian, T. Kumamar, S. Hamada, T. W Okita.
Multifunctional RNA binding protein OsTudor-SN in storage protein mRNA transport and localization.
Plant Physiology, **175**, 16028-1623 (2017)

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Genotype by environment interactions (GEIs) for barley grain yield under salt stress condition.
Journal of Crop Science and Biotechnology, **20**, 193-204, (2017)

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Inheritance pattern of earliness and yield related-traits in spring barley (Hordeum vulgare L.).
Journal of Agricultural Science, **9**, 142-153 (2017)

6) Matsumoto H., Y. Yasui, T. Kumamaru, H. Hirano.
Characterization of a half-pipe-like leaf1 mutant that exhibits a curled leaf phenotype.
Genes Genet. Syst., **92**, 287-291, (2017)

7) Kubo T., Yoshimura A., and Kurata N.
Genetic characterization and fine-mapping of S25, a hybrid male sterility gene, on rice chromosome 12.
Genes & Genetics Systems, **92**: 205-212 (2017)

B. 著書・総説

無し

C. 学会発表

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International Symposium on Rice Functional Genomics, 2017年9月, Suwon (Korea).

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糊粉層の肥厚化による高脂質含量イネ系統の開発と評価.
日本育種学会，2017 年 10 月，岩手大学.

3) 和田 卓也, 熊丸 敏博, 宮原 克典, 宮崎 真行.
玄米のタンパク質含有率 QTL のマッピングおよび QTL 保有 NIL の遺伝子発現解析.
日本育種学会，2017 年 10 月，岩手大学.

4) 福田真子，小川雅広，熊丸敏博.
コメグルテリンの生合成･蓄積に関わる因子の欠損による貯蔵タンパク質の局在部位の変化.
九州育種談話会，2017年12月，佐賀大学.

5) Sobhy A., Fukuda M., and Kumamaru T.
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6) 中村哲洋，熊丸敏博.
イネ *ae/sug1* 二重変異体における胚乳澱粉生合成解析.
九州育種談話会，2017 年 12 月，佐賀大学.

7) Elakhdar A., Ushijima T, Fukuda M, Yamashiro N, Kawagoe Y, Kumamaru T.
The Recognition Assay of Termination Codon by ESP1/ Eukaryotic Releases Factor-1 (eRF1) in Rice.
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日本育種学会, 2018年3月, 九州大学

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【微生物遺伝子開発分野】

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無し

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2.　原著論文要旨

家蚕遺伝子開発分野

Journal of Insect Biotechnology and Sericology 86, 95-103 (2017)

Unique phenotype of the metamorphosis-defective mutant Ishigameyoh (*gap*): Establishment of a PCR-based marker for efficacious mutant maintenance in *Bombyx mori*

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Ishigameyoh (*gap*) was identified as a spontaneous mutant of *Bombyx mori* with an apterous and sterile phenotype. Phenotypic observations revealed that the *gap* mutation affected the development of imaginal discs and the primordia of adult organs, including the wings, legs, compound eyes, and reproductive organs. Therefore, Ishigameyoh (*gap*) is a metamorphosis-defective mutant. We found that oogenesis depends on the *gap* gene, whereas spermatogenesis is independent. Ovary transplantation experiments suggest a cell-autonomous requirement for the *gap* gene in the process of oogenesis. The *gap* mutation has been maintained by crossing $+^{gap}$/*gap* moths as the u90 strain. However, the u90 strain is difficult to maintain because $+^{gap}/+^{gap}$ and $+^{gap}$/*gap* moths cannot be distinguished phenotypically. For efficacious maintenance, we established a molecular marker that is closely linked to the *gap* locus. First, we localized the *gap* locus to chromosome 8 using phenotypic markers. Second, the *gap* locus was narrowed down to within a 1-Mb region using PCR-based markers. Within this 1-Mb region, we finally established a PCR-based marker that amplified different-sized products from $+^{gap}/+^{gap}$ and *gap*/*gap* offspring of the u90 strain. This marker allows the distinction of $+^{gap}/+^{gap}$ and $+^{gap}$/*gap* moths with an error rate of <1% and therefore reduces the labor needed to maintain the u90 strain. Moreover, this marker helps to identify $+^{gap}$ and *gap* individuals before pupation, which is necessary for larvae-based genetic and physiological analyses of the *gap* gene.

Key words: Metamorphosis, Imaginal disc, Primordia, PCR-based marker, Ovary transplantation, Sterility

INTRODUCTION

During the metamorphosis of Lepidopteran insects, the imaginal discs develop rapidly to form adult organs during the last instar-to-pupal period. Early in the larval stage, the imaginal discs corresponding to the wings and reproductive organs become distinct from those of the larval epidermis (Truman and Riddiford, 2002; Ikeda, 1913). However, the imaginal discs corresponding to the antenna, legs, and compound eyes are late-forming and derived from the primordia that constitute the larval epidermis, and are not differentiated before the last instar stage (Svácha, 1992; Singh *et al.*, 2007; Allee *et al.*, 2006; Truman and Riddiford, 2002). In the moth *Manduca sexta*, imaginal disc development is hormonally regulated and dependent on the larval nutritional condition (Truman *et al.*, 2006). However, many of the endocrinal requirements for the metamorphosis of imaginal discs in Lepidopteran species remain to be elucidated.

At Kyushu University, as many as 500 silkworm strains have been maintained as inbred strains via sibling mating. Most of these strains are reared by mixing larvae hatched from approximately 15 batches because larger numbers of larvae simplify the process of rearing the first and second instar larvae to escape the bottleneck effect of accumulated mutations on viability. By contrast, strains harboring lethal or sterile mutations require special maintenance. These mutants should be maintained by crossing with individuals heterozygous for the target mutation. Generally, normal individuals that do and do not harbor a recessive mutant allele cannot be distinguished phenotypically. Therefore, to maintain these mutant strains, single-batch rearing should be performed until a particular phenotype is observed. For example, to maintain the a60 strain harboring the lethal albino mutation (*al*), about nine batches are raised as single batches until the second instar stage, as mutants exhibit a whitish, unpigmented integument and die (Doira *et al.*, 1973; Fujii *et al.*, 2013). After the second instar, batches yielding albino larvae are selected, mixed, and reared.

Among the roughly 500 strains preserved at Kyushu University, approximately 50 harbor lethal or sterile mutations. Of these, the u90 strain is the most challenging to maintain (Banno, personal communication). This strain has been maintained by crossing with +/*gap* moths. "Ishigameyoh", denoted by the gene symbol "*gap* (apterous and rudimentary gonads)", is a spontaneous mutant with an apterous and sterile phenotype (Doira, 1992; Kawaguchi *et al.*, 2009). The phenotypic features of *gap* mutants cannot be identified during the larval stage. Therefore, each batch of the u90 strain must be raised in a single batch until the spinning stage. Traditionally, we have reared more than

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Genetic studies on two egg mutants, "*small size egg*" and "*lethal non- diapausing egg*" in the silkworm, *Bombyx mori*

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Key words: *Bombyx mori*, oogenesis, lethal gene, mutant, embryogenesis

INTRODUCTION

So far, many mutants related with egg character, such as size, shape, color, so forth, have been reported in the silkworm (Doira, 1978; Goldsmith, 1995; and Banno *et al.*, 2005). We found two irregular egg characters in one hybrid strain, which had been sold as a commercial strain by the Katakura Company. One phenotype is small size eggs. In another mutant, the egg size was normal, but the hatching was not recognized. Preliminary tests showed that both mutants were controlled by recessive genes, which have non-allelic relationship. In this study, we analyzed the two mutants genetically and determined the two gene loci on linkage maps.

MATERIALS AND METHODS

Insect

One strain derived from an offspring obtained in a cross between Shukou and Ryuhaku was provided to Yamaguchi University by the Katakura Company. After 2011, it has been preserved as xe51 strain at the institute of genetic resources, Kyushu University. The other original strains used in this study were d30, e80, e81, and p50, which are preserved at Kyushu University, supported by the National BioResources Project. The silkworm larvae were reared on mulberry leaves at 25°C.

Linkage analysis

Linkage analysis of the small size egg mutant was performed applying a single nucleotide polymorphism linkage map (Yamamoto *et al.*, 2006). Practically, a single nucleotide polymorphism based analysis (SNP analysis) was done via PCR amplification of the SNP region and sequencing of PCR products. Genomic DNA was isolated from the anterior legs of adult moths from grandparental strains (p50 female and xe51 male) and their F_1 generations using DNAzol (Invitrogen, Carlsbad, CA, USA). Sixteen segregants of F_2 female moths and their laid small eggs were used for the linkage analysis. A SNP marker was used for each of the 28 chromosomes (Table 1). The SNP marker primer sequences have been described by Yamamoto *et al.* (2006). The PCR products were directly sequenced via BigDye terminator cycle sequencing (Applied Biosystems) with the same primers used for PCR.

RESULTS AND DISCUSSION

1. Lethal non-diapausing egg

・Origin and phenotype

Jun Kobayashi, one of the authors obtained one commercial strain from the Katakura Company that originated from a cross between Shukou and Ryuhaku. When approximately three generations had passed for the preservation of this strain at Yamaguchi University, we recognized that non-pigmented eggs (yellow) were segregated with normal pigmented eggs in the same batch (Fig. 1). Usually, fertilized egg colors will change to dark blue from yellow within 2-3 days after oviposition under 25°C. However, even though one month had passed, color changing observed in normal eggs was not shown. In those eggs, embryos were formed and demonstrated a small progressive stage of development that stopped around blastokinesis.

On the other hand, the normal pigmented eggs, which segregated with the yellow colored eggs, hatched and developed into moths. In four bloods of six from the sib cross of those moths, non-pigmented eggs segregated again (Table 2). In each of these four lines, non-pigmented eggs were observed at a rate of approximately 25% among the total eggs. Because the segregated, non-pigmented eggs did not hatch, the gene controlling this trait is estimated to be a recessive lethal gene, and this trait is inherited to the next generation by only heterozygous individuals. For

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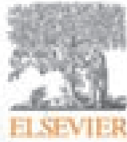

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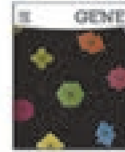

Research paper

Bm-muted, orthologous to mouse *muted* and encoding a subunit of the BLOC-1 complex, is responsible for the *otm* translucent mutation of the silkworm *Bombyx mori*

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ABSTRACT

"Tanaka's mottled translucent" (*otm*) is a mutation of the silkworm *Bombyx mori* that exhibits translucent skin during larval stages. We performed positional cloning of the gene responsible for *otm* and mapped it to a 364-kb region on chromosome 5 that contains 22 hypothetical protein-coding genes. We performed RNA-seq analysis of the epidermis and fat body of *otm* larvae and determined that the gene *BGIBMGA002619* may be responsible for the *otm* mutation. *BGIBMGA002619* encodes the biosynthesis of lysosome-related organelles complex 1 (BLOC-1) subunit 5, whose ortholog is responsible for the *Muted* mutant in mouse. Accordingly, we named this gene *Bm-muted*. We discovered that the expression of *Bm-muted* in the epidermis and fat body of *otm* mutants was dramatically suppressed compared with the wild type. We determined the nucleotide sequences of the full-length cDNA and genomic region corresponding to *Bm-muted* and found that a 538-bp long DNA sequence similar to *B. mori* transposon *Organdy* was inserted into the 3′ end of the first intron of *Bm-muted* in two *otm* strains. The *Bm-muted* cDNA of *otm* mutants lacked exon 2, and accordingly generated a premature stop codon in exon 3. In addition, short interfering RNA (siRNA)-mediated knockdown of this gene caused localized partial translucency of larval skin. These data indicate that the mutation in *Bm-muted* caused the *otm*-mutant phenotype. We propose that the insertion of *Organdy* caused a splicing disorder in *Bm-muted* in the *otm* mutant, resulting in a null mutation of *Bm-muted*. This mutation is likely to cause deficiencies in urate granule formation in epidermal cells that result in translucent larval skin.

1. Introduction

The silkworm *Bombyx mori* synthesizes uric acid in the fat body (Hayashi, 1960; Bray et al., 1996) and stores it in the form of crystals that form urate granules in epidermal cells (Bursell, 1967). The urate granules make the larval skin opaque white and protect the larval body from ultraviolet radiation from the sun (Hu et al., 2013). Deficiencies in uric acid accumulation or urate granule formation result in a translucent epidermis resembling oiled paper that characterizes the larval translucent or "oily" mutants (Tamura and Sakate, 1983). The oily mutants are visible and can be easily distinguished. They have been utilized to characterize some geographic strains in the history of sericulture and recently as very convenient phenotypic markers for genetic and molecular genetic studies (Abe et al., 2008; Fujii et al., 2010a; Ma et al., 2012, 2014).

Approximately 30 independent loci have been identified to be associated with the larval translucent mutations (http://www.shigen.nig.ac.jp/silkwormbase/). Translucency of the larval epidermis varies among mutants, indicating that mutations of different loci have different effects on uric acid accumulation. To date, 10 loci had been reported to be responsible for translucent mutations (Fujii et al., 2016); these were categorized into three types according to their functions

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Abbreviations: AA, amino acid; ABC, ATP-binding cassette; AP-3, Adaptor Protein-3; BC, backcross; BLOC, biosynthesis of lysosome-related organelles complex; EGFP, enhanced green fluorescent protein; HotSHOT, hot sodium hydroxide and Tris; KD, knockdown; LROs, lysosome-related organelles; MITE, miniature inverted-repeat transposable element; MoCo, molybdenum cofactor; ORF, open reading frame; *otm*, Tanaka's mottled translucent; PCR, polymerase chain reaction; qPCR, quantitative reverse transcript PCR; RACE, rapid amplification of cDNA ends; RNAi, RNA interference; *rp49*, *ribosomal protein 49 gene*; RPKM, Reads Per Kilobase of exon model per Million mapped reads; siRNA, short interfering RNA; RT-PCR, reverse transcription PCR; TIRs, terminal inverted repeats; TSD, target site duplication; XDH, xanthine dehydrogenase

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Toll ligand Spätzle3 controls melanization in the stripe pattern formation in caterpillars

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A stripe pattern is an aposematic or camouflage coloration often observed among various caterpillars. However, how this ecologically important pattern is formed is largely unknown. The silkworm dominant mutant *Zebra* (*Ze*) has a black stripe in the anterior margin of each dorsal segment. Here, fine linkage mapping of 3,135 larvae revealed a 63-kbp region responsible for the *Ze* locus, which contained three candidate genes, including the Toll ligand gene *spätzle3* (*spz-3*). Both electroporation-mediated ectopic expression and RNAi analyses showed that, among candidate genes, only processed *spz-3* induced melanin pigmentation and that *Toll-8* was the candidate receptor gene of *spz-3*. This Toll ligand/receptor set is also involved in melanization of other mutant *Striped* (p^S), which has broader stripes. Additional knockdown of 5 other *spz* family and 10 *Toll*-related genes caused no drastic change in the pigmentation of either mutant, suggesting that only *spz-3*/*Toll-8* is mainly involved in the melanization process rather than pattern formation. The downstream pigmentation gene *yellow* was specifically upregulated in the striped region of the *Ze* mutant, but *spz-3* showed no such region-specific expression. Toll signaling pathways are known to be involved in innate immunity, dorsoventral axis formation, and neurotrophic functions. This study provides direct evidence that a Toll signaling pathway is co-opted to control the melanization process and adaptive striped pattern formation in caterpillars.

Spätzle3 | Toll signaling pathway | melanization | striped pattern | *Bombyx mori*

Animals have various color patterns: spot and stripe patterns are frequently observed. These body patterns are often used for camouflage or aposematic coloration to avoid predators (1, 2). In aposematism, the contrast created by the combination of bright and dark colors, such as yellow/red and black (Fig. 1*A*), is important to facilitate detection by predators and hasten avoidance learning (3). The molecular mechanism underlying spot formation in adult and larval insects has become more apparent via many studies in *Drosophila* (4, 5), *Bombyx mori* (6, 7), and several butterflies (8–10). Although stripe pattern formation is well-studied in vertebrates, such as the zebra (11), rodents (12), and zebrafish (13), the molecular backgrounds of this pattern in insects are largely unknown. Because the stripe pattern is often observed in lepidopteran larva and its biological roles are more evident than in other animals (14, 15), it is intriguing to study the mechanism and evolutionary origin of caterpillar stripe formation.

The silkworm *B. mori* is a suitable model organism to study the genetic and molecular mechanisms of color pattern formation. There are extensive stocks of larval marking mutants, whole-genome information, and readily available functional analysis tools (16, 17). The silkworm mutant *Zebra* (*Ze*), which occurred spontaneously in ancient China, has a black stripe on one-fourth of the anterior part of the dorsal side of each larval segment (Fig. 1*B*, *Left*) and provides a good object to study to understand the mechanism of striped pattern formation. Previous genetic studies revealed that the *Ze* allele is dominant over the WT and mapped to 20.8 cM of linkage group 3 (18) (Fig. 1*C*).

In this study, a 63-kbp region responsible for *Ze*, including three predicted genes, was identified by linkage analysis. Additional observation of expression profiles and functional analysis of these three genes showed that the *Ze* allele is caused by a *spätzle* (*spz*) family gene *Bmspätzle3* (*Bmspz-3*). A cytokine-like ligand for the Toll receptor, Spz, is a key molecule in the activation of the Toll signaling pathway, which is involved in both dorsoventral axis formation during embryogenesis (19) and innate immunity (20). In addition, among *spz* paralogous genes in *Drosophila melanogaster*, some have been suggested to be involved in innate immunity and neurotrophic functions (20). However, to date, no evidence has been provided to show that this *spz* family gene regulates the melanin synthesis pathway directly. Therefore, the findings in this study shed light on not only the unique function of this *spz* family gene but also, the molecular mechanism of color pattern formation, which furthers our understanding of the whole melanin synthesis pathway involved in body marking and innate immunity.

Results

Fine Linkage Mapping of the *Ze* Locus in *B. mori*. Each larval segment of the *Ze* mutant strain f40 [*Ze*/*Ze*; *plain* (*p*)/*p*] has a black striped marking in the anterior margin on the dorsal side and a twin spot marking on the ventral side (Fig. 1*B*, *Left*), in contrast to the WT strain N4 ($+^{Ze}/+^{Ze}$; *p*/*p*) without markings (Fig. 1*B*, *Right*). Chemical analysis suggested that the pigments accumulated in the black stripes on the cuticle of the *Ze* mutant

Significance

A stripe pattern is widely observed among animals and often used for warning or camouflage in caterpillars. However, its genetic background is largely unknown. This study showed that the Toll ligand Spätzle3 (Spz-3) is responsible for the silkworm *Zebra* locus, which causes black stripes on the anterior margin of each segment. Exhaustive knockdown experiments of *spz*- and *Toll*-related genes clarified that *spz-3* and *Toll-8* are involved in the melanin pigmentation of *Zebra* and another mutant. The Spz-3/Toll-8 signaling pathway is suggested to induce *Zebra* stripe-specific expression of the pigmentation gene *yellow*. This study sheds light on not only the unique aspect of Spz/Toll functions but also, stripe pattern pigmentation in caterpillars through co-option of a Toll signaling pathway.

Author contributions: J.Y. and H.F. designed research; Y.K., S.Y., T.M., T.A., J.Y., and K.Y. performed research; Y.K., S.Y., Y.B., and H.F. analyzed data; and Y.K., S.Y., and H.F. wrote the paper.

The authors declare no conflict of interest.

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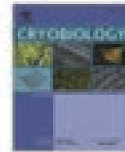

Long-term preservation of eri and ailanthus silkworms using frozen gonads

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A B S T R A C T

Cryopreservation of eri and ailanthus silkworms using frozen gonads was investigated. First, we evaluated the freeze tolerance of ovary and testis in the eri silkworm, which showed high tolerance. Mating between frozen ovary-transplanted females and frozen testis-transplanted males produced 163.0 eggs, yielding 105.7 larvae per moth. In a second experiment, we tested the use of the eri silkworm as a host insect for gonad transplantation from ailanthus silkworm donors. A high success ratio for laid and hatched eggs was demonstrated for ovary transplantation (97.8 and 51.3 eggs per moth, respectively). For testis transplantation, however, the average number of hatched larvae was low (12.0). Mating between host eri females and males in which both frozen ovary and testis of the ailanthus silkworm had been transplanted produced 6.4 fertilized eggs per host moth. Our success in using cross subspecies cryopreservation between these wild silkworms could lead to the alternative use of hosts between species in other insects.

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1. Introduction

Long-term and secure preservation are required for bio-resources. In mammals, such as cows, mice, and humans, cryopreservation methods have been developed and are used as routine tools [2,4]. On the other hand, the development of similar methods was rather late in insects. Recently, we succeeded in establishing a technique for using frozen ovaries in *Bombyx mori* [1]. However, this method has not yet been evaluated in any other insect nor has it been applied to testis. In the present study, we first investigated the possibility of applying our established technique to the eri silkworm. The eri silkworm (*Samia cynthia ricini*), which is primarily cultivated in India, Thailand, and South China, is one of the main domesticated silkworms used for producing fiber material. This insect is easy to handle because of its long-term selective breeding; thus, it is also used as an experimental animal. The eri silkworm is, however, multivoltine; therefore, continuous rearing and reproduction are needed for its preservation. For these reasons, we selected the eri silkworm for our first experiments. We also evaluated the suitability of frozen testes, in addition to ovaries, in this species.

Secondly, we investigated the possibility of using the eri silkworm as a host insect for transplantation of gonads from ailanthus silkworm (*S. cynthia pryeri*) donors. This species, which inhabits a broad territory from South to East Asia, is considered an ancestor of the eri silkworm. Further, taxonomically, they are considered a subspecies. Although the two insects have a very close taxonomic relationship, the ailanthus silkworm has not been domesticated and thus, is difficult to rear in captivity or under controlled conditions. Hence, it is problematic to apply a cryopreservation method to the ailanthus silkworm. If the eri silkworm could be used as a host for the ailanthus silkworm, cryopreservation of the latter might be possible.

2. Materials and methods

2.1. Insects

Eri and ailanthus silkworm larvae were obtained from Shinshu University. Eri silkworms were reared on Tree of Heaven (*Ailanthus*

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ORIGINAL PAPER

Characterization of Recombinant *Thermococcus kodakaraensis* (KOD) DNA Polymerases Produced Using Silkworm-Baculovirus Expression Vector System

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Abstract The KOD DNA polymerase from *Thermococcus kodakarensis* (Tkod-Pol) has been preferred for PCR due to its rapid elongation rate, extreme thermostability and outstanding fidelity. Here in this study, we utilized silkworm-baculovirus expression vector system (silkworm-BEVS) to express the recombinant Tkod-Pol (rKOD) with N-terminal (rKOD-N) or C-terminal (rKOD-C) tandem fusion tags. By using BEVS, we produced functional rKODs with satisfactory yields, about 1.1 mg/larva for rKOD-N and 0.25 mg/larva for rKOD-C, respectively. Interestingly, we found that rKOD-C shows higher thermostability at 95 °C than that of rKOD-N, while that rKOD-N is significantly unstable after exposing to long period of heat-shock. We also assessed the polymerase activity as well as the fidelity of purified rKODs under various conditions. Compared with commercially available rKOD, which is expressed in *E. coli* expression system, rKOD-C exhibited almost the same PCR performance as the commercial rKOD did, while rKOD-N did lower performance. Taken together, our results suggested that silkworm-BEVS can be used to express and purify efficient rKOD in a commercial way.

Keywords DNA polymerase · KOD · Silkworm · Baculovirus expression system

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Introduction

Thermostable DNA polymerases (DNAPs) have been widely used for in vitro DNA manipulations, such as polymerase chain reaction (PCR), DNA sequencing and genotyping. Generally, there are seven classified DNAP families (A, B, C, D, X, Y, and reverse transcriptase/RT) according to the sequence similarity of amino acids [1]. Family-B DNAPs were discovered in all archaeal lineages, and recently they have also been revealed as a co-operating member of integrase Cas1-dependent self-synthesizing mobile elements (termed casposons) in archaeal and bacterial [2, 3]. Among those Family-B DNAPs, several archaeal family-B DNAPs, such as *Pyrococcus furiosus* Pfu-Pol and *Thermococcus kodakarensis* Tkod-Pol, have been preferred to be used for PCR, especially for long-fragment amplifications because of their extreme thermostability and outstanding fidelity along with integrated

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Short Communication

OXFORD

Localization of an Aldo-Keto Reductase (AKR2E4) in the Silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

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Abstract

The aldo-keto reductase AKR2E4 reduces 3-dehydroecdysone to ecdysone in the silkworm *Bombyx mori* L. In this study, a quantitative polymerase chain reaction analysis revealed that the level of *AKR2E4* mRNA was higher in the testes than in other tissues, and a western immunoblot analysis revealed that the AKR2E4 content in the testes was stage-specific from the fifth larval instar to the pupal stage. Immunohistochemical analysis showed that the AKR2E4 protein was present in cyst cells associated with sperm cells and spermatocytes. These results indicate that AKR2E4 plays an important role in 3-dehydroecdysone conversion to ecdysone and spermatogenesis in silkworm testes.

Key words: Aldo-keto reductase, Lepidoptera, testes

In insects, ecdysteroids affect molting, metamorphosis, and reproduction (Gilbert et al. 2002). The ecdysteroid, ecdysone, was first isolated in 1954 (Karlson 1996) and shown to be produced through the reduction of 3-dehydroecdysone (3DE) by a 3DE 3β-reductase (Sakurai et al. 1989); the ecdysone is then hydroxylated at the 20 position to form the active steroid hormone, 20-hydroxyecdysone. Thus, close regulation of the 20-hydroxyecdysone titer through production and inactivation is needed to mediate precise molting in insects.

Previously, we identified a 3DE 3β-reductase in the silkworm *Bombyx mori* L. and determined its X-ray structure (Yamamoto and Wilson 2013). The 3DE 3β-reductase of *B. mori* belongs to the aldo-keto reductase (AKR) family 2, and the enzyme was named AKR2E4. This enzyme catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reduction of ketones and aldehydes, and reduces 3DE to ecdysone in the presence of NADPH. The structure of AKR2E4 in a complex with an $NADP^+$ co-substrate has been determined (Yamamoto and Wilson 2013). The amount of AKR2E4 in the silkworm hemolymph is likely to change in response to the hormonal activities of ecdysteroids (Yamamoto and Wilson 2013).

Ecdysteroids stimulate spermatogenesis in insect testes (Kambysellis and Williams 1972, Dumser and Davey 1975). *B. mori* testes contain ecdysteroids, the concentrations of which fluctuate during development (Fugo et al. 1995). Although the mechanisms involved in ecdysteroid regulation in *B. mori* ovaries have been established (Ohnishi 1990), those in the testes remain uncertain; we initiated the present study to investigate these mechanisms through an immunohistochemical analysis of AKR2E4 in the testes.

Materials and Methods

Insect Culture and Tissue Dissection

B. mori larvae (p50T strain) were reared at 25°C and fed on mulberry leaves. For a real-time polymerase chain reaction (PCR) analysis, Day 3 fifth-instar larvae were dissected on ice, and the fat body, midgut, silk gland, ovaries, and testes were stored at −80°C until use. For western blot analysis, Days 1, 3, 5, 7, 9, and 10, fifth-instar larvae were dissected on ice, and the testes were stored at −80°C until use.

Quantitative PCR Analysis

Total RNA was extracted from the dissected tissues using an RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNAs were prepared as described by Yamamoto and Wilson (2013). qPCR primer sets for *AKR2E4* and *B. mori* ribosomal protein 49 (*Bmrp49*) were designed. The primer sequences were as follows.

AKR2E4F (forward), 5′-CCGAAATCCACAAACAAGCA-3′, and AKR2E4R (reverse), 5′-TGGCCGCTACCTCTTCAAAC-3′; Bmrp49F (forward), 5′-GATGTGTTTTATATTC-3′, and Bmrp49R (reverse), 5′-GCATCATCAAGATTTCCAGCTC-3′. qPCR was performed on a Dice Real Time System TP-800 Thermal Cycler (Takara, Shiga, Japan) using SYBR *Premix Ex Taq* (Takara). PCR amplification was initiated with a 10-s denaturation step at 95°C and then 40 cycles of denaturation at 95°C for 5 s, annealing at 55°C for 20 s, and an extension at 72°C for 20 s. The samples were analyzed in triplicate, and *AKR2E4* levels were normalized against corresponding *Bmrp49* levels and expressed as the *AKR2E4*/*Bmrp49* ratio.

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WILEY INSECT BIOCHEMISTRY AND PHYSIOLOGY

ARTICLE

Enzymatic characterization of two epsilon-class glutathione *S*-transferases of *Spodoptera litura*

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Abstract

Two cDNAs encoding glutathione *S*-transferase (GST) of the tobacco cutworm, *Spodoptera litura*, were cloned by reverse transcriptase-polymerase chain reaction. The deduced amino acid sequences of the resulting clones revealed 32–51% identities to the epsilon-class GSTs from other organisms. The recombinant proteins were functionally overexpressed in *Escherichia coli* cells in soluble form and were purified to homogeneity. The enzymes were capable of catalyzing the bioconjugation of glutathione with 1-chloro-2,4-dinitrobenzene, 1,2-epoxy-3-(4-nitrophenoxy)-propane, and ethacrynic acid. A competition assay revealed that the GST activity was inhibited by insecticides, suggesting that it could be conducive to insecticide tolerance in the tobacco cutworm.

KEYWORDS
glutathione, glutathione transferase, Lepidoptera, Spodoptera litura

1 | INTRODUCTION

Glutathione conjugation is a major pathway for detoxification of xenobiotics as well as for homeostasis of endogenous compounds. Glutathione *S*-transferases (GSTs, EC 2.5.1.18) are widespread in both prokaryotic and eukaryotic cells, and catalyze the conjugation of reduced glutathione (GSH) with substrates (Armstrong, 1997; Listowsky, Abramovitz, Homma, & Niitsu, 1988). We previously identified several GSTs (delta, epsilon, omega, sigma, theta, zeta, and an unclassified isoform) in the silkworm, *Bombyx mori*, a lepidopteran model insect (Yamamoto et al., 2011; Yamamoto et al., 2009b; Yamamoto et al., 2005; Yamamoto, Aso, & Yamada, 2013a; Yamamoto, Fujii, Aso, Banno, & Koga, 2007; Yamamoto, Nagaoka, Banno, & Aso, 2009a; Yamamoto, Zhang, Banno, & Fujii, 2006). There are six classes of GST (delta, epsilon, omega, sigma, theta, and zeta) found in dipteran insects, such as *Anopheles gambiae* (Ranson & Hemingway, 2005) and *Drosophila melanogaster* (Sawicki, Singh, Mondal, Benes, & Zimniak, 2003; Tu & Akgul, 2005). In addition, we also characterized a sigma-class GST from the fall webworm, *Hyphantria cunea*, which is one of the most serious lepidopteran pests of broad-leaved trees (Yamamoto et al., 2007), and a delta-class GST from *Nilaparvata lugens*, which is a

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; CDS, complete coding sequence; GSH, glutathione; GST, glutathione transferase; ORF, open reading frame; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SlituGST, *Spodoptera litura* GST

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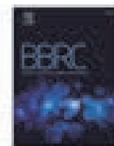

Molecular structure of a prostaglandin D synthase requiring glutathione from the brown planthopper, *Nilaparvata lugens*

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ABSTRACT

Prostaglandins are involved in many physiological processes, and prostaglandin synthases facilitate the detoxification of xenobiotics as well as endogenous compounds, such as through glutathione conjugation. Specifically, prostaglandin D synthase (PGDS) catalyzes the isomerization of PGH_2 to PGD_2. Here we report the identification and structural analysis of PGDS from the brown planthopper rice pest *Nilaparvata lugens* (nlPGDS), which belongs to the sigma-class glutathione transferases. The structure of nlPGDS in complex with glutathione was determined at a resolution of 2.0 Å by X-ray crystallography. Bound glutathione was localized to the glutathione-binding site (G-site). Enzyme activity measurements following site-directed mutagenesis of nlPGDS indicated that amino acid residues Tyr8, Leu14, Trp39, Lys43, Gln50, Val51, Gln63, and Ser64 in the G-site contribute to its catalytic activity. To our knowledge, this represents the first report of a PGDS in insects. Our findings provide insights into the mechanism of nlPGDS activity and potentially that of other insects and therefore may facilitate the development of more effective and safe insecticides.

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1. Introduction

Prostaglandins (PGs) are involved in a variety of physiological and pathological processes in mammals. PG isomers have also been found, such as PGH_2, PGD_2, and PGE_2 [1–3]. We previously reported the identification and structural characterization of *Bombyx mori* prostaglandin E synthase (bmPGES), which catalyzes the isomerization of PGH_2 to PGE_2 in silkworm. PGES enzymes in mammals are homologs of sigma-class glutathione transferases (GSTs) [4,5]. Glutathione (GSH) conjugation is essential for the detoxification of xenobiotics as well as endogenous compounds including unsaturated aldehydes and PGs [6,7].

To further characterize prostaglandin synthases in insects, we isolated mRNA encoding a prostaglandin D synthase of the brown planthopper *Nilaparvata lugens* (nlPGDS), which is a worldwide insect pest for rice. nlPGDS was found to belong to sigma-class GSTs. Examination of the structure-function relationships involved in nlPGDS catalytic action indicated that it participates in the conversion of PG H_2 into its D_2 form. Additionally, similarities were identified between the structures of bmPGES and nlPGDS [8]. The active sites in nlPGDS were then determined to better understand the structural basis for conversion of PG H_2 into D_2. The reported high resolution (2.0 Å) crystal structure of nlPGDS complexed with GSH and identification of the amino acid residues involved in catalytic function reported herein may provide insights into the mechanism of PGDS activity and might facilitate the development of more effective and safer insecticides.

2. Materials and methods

2.1. Insects and RNA extraction

The brown planthopper, *N. lugens* (strain: Izumo), was reared and maintained at the National Institute of Agrobiological Sciences (Tsukuba, Japan). Total RNA was isolated from the adults using the SV Total RNA Purification kit (Promega).

Abbreviations used: GSH, glutathione; GST, glutathione transferase; GSTS, Sigma class GST; PG, prostaglandin; PGDS, prostaglandin D synthase; PGES, prostaglandin E synthase; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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ARTICLES

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OPEN

Genomic adaptation to polyphagy and insecticides in a major East Asian noctuid pest

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The tobacco cutworm, *Spodoptera litura*, is among the most widespread and destructive agricultural pests, feeding on over 100 crops throughout tropical and subtropical Asia. By genome sequencing, physical mapping and transcriptome analysis, we found that the gene families encoding receptors for bitter or toxic substances and detoxification enzymes, such as cytochrome P450, carboxylesterase and glutathione-S-transferase, were massively expanded in this polyphagous species, enabling its extraordinary ability to detect and detoxify many plant secondary compounds. Larval exposure to insecticidal toxins induced expression of detoxification genes, and knockdown of representative genes using short interfering RNA (siRNA) reduced larval survival, consistent with their contribution to the insect's natural pesticide tolerance. A population genetics study indicated that this species expanded throughout southeast Asia by migrating along a South India–South China–Japan axis, adapting to wide-ranging ecological conditions with diverse host plants and insecticides, surviving and adapting with the aid of its expanded detoxification systems. The findings of this study will enable the development of new pest management strategies for the control of major agricultural pests such as *S. litura*.

The tobacco cutworm, *Spodoptera litura* (Lepidoptera, Noctuidae), is an important polyphagous pest; its larvae feed on over 100 crops[1]. This pest is widely distributed throughout tropical and subtropical areas of Asia including India, China and Japan. In India particularly, *S. litura* causes heavy yield loss varying between 10 and 30%[2]. High fecundity and a short life cycle under tropical conditions result in a high rate of population increase and subsequent population outbreaks. In addition, it has evolved high resistance to every class of pesticide used against it[2,3], including the biopesticide Bt[4]. Few complete genome sequences have been reported for noctuids, which include many serious agricultural pests. Asian researchers launched the *S. litura* genome project as an international collaboration in cooperation with the Fall armyworm International Public Consortium (FAW-IPC), for which a genome project is coordinately underway[5]. By comparative genomic studies with the monophagous species *Bombyx mori* and other *Spodoptera* species such as *S. frugiperda* (which has a different geographical distribution), *S. litura* genome information can provide new insights into mechanisms of evolution, host plant specialization and ecological adaptation, which can serve as a reference for noctuids and lead to selective targets for innovative pest control.

Results and discussion

Genome structure and linkage map of *S. litura*. We sequenced and assembled a genome for *S. litura* comprising 438.32 Mb, which contains 15,317 predicted protein-coding genes analysed by GLEAN[6] and 31.8% repetitive elements (Supplementary Tables 1–4). Among four representative lepidopteran species with complete genome sequences[7–11], *S. litura* harbours the smallest number of species-specific gene families (Supplementary Fig. 1a and Supplementary Table 9). A phylogenetic tree constructed by single-copy orthologous groups showed that *S. litura* separated from *B. mori* and *Danaus plexippus*

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植物遺伝子開発分野

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RESEARCH ARTICLE

Rapid semi-quantification of triacylglycerols, phosphatidylcholines, and free fatty acids in the rice bran of one grain

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Abstract

We developed a microplate assay method for determining the contents of triacylglycerols (TAGs), phosphatidylcholines (PCs), and free fatty acids (FFAs) in the rice bran of one grain using enzymatic reactions. In this method, enzymes from commercially available kits were used. Optimum reaction conditions were established. It was found that Nonidet P-40 was the optimal among the three surfactants used (Triton X–100, TWEEN 40, and Nonidet P-40) when lipid was dissolved in a reaction solution. Using this method, it was possible to quantify TAGs, PCs, and FFAs in concentration ranges of 7–150, 5–70, and 8–200 mg L^{-1}, respectively. Furthermore, when the TAG contents in the rice bran were measured, the values closely corresponded to those obtained by extracting from large amounts of rice bran. However, sufficient data on the PC and FFA contents in rice bran are not available for valid comparisons. Although this method can accurately quantify the TAG contents in the rice bran of one grain, the accuracy of the PC and FFA contents has not been verified. Hence, future study is necessary.

Keywords: rice bran, triacylglycerols, phosphatidylcholines, free fatty acids, surfactants

1. Introduction

In Asia, rice bran oil is extracted from rice bran, which is composed of the embryo and aleurone layer, using hexane and then used as cooking oil (Pal and Pratap 2017). However, when triacylglycerols (TAGs), the main component of rice bran oil, are excluded, the crude oil contains only phospholipids and free fatty acids (FFAs) that cannot be used as refined oil. FFAs are produced by lipase present in rice bran (Bhattacharyya and Bhattacharyya 1989). It is possible to produce rice oil more efficiently when rice bran is rich in TAG but poor in phospholipids and FFAs. For screening rice lines suitable for rice oil production, we attempted to rapidly determine the TAG; phosphatidylcholine (PC), which is a major phospholipid class; and FFA contents in the rice bran obtained from one grain using a microplate assay method.

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RESEARCH PAPER

Impairment of Lhca4, a subunit of LHCI, causes high accumulation of chlorophyll and the stay-green phenotype in rice

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Abstract

Chlorophyll is an essential molecule for acquiring light energy during photosynthesis. Mutations that result in chlorophyll retention during leaf senescence are called 'stay-green' mutants. One of the several types of stay-green mutants, Type E, accumulates high levels of chlorophyll in the pre-senescent leaves, resulting in delayed yellowing. We isolated *delayed yellowing1-1* (*dye1-1*), a rice mutant whose yellowing is delayed in the field. *dye1-1* accumulated more chlorophyll than the wild-type in the pre-senescent and senescent leaves, but did not retain leaf functionality in the 'senescent green leaves', suggesting that *dye1-1* is a Type E stay-green mutant. Positional cloning revealed that *DYE1* encodes Lhca4, a subunit of the light-harvesting complex I (LHCI). In *dye1-1*, amino acid substitution occurs at the location of a highly conserved amino acid residue involved in pigment binding; indeed, a severely impaired structure of the PSI-LHCI super-complex in *dye1-1* was observed in a blue native PAGE analysis. Nevertheless, the biomass and carbon assimilation rate of *dye1-1* were comparable to those in the wild-type. Interestingly, Lhcb1, a trimeric LHCII protein, was highly accumulated in *dye1-1*, in the chlorophyll–protein complexes. The high accumulation of LHCII in the LHCI mutant *dye1* suggests a novel functional interaction between LHCI and LHCII.

Keywords: Chlorophyll, Lhca4, light-harvesting complex, long-term acclimation, rice, state transition, stay-green.

Introduction

Chlorophyll synthesis and breakdown are strictly regulated in plants not only because it is an essential photosynthetic molecule, but also because in its free form it photo-oxidatively damages cells (Tanaka *et al.*, 2011). Mutants that retain the greenness of leaves under senescence-inducing conditions are called stay-green mutants, and they are classified into two

Multifunctional RNA Binding Protein OsTudor-SN in Storage Protein mRNA Transport and Localization[1[OPEN]]

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The multifunctional RNA-binding protein Tudor-SN plays multiple roles in transcriptional and posttranscriptional processes due to its modular domain structure, consisting of four tandem *Staphylococcus* nuclease (SN)-like domains (4SN), followed by a carboxyl-terminal Tudor domain, followed by a fifth partial SN sequence (Tsn). In plants, it confers stress tolerance, is a component of stress granules and P-bodies, and may participate in stabilizing and localizing RNAs to specific subdomains of the cortical-endoplasmic reticulum in developing rice (*Oryza sativa*) endosperm. Here, we show that, in addition to the intact rice OsTudor-SN protein, the 4SN and Tsn modules exist as independent polypeptides, which collectively may coassemble to form a complex population of homodimer and heteroduplex species. The 4SN and Tsn modules exhibit different roles in RNA binding and as a protein scaffold for stress-associated proteins and RNA-binding proteins. Despite their distinct individual properties, mutations in both the 4SN and Tsn modules mislocalize storage protein mRNAs to the cortical endoplasmic reticulum. These results indicate that the two modular peptide regions of OsTudor-SN confer different cellular properties but cooperate in mRNA localization, a process linking its multiple functions in the nucleus and cytoplasm.

mRNA localization is readily evident in structurally polarized somatic cells (Singer, 1993, 1996; St Johnston, 1995; Hesketh, 1996; Bassell and Singer, 1997) and during early embryo development (St Johnston, 1995; Bogucka-Glotzer and Ephrussi, 1996; Grünert and St Johnston, 1996; King, 1996; Lécuyer et al., 2007), where it is responsible for embryonic polarity and cell fate determination (St Johnston, 1995; Bogucka-Glotzer and Ephrussi, 1996; King, 1996). The localization of many mRNAs is dependent on the cytoskeleton (Wilhelm and Vale, 1993; St Johnston, 1995; Hesketh, 1996; López de Heredia and Jansen, 2004) and requires cis-determinant zip code sequences located predominantly at the 3′ untranslated region (UTR; Oleynikov and Singer, 1998; Hamilton and Davis, 2011) but also found less frequently in upstream coding sequences of the mRNA. RNA-binding proteins (RBPs) that recognize these 3′ UTR zip code signals and facilitate mRNA localization have been identified (St Johnston, 2005; Martin and Ephrussi, 2009; Macdonald, 2011; Fritzsche et al., 2013).

RNA localization is initiated in the nucleus, where cis-determinants or zip codes (Singer, 1993) are recognized by specific RBPs, mainly heterogenous nuclear ribonucleoprotein (RNP), to form an RNP complex (Mowry and Melton, 1992; Cohen, 2005; Schmid et al., 2006; Irion and St Johnston, 2007; Gerst, 2008; Fundakowski et al., 2012; Hermesh and Jansen, 2013). Upon export from the nucleus, the RNP complex is remodeled, where RBPs are removed and others added, enabling transport to selective intracellular sites where further remodeling enables anchoring to specific intracellular locations, activation for translation, storage, or processing (Aronov et al., 2007; Crofts et al., 2010; Doroshenk et al., 2010).

We have demonstrated previously that the rice (*Oryza sativa*) storage protein mRNAs are localized to subdomains of the cortical endoplasmic reticulum (ER) membrane complex by at least three distinct mRNA transport pathways in developing endosperm tissue (Li et al., 1993; Choi et al., 2000; Crofts et al., 2005; Washida et al., 2009a). In addition to a default pathway, two regulated pathways exemplified by the localization of rice storage protein mRNAs for prolamines and glutelins are evident. Prolamine mRNAs are targeted to the protein body (PB)-ER that delimits the prolamine intracisternal granules, whereas

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H.-L.C. designed and conducted the experiments; L.T. performed the BiFC study; S.H. examined the protein profile of OsTudor-SN via blue native gel electrophoresis; T.K. conducted the rice mutant population screens; T.W.O. supervised the research; H.-L.C. and T.W.O. wrote the article with critical inputs from the other coauthors.

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Inheritance Pattern of Earliness and Yield Related-Traits in Spring Barley (*Hordeum vulgare* L.)

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Abstract

To understand the genetic patterns of the physio-morphological traits for barley grain yield, six-generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) were used to determine the type of gene action in the four barley crosses. Grain yield showed a strong positive association (r = 0.83 and 1) with Grain Filling Rate in Giza121/RIL1 and Giza126/RIL2 crosses, respectively. The relationship between yield and earliness was not consistent with crosses and positive (r) values were quite low. It should be possible to select early-maturing and high-yielding segregates with high 100- kernel weight. The results indicated that the dominance effect [dd] was more important and greater than the additive effect [aa] and [ad] for most traits. Positive heterosis over the mid- and better- parent was quite similar for the most traits, except for heading and maturity dates, that showed negative heterotic effects. The inbreeding depression was high significant and positive for Grain Filling Rate, chlorophyll contents, Flag Leaf area and 100- kernel weight. On the other hand, it was a negatively significant for the earliness trait (HD, MD, and GFP). The lack of uniformity for estimates of inbreeding depression can be explained by environmental variation and to its influence on the type of gene action. Narrow-sense heritability ranged from 13.3% for Grain Filling Period in Giza12/RIL1 to 66.6% for heading dates in Giza121/RIL2 crosses. Genetic advance estimates were low due to lack of additive variance. The crosses Giza121/RIL1 and Giza126/RIL2 would be of interest in a breeding program, for improving characteristics of earliness, yield, and its components.

Keywords: *Hordeum vulgare* L., type of gene action, heterosis, heritability

1. Introduction

Barley (*Hordeum vulgare* L.) is a crop with great adaptive potential in many regions of the world. In areas which have only a brief rainy season, growers can obtain a harvest mainly because this crop has advantages in aspects such as salt tolerance, frost tolerance in the early period of development, drought tolerance, etc. Breeding for quantitative traits in early generations is impeded by several factors such as polygenic nature and low heritability of a trait (grain yield, the number of spikes per plant, etc.), linkage, non-additive gene effects and environmental effects (Harlan, 1976).

In order to overcome these difficulties, it is necessary to get as much information as possible about the genetic structure of breeding population undergoing selection (Sharma et al., 2003). This means identifying the gene effects that control the inheritance of a trait of interest and contributing to the exploitable genetic variance of the population.

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RESEARCH ARTICLE

Genotype by Environment Interactions (GEIs) for Barley Grain Yield Under Salt Stress Condition

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Abstract

Changes in the relative genetic performance of genotypes across environments are referred to as genotype × environment interactions (GEIs). GEIs can affect barley breeding improvement for salt tolerance because it often complicates the evaluation and selection of superior genotypes. The present study evaluated the GEIs over 60 barley genotypes for yield components and grain yield in six salinity environments in North Delta, Egypt. Data were analyzed using the additive main effects and multiplicative interaction (AMMI) and Tai's stability parameters. GEIs effects on yield explained 20.3, 20.1, 14.6, and 33.0% of the total variation besides, the first two principal components account for 67.3, 56.3, 64.3, and 83.7% of the explained variance in the four sets, respectively. Six genotypes namely G-4, G-7, G-20, G-34, G-36, and G-39 were found to be most stable and high yielding across environments (GY > 2.00 t ha^{-1}), and located close to zero projection onto the AEC ordinate. Tai's stability parameters demonstrated that these genotypes were more responsive to the environmental changes. The genotypes G-50 and G-53 showed perfect/static stability (α = -0.95, -0.91, respectively). In contrast, the genotype; G-36 had α = 0 and λ = 1.10, indicating parallel with the environmental effects followed by G-44. Overall, we found that GEIs for grain yield are highly significant in all sets, suggesting that responded differently across environments. This interaction may be a result of changes in genotypes' relative performance across environments, due to their differential responses to various abiotic factors.

Key words : *Hordeum vulgare*, G × E interaction, AMMI, Tai's stability parameters, salt stress

Introduction

Climate change affects agricultural productivity globally and may outcome in strong influence on agriculture, particularly on crop yield improvement. Crops are largely certain by climatic situation during growing season, thus even slight deviations from optimal conditions can seriously overhang yield (Kole et al. 2015; Lobell et al. 2011). Therefore, understanding the effects of environmental factors on crop production could reduce the possibilities of significant yield losses and improve the selection of elites cultivars for using in the target aspects and area (Smith and Tirpak 1989). Soil salinity is one of the most important abiotic stresses worldwide, affecting crops production and acts as a major obstacle to increasing barley production in growing areas, particularly in the Mediterranean region ((Elakhdar et al. 2016a; Rharrabti et al. 2013; Rodriguez et al. 2008; Sayar et al. 2010). Globally, it is estimated that 19.5% of irrigated land (about 230 million ha) and almost 2.1% of dry land agriculture (about 45 million ha) is affected by salinity (FAO 2015).

Today, cultivated barley has become the fourth most abundant cereal in term of area and harvested tonnages as well (FAO 2015). Barley is widely adapted to various

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Characterization of a *half-pipe-like leaf1* mutant that exhibits a curled leaf phenotype

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Leaf forms are diverse in angiosperms, and different types of cells are differentiated depending on the species. Rice leaves are composed of a leaf blade, a leaf sheath and the junction region between them. Cells with characteristic features, such as bulliform cells and sclerenchyma cells, are differentiated in the leaf blade, together with standard epidermal and mesophyll cells. To understand the genetic mechanism underlying leaf morphogenesis in rice, we focused on a mutant, *half-pipe-like leaf1* (*hal1*), whose leaves are adaxially curled. Histological observation revealed that the bulliform cells, which are responsible for leaf rolling under dry conditions, were small in size and abnormal in shape in a semidominant *hal1-d* mutant. Bulliform cell files were often ambiguous in semi-transparent *hal1-d* leaves cleared by the TOMEI method, suggesting that the bulliform cells were undeveloped. Therefore, a reduction in the growth of the bulliform cells seemed to be a major cause of leaf curling in the *hal1-d* mutant. The *hal1-d* mutation also affected the size of the leaf blade and the spikelet.

Key words: bulliform cell, curled leaf, leaf development, rice (*Oryza sativa*)

Plants successively generate leaves that perceive sunlight for photosynthesis. Leaf primordia initiate at the peripheral region of the meristem, where the leaf founder cells are recruited from undifferentiated cells supplied from the stem cells (Aichinger et al., 2012). In the leaf primordia, three developmental axes (apical-basal, adaxial-abaxial and centrolateral axis) are established and specific cell types then differentiate along these axes (Kuhlemeier and Timmermans, 2016). At the peripheral region of the meristem, *KNOX* genes, such as *SHOOT MERISTEMLESS* (*STM*) of *Arabidopsis thaliana* and *OSH1* of *Oryza sativa* (rice), are down-regulated to specify the fate of leaf founder cells (Long et al., 1996; Sentoku et al., 1999). The HD-ZIPIII and *ETTIN* genes and small RNAs targeting these genes are involved in the establishment of adaxial-abaxial cell fate (Chitwood et al., 2007; Husbands et al., 2009). These initial developmental processes and the genes that regulate them in leaf development are likely to be conserved in eudicots and monocots.

In contrast to this conservation, the morphology and size of the leaves are highly diverse in angiospems. Venation pattern also differs in eudicots and monocots. Therefore, developmental mechanisms after leaf primordium formation seem to be diversified in angiosperms. However, our understanding of gene function involved in the formation of diverse leaf forms or cell differentiation specific to each species is still poor.

In rice, like other grasses such as *Zea mays* (maize) and *Brachypodium distachyon*, the leaf is composed mainly of two parts, the leaf blade and leaf sheath. Between these two distinct structures, an auricle and a ligule are formed. Two types of vascular bundles, large and small, are formed in parallel in both the leaf blade and the leaf sheath. The two types are composed of partially different cell types. In the central region of the leaf blade, a strong structure, the midrib, is formed. The midrib develops from cells that proliferate in the central region of early leaf primordia (Yamaguchi et al., 2004; Ohmori et al., 2011). The *DROOPING LEAF* (*DL*) gene plays a central role in midrib formation by promoting cell proliferation (Yamaguchi et al., 2004; Ohmori et al., 2008). The midrib is indispensable for erection of the leaf blade, which is thin and very long in rice. In the *dl* mutant, the leaves fail to erect due to the lack of a midrib.

A number of genes are involved in the formation and elaboration of the leaf in rice. Failures in the establishment of the adaxial-abaxial axis generate abnormal leaves in mutants such as *shoot organization2* and *wavy leaf1* (Itoh et al., 2008; Abe et al., 2010). These abnormalities are often closely associated with partial defects

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Genetic characterization and fine mapping of *S25*, a hybrid male sterility gene, on rice chromosome 12

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Hybrid male sterility genes are important factors in creating postzygotic reproductive isolation barriers in plants. One such gene, *S25*, is known to cause severe transmission ratio distortion in inter-subspecific progeny of cultivated rice *Oryza sativa* ssp. *indica* and *japonica*. To further characterize the *S25* gene, we fine-mapped and genetically characterized the *S25* gene using near-isogenic lines with reciprocal genetic backgrounds. We mapped the *S25* locus within the 0.67–1.02 Mb region on rice chromosome 12. Further genetic analyses revealed that *S25* substantially reduced male fertility in the *japonica* background, but not in the *indica* background. In first-generation hybrid progeny, *S25* had a milder effect than it had in the *japonica* background. These results suggest that the expression of *S25* is epistatically regulated by at least one partially dominant gene present in the *indica* genome. This finding supports our previous studies showing that hybrid male sterility due to pollen killer genes results from epistatic interaction with other genes that are hidden in the genetic background.

Key words: epistasis, fine mapping, hybrid male sterility, *Oryza sativa*, reproductive isolation

INTRODUCTION

Hybridization between genetically divergent populations and species often produces hybrid offspring with reproductive abnormalities such as hybrid inviability or hybrid sterility. These phenomena play pivotal roles in speciation by acting as postzygotic reproductive isolation barriers. Two major genetic elements, duplicated genes and gamete killers (including egg killers and pollen killers), are known to be involved in hybrid sterility. Reciprocal loss of duplicated genes between two species (including gene transposition as a similar mechanism) has been reported to be the cause of hybrid sterility in *Drosophila* (Masly et al., 2006), *Arabidopsis* (Bikard et al., 2009) and rice (Mizuta et al., 2010; Yamagata et al., 2010). Duplication of genes essential for reproductive development allows subsequent loss of one copy without any reduction in fitness, and reciprocal gene loss in both species leads to hybrid sterility. This is a logical and simple mechanism that can lead to hybrid sterility. On the other hand, the genetic basis of gamete killers is not so simple. Gamete killers are selfish genetic elements that kill non-carrier gametes in heterozygous hybrid progeny. Initial examination of the genetics of gamete killers suggested that this phenomenon was caused by a simple allelic interaction at a single heterozygous locus; however, the molecular mechanism is far more complex than expected. Some examples include the *t*-complex in mice (Lyon, 2003), *Segregation distorter* (*Sd*) in *Drosophila* (Larracuente and Presgraves, 2012) and egg killer in rice (Yang et al., 2012). All three of these examples involve a complex interaction between multiple genes located within the same chromosomal region.

Since rice improvement is largely dependent on cross breeding, hybrid sterility is considered a critical issue for breeding programs using remote relatives. Seven species are known in the *Oryza sativa* complex (also known as AA genome species): two cultivated rice species (Asian rice *O. sativa* and African rice *O. glaberrima*) and five wild relatives (*O. rufipogon*, *O. barthii*, *O. glumaepatula*, *O. longistaminata* and *O. meridionalis*). *Oryza rufipogon*, a close wild relative of Asian cultivated rice *O. sativa*, is divided into two subgroups, an annual type (also referred to as a new species, *O. nivara*) and a perennial type (*O. rufipogon*) (Sharma and Shastry, 1965). All of the *Oryza sativa* complex species are diploid ($2n = 24$) and are distributed broadly in tropical areas including Asia, Africa

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最終頁に続く

(54)【発明の名称】植物の貯蔵タンパク質の集積に関与する遺伝子およびその利用

(57)【特許請求の範囲】

【請求項１】

下記の（a）、（b）、（c）、（d）、または（e）のポリヌクレオチドを機能可能に有し、貯蔵タンパク質を貯蔵型液胞に集積する植物において、該ポリヌクレオチドを機能不能とすることにより、貯蔵タンパク質の集積性が改変された植物を生産する方法：

（a）SEQ ID NO: 1、SEQ ID NO: 3、SEQ ID NO: 5、SEQ ID NO: 11またはSEQ ID NO: 12に記載の塩基配列からなるポリヌクレオチド；

（b）SEQ ID NO: 1、SEQ ID NO: 3、SEQ ID NO: 5、SEQ ID NO: 11またはSEQ ID NO: 12に記載の塩基配列と少なくとも90%の同一性を有し、かつ貯蔵タンパク質を貯蔵型液胞に集積する機能を有するタンパク質をコードするポリヌクレオチド；

（c）SEQ ID NO: 2またはSEQ ID NO: 6に記載のアミノ酸配列をコードするポリヌクレオチド；

（d）SEQ ID NO: 2またはSEQ ID NO: 6に記載のアミノ酸配列において１〜９個のアミノ酸が欠失、置換、付加および／または挿入したアミノ酸配列からなり、かつ貯蔵タンパク質を貯蔵型液胞に集積する機能を有するタンパク質をコードするポリヌクレオチド；

（e）SEQ ID NO: 2またはSEQ ID NO: 6に記載のアミノ酸配列と少なくとも97%の同一性を有するアミノ酸配列からなり、かつ貯蔵タンパク質を貯蔵型液胞に集積する機能を有するタンパク質をコードするポリヌクレオチド。

【請求項２】

請求項１に定義された（a）、（b）、（c）、（d）、または（e）のポリヌクレオチド

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最終頁に続く

(54)【発明の名称】イネ変異体、食品、及びイネ変異体の作出方法

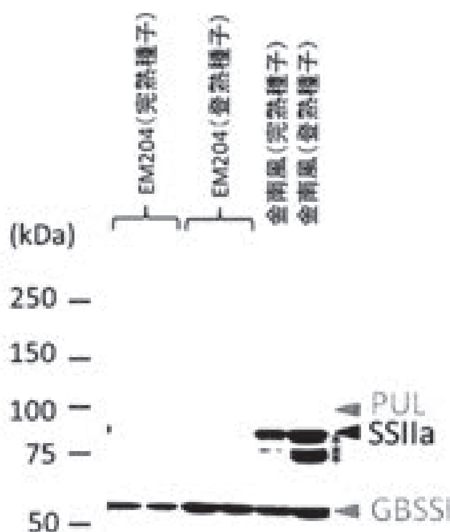
(57)【要約】

【課題】遺伝子組み換え手法を用いず、高水分吸収且つ低糊化温度の胚乳澱粉をもつ新規イネ変異体を提供する。

【解決手段】

イネスターチシンターゼＳＳＩＩａの遺伝子座が劣性ホモであり、非遺伝子組み換え体のイネ変異体を得る。当該イネ変異体は、野生型と比べて、種子重量が８割以上維持され、農業形質が維持されている。また、精米を、過剰に水分を与えて簡易炊飯した際、炊飯後の水分増加率が野生型の１．１倍以上である。また、この変異体は、ＳＳＩＩａタンパク質を欠損する。さらに、このイネ変異体のイネ種子は、イネでは類い希な高水分吸収かつ低糊化温度の胚乳澱粉を含んでおり、炊飯した際、良食味である。

【選択図】図１



III. センターの活動状況

1.　教育活動

【家蚕遺伝子開発分野】
大学院生物資源環境科学府（修士課程）

吉永　啓恵	カイコの生殖細胞を用いた凍結保存に関する研究

大学院生物資源環境科学府（博士課程）

Md. Rezwanul Haque	Study on serine synthesis pathway in silkworm
池永照美	テキスタイル素材としてのカイコ遺伝資源特性の評価に関する研究

【植物遺伝子開発分野】
大学院生物資源環境科学府（博士課程）

Ammar El-Akhdar	The recognition assay of termination codon by ESP1/eRF1
加藤 和直	登熟温度が異なる米の食味と胚乳澱粉の特性解析

大学院生物資源環境科学府（修士課程）

林　挺	Mutmap法による*glup7*遺伝子の連鎖地図構築
Saw Myat Nwe	The role of the Protein Disulfide Isomerase Like (PDIL) 2-3 in the accumulation of the storage proteins in rice endosperm
Rahma Siti Nur Azizah Fauziyati	Functional analysis of rice DUF1618 proteins
Nguyen Thi Phuong Tho	Cloning and characterization of rice *sugary2* gene

【微生物遺伝子開発分野】
農学部生物資源環境科学科（学士課程）

白澤　拓海	ガン細胞アポトーシス誘導におけるHolin種の影響
合田　明日香	*Lactobacillus otakiensis*によるD-分岐鎖アミノ酸生産機構の解明
廣瀬　万優	ϕOH2由来溶菌酵素Endolysinのドメイン機能解析

大学院生物資源環境科学府（修士課程）

千羽　啓太	各種ガン細胞における好熱性ファージϕOH2溶菌酵素Holinのアポトーシス誘導機序の解明
山迫　彩華	*Lactococcus lactis* subsp. *cremoris*に感染するファージQ1の特性およびゲノム解析

渡邉　修平	地熱環境より分離した*Thermus*属ファージの性状解析
Xayapatha Souliya	Identification of plant pathogenic microorganisms in Laos.

大学院生物資源環境科学府（博士課程）

Kim Mina	好熱性ファージ由来耐熱性溶菌酵素を用いたファージセラピー技術の開発
Nguyen Cong Thanh	Application of phage therapy to plant disease in Vietnam
土谷　直史	地熱発電所における好熱性微生物の網羅的解析

研究生

Sirinthorn Sunthornthummas	Characterization of *Lactobacillus paracasei* phage ϕT25 from fermented milk in Thailand

2. 科学研究費・共同、受託研究等

【家蚕遺伝子開発分野】

ナショナルバイオリソースプロジェクト中核的拠点整備プログラム
課題管理者　伴野　豊
「カイコバイオリソースの収集・高品質化と効率的保存・供給体制の整備」

挑戦的萌芽研究　研究代表　伴野 豊
「CAS 冷却を用いたカイコの永久保存システムの構築に関する基盤研究」

挑戦的研究　研究代表　山本 幸治
「Ｘ線結晶構造を用いた農薬開発」

基盤研究（B）研究代表　山本 幸治
「プロスタグランジンは新たな昆虫成長制御因子となるか？」

国際共同研究加速基金　研究代表　山本 幸治
「タンパク質構造情報を基礎とした昆虫成長阻害剤の創出」

【植物遺伝子開発分野】
ナショナルバイオリソース中核的拠点整備プログラム
機関代表　熊丸敏博 「イネ属の多様性を生かすリソース基盤の構築（多様な高品質イネ実験系統の整備）」

農林水産業・食品産業科学技術研究推進事業、発展融合ステージ
研究統括者　熊丸敏博 「米油原料用イネの作出と利用に関する研究・開発」

National Science Foundation, Plant Genome Research Project, Co-PI, Toshihiro Kumamaru, ”Deciphering the role of RNA binding proteins in RNA transport, localization and post-transcriptional processes in plants”

基盤研究(C)　研究分担　熊丸敏博
「水稲玄米の登熟過程における貯蔵タンパク質蓄積に関する遺伝的制御機構の解明」

JFC インターナショナル国際共同研究(米国)加州米品種改良プロジェクト研究分担　熊丸敏博

基盤研究(C)　研究代表　久保 貴彦
「花粉キラーを制御する分子ネットワークの解明」

【微生物遺伝子開発分野】

基盤研究(B), 研究分担　土居　克実
「ネパール野生キノコのライブラリーと健康機能を含むデータベースの構築」

基盤研究(B), 研究代表　土居　克実
「地熱環境における好熱性微生物によるシリカバイオミネラリゼーション形成の統合解析」

高橋産業経済研究財団助成, 研究代表　土居　克実
「地熱発電の高次利用に資するバイオ素材の開発」

NEDO地熱発電技術研究開発プログラム, 研究分担　土居　克実
「シード循環法によるシリカスケール防止技術の研究開発」

若手研究(B), 研究代表　藤野　泰寛
「バイオテクノロジーを利用した耐熱化シリカ重合・分解酵素の創製」

3. 講演会・セミナー・講習会

【家蚕遺伝子開発分野】

伴野 豊
九州大学　福岡県箱崎中学校職場体験学習協力　2017 年 8 月
九州大学　学振「ひらめきときめきサイエンス」協力　2017 年 7 月
科学未来館　JST 主催サイエンスアゴラ企画出展　2017 年 11 月

【植物遺伝子開発分野】

熊丸敏博

東北醸友会講演会　2017 年 11 月仙台

4. 海外渡航

【家蚕遺伝子開発分野】
山本 幸治
USA，California，カリフォルニア大学, 2018 年 1 月

【植物遺伝子開発分野】
無し

【微生物遺伝子開発分野】

5. 訪問研究員等
【家蚕遺伝子開発分野】
なし

【植物遺伝子開発分野】
Abdelghany Sobhy Abdelghany Shaban, 訪問研究員, Faculty of Science, Al-Azhar University, 2017 年 4 月～2018
年 1 月, エジプト

【微生物遺伝子開発分野】
Sirinthorn Sunthornthummas, 訪問研究員, 訪問研究員, Faculty of Science, Srinakharinwirot University, 2017.02 ~ 2017.09, タイ

IV. 遺伝子資源の保存、収集の状況

【家蚕遺伝子開発分野】

本センター保存の家蚕（カイコ）系統は、アカデミックリソースとしては、世界最大のコレクションであり、カイコ研究の拠り所として国の内外の研究者から利用されている。2002 年 7 月からスタートした文部科学省ナショナルバイオリソース（NBRP）のカイコの中核拠点として本分野は指定され、本センターの果たすべき役割は益々高まっている。保存系統はまずその主要目的形質によってアルファベットで分類し、それに 2 位数を附し系統番号としている（同一起源の分枝系は 3 位数）。分類記号の内容及びおよび、記号別保有数は以下の如くである。それらは、下記の系統約 500 系統がコアとなっている。コア系統の遺伝子情報の詳細はナショナルバイオリソースプロジェクトのホームページに掲載されている。
http://www.shigen.nig.ac.jp/silkwormbase/index.jsp

コア系統以外に TG（ゲノム改変カイコ）系統 138 系統、クワコへカイコを連続戻し交配して作成した染色体置換系統 53 系統、ケミカルミュータジェネシス(ENU)25 系統、他機関から寄託された系統等が維持されている。

p	（地域型品種）	２３	a	（胚子，幼虫期致死）	２０
b	（繭形・繭質）	１７	c	（繭色）	２５
d	（卵形・卵殻色）	３５	e	（卵色）	２８
f	（幼虫肢・斑紋）	３８	g	（幼虫斑紋）	１７
i	（幼虫眼紋・頭尾斑）	１３	k	（幼虫体色）	２４
l	（幼虫体色）	２８	m	（モザイク・畸形）	１７
n	（幼虫体形）	２８	o	（油蚕）	４０
r	（染色体異常・交叉率）	１６	t	（発育・眠性）	２５
u	（蛹・成虫）	２１	w	（連関分析用合成系）	２７
x	（分析未了の新突然変異）	１４			

（提供：系統の分譲件数）

本分野の過去５年間のカイコ系統の分譲件数を示す。分譲依頼者は、研究、教育関係が大半である。農学部、理学部系から依頼が多いが近年は薬学関係からの依頼も増えている。哺乳類等の代替の実験生物としての利用が創薬分野で広がっている。

		2013	2014	2015	2016	2017
生物体での分譲	国内	973 件	1295 件	609 件	680 件	763 件
	国外	178 件	75 件	128 件	115 件	163 件
DNA での分譲	国内	470 件	20 件	205 件	64 件	30 件

（カイコバイオリソースに関する専門知識・情報の提供）

リソース分譲の増加と共にカイコに関する生物学的知識、利活用に関する専門知識、技術相談、研修依頼、また来訪者への対応が増大している。その主な項目を下記に列挙する。括弧内は主な対象者。

・ カイコ突然変異体を中心とした形質特性、起源に関する情報提供（研究者、院生）
・ 研究に適した系統の選出依頼や、研究計画に対する助言依頼（研究者、院生）
・ カイコバイオリソースに関する遺伝を中心とした文献や知識の提供（研究者、院生）
・ カイコの系統維持に関する専門知識の提供（研究者、教育関係者）
・ カイコ系統の凍結保存に関する技術移転に関する相談（研究者）

・ 桑の分譲、栽培に関する専門知識の提供（研究者、一般）
・ カイコ全般に関する知識提供（教員、一般）
・ 養蚕に関する知識、技術の提供（農業関係者、一般）
・ カイコの教材としての活用方法に関する相談（教員、教育関係者）
・ 報道、出版機関からのカイコ、養蚕に関する問い合わせや専門用語の解説依頼や知識の提供、監修依頼（報道、出版関係者）
・ カイコを用いたイベント開催に関するアドバイスや講演依頼（教員、自治体関係者、一般）

（カイコ系統の保存事業）
文部科学省研究振興局ライフサイエンス課研究開発施設共用等促進費補助金（ナショナルバイオリソースプロジェクト）の支援を受け実施

①カイコバイオリソースの収集と高品質化
・ゲノム改変系統を１０系統収集した。
・ENU 誘発新規突然変異系統を５系統収集した。
・ユーザが扱いやすい大型系統を１系統収集した。
・１本の常染色体のみをｸﾜｺの染色体で置換することを最終目的としたｺﾝｿﾐｯｸ系統を 26 系統収集した。
・DNA リソースの収集は３０ 個体（カイコ３０ 個体）であった。
②カイコバイオリソースの保存
・700 系統のカイコ系統を飼育し、卵、幼虫、蛹時期に形質の評価を行い、平成 30 年度へと継代する保存業務を行った。
・凍結保存の実用化に取組み、新規に 50 系統の凍結保存を行った。累計の凍結保存数は 860 系統となった。
・凍結精子の受精能力を向上させる効果のある 3 倍体の精子を 50 ストロー分凍結した（人工授精 100 回分）
・新たに 1 種類の致死遺伝子について、効率的に系統維持を行うために分子マーカーを設計した。
・DNA リソース１６１０個体を保存した。
・カイコで実施している凍結卵巣による保存方法が野蚕リソースにも有効であるか、分担機関と協力して試験を行った。本年度はサクサンとエリサンについて凍結卵巣の移植実験を行った。移植個体が蛹で休眠したため、実験結果は平成３０年度に判明する。
・バックアップ体制の整備として以下のことを実施した。九州大学保存のコアリソース 495 系統については保管の委託契約先である長野県松本市の風穴で、信州大学の野蚕系統は九州大学でバックアップ保存する為に、リソースの移動、管理を行なった。
・分担機関東京大学で管理されてきたイチジクカサンの保存・提供業務を平成 31 年度から中核機関（九大）へ集約するため、イチジクカサンの食樹であるガジュマルの整備を行った。
③カイコバイオリソースの提供
・日常の飼育室管理、栄養状態の良い桑葉を確保するための桑園管理（福岡市 4 ヶ所、指宿市 1 ヶ所）を行った。
・提供に備えるため、第 1 期の飼育（5 月～6 月）において、浸酸種、冷浸種、人工越年種を、コア系統を中心に約 500 系統で採卵を行った。その卵を基本に、年間計５回の桑葉飼育を行い、幼虫、蛹、成虫を準備し、提供事業を行った。
・提供件数は予定数 1,220 件（個体 1120 件、DNA100 件）に対し、950 件（個体 920 件、DNA30 件）
④ﾌﾟﾛｼﾞｪｸﾄの総合的推進
④プロジェクトの総合的推進
・プロジェクト推進のため、基盤設備整備を行ない、分光光度計、電子天秤、乾燥機、遠心機、サーマルサイクラーを購入した。
・事業運営の円滑化のため、運営委員会（千代田区 2018 年３月７日）を開催した。

・広報誌としてニュースレター“おかいこさま”を3回（4月、８月、12月）発行し、研究コミュニティに配布した。第62回日本応用動物昆虫学会 (鹿児島市、3月27日)、日本蚕糸学会第88回大会（名古屋市、3月19〜20日）、CRYOPRESERVATION CONFERENCE 2017（つくば市、11月1～2日）においてポスター発表や口頭発表を行い、NBRPの事業紹介や活動成果の発表、凍結保存技術の広報を行った。JST主催のサイエンスアゴラ（東京都、11月24〜26日）、第41回日本分子生物学会年会 (神戸市、12月6日〜12月８日)において、ポスター発表や生体展示を行い、事業を広く紹介した。大学共同利用機関法人情報システム研究機構国立遺伝学研究所川本祥子准教授と連携して、データベース SilkwormBase の更新、データの拡充を行った。分子マーカーを利用してカイコの突然変異石亀蛹(*gap*)を効率的に維持する方法をJIBS誌(Journal of Insect Biotechnology and Sericology)で発表した。当該論文は平成29年度日本蚕糸学会進歩賞（奨励賞）を受賞した。

⑤桑園管理

カイコ飼育には餌となる桑の確保が必要で、本分野の業務は桑園管理から行われている。桑園管理は、施肥、除草、病害虫、剪定、収穫など幅広い分野に関する知識と経験が必要な業務であり、本分野の技術職員が主導して行うと共に農場職員の協力のもと行なわれている。大学移転がH30年秋に迫り、伊都での桑園作りが急がれている。しかし、桑の伸長が悪く、施設部、工事業者、コンサルタント業者、農学研究院長、同圃場計画専門委員会を始めとした関係各位と頻繁に対応を協議して改善をはかる状況が昨年度と同様続いている。

◎H29年度の桑園移転に伴う管理業務の主なものは以下の通りである。

4月

第4工区20、21、22圃場の桑園にH29年3月新植した桑苗の管理を開始する。伊都地区の桑植付け面積は3工区の0.9 ha、昨年度新植の第4工区17、18、19と合わせ約2.4 haとなる。残りは新築予定の建物前の0.2 haとなる。飼育は箱崎の建物で従来通り行っており、その飼育用桑は、箱崎地区桑園、留学生会館横の香椎浜桑園、原町農場の計3 haから収穫しているので、管理面積は5.4ha となっている。

5月

業務多忙で、福岡市西区シルバー人材センターに委託し、伊都地区の桑苗植え付け後の全桑園の株間の除草を依頼しての管理（週3日5時間×6名）。十分な管理にはほど遠く、技術職員が交代で伊都に入り、トラクタ耕運及び乗用草刈機で草刈りを行う。しかし、本年は雨が多く、高温で雑草の勢い続く。また、圃場の水はけが悪い部分が残る（暗渠工事で軽減はしたが）。朝倉地方では大水害。

7月

昨年までに植え付けていた桑園を含め、ほぼ全桑園の生育不良。大日本蚕糸会蚕業技術研究所市橋氏に伊都桑園の現地調査を依頼し来福頂く。土壌診断、作土層の土壌改良・肥沃度の維持増進が重要との助言を得る。

8月

日照良く、高温も続いたので、雑草の生育が頗る旺盛で株間の除草が間に合わない。教職員及び蚕学分野の学生アルバイト等の応援も得て、総出で株間の除草作業を行う。１月程の人海戦術で草の状態は落ち着く。

10月

微量要素欠乏により桑葉色が薄いため宗像緑地により桑株から40cm離し、施肥（尿素、バーク堆肥）を行いトラクタ撹拌。また、18,19圃場が排水不良のため砂利暗渠を18,19圃場中央に施工。

1 月
桑の生育増進を図るため全桑園春刈を行う（この作業は市橋氏から特に推奨された）。指宿試験地にて昨年度挿し木を行ったシマ桑（沖縄グワ）苗をハウス内に植付けを予定していたが育苗がうまく行えずシマ桑苗が確保できず植え付け一部中止。シマ桑苗の育苗を見直す必要があり。

2 月
来春、建物前見本桑園に納入予定の桑苗の穂木を群馬大竹桑園へ送付。
第 3 工区、第 4 工区土壌改良のため、牛糞堆肥を搬入撹拌。
冬季の蚕飼育の桑葉確保の必要がある為建物前圃場にパイプハウス設置。

3 月
群馬大竹桑園より桑苗納入。建物前見本桑園に植え付け行う。

次ページに伊都桑園様子の写真を示すが、草刈り直後は奇麗になるものの、草の勢いが強く、雑草との戦いであった。また、暗渠、弾丸暗渠の効果は薄く、明渠を自作して圃場整備をはかった。施設部はじめ、大学、農学部関係者から多大な援助を得て、管理が行なわれた。

春刈後の20桑園

雑草の生育旺盛

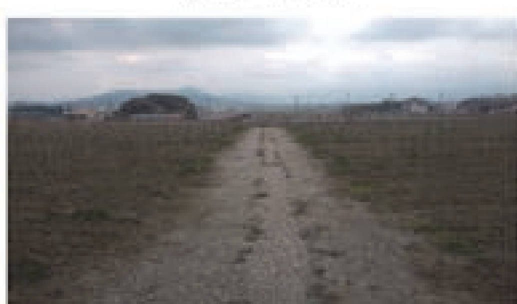
株間の除草

ハウス内シマ桑

除草作業前

除草作業後

排水不良桑園


19桑園の砂利暗渠

【植物遺伝子開発分野】

現在保存している品種系統の分類基準とその数を以下に示す。

HO系統　国内外の品種系統　1,398 系統

LO系統　1962–1965年収集したわが国在来品種　1,341 系統

IBP系統 FAO 国際共同研究供試品種　276 系統

UP系統　国内外の陸稲品種　342 系統

CM系統 化学変異源処理突然変異系統　5,715 系統

EM系統 胚乳形質に関する突然変異系統　1,764 系統

計 10,836 系統

これらの系統の一部をデータベースとして公開している。

http://w3.grt.kyushu-u.ac.jp/Rice_Kyushu/rice-kyushu/htdocs/main.html

http://www.shigen.nig.ac.jp/rice/oryzabase/

NBRPにおいて開設したTILLINGオープンラボに国内外の研究者を受け入れている。

http://www.shigen.nig.ac.jp/rice/oryzabaseV4/tilling/openLab

遺伝子資源系統の導入と分譲

年次	開発系統	導入(件数-系統数)		分譲(件数-系統数)	
		国内	国外	国内	国外
2016	569			6-70	1-5

【微生物遺伝子開発分野】

微生物遺伝子開発分野における菌株の収集と保存は、発酵学講座、微生物工学講座など応用微生物関連講座での有用微生物の探索とその研究過程で得られた分離株及び変異株の収集・保存に始まる。これら菌株の多くはアルコール、有機酸、アミノ酸、核酸、抗生物質、酵素等の発酵、食品、医薬、化学工業にまたがる広範囲の各種有用物質の生産に利用されている。また、産業廃棄物の処理と資源化、炭酸ガス処理を含む地球環境の改善に係わる環境科学の基礎的・応用的研究にも大きく貢献している。

現在、以下のような菌株を保存している。

I.　細菌

(A) 基準株　*Bacillus* 属, *Cellulomonas* 属, *Lactobacillus* 属, *Lactococcus* 属, *Pseudomonus* 属, *Thermus* 属および大腸菌
66 種 147 株

(B) 分離株　*Bacillus* 属, *Geobacillus* 属, *Ureibacillus* 属, *Lactobacillus* 属, *Lactococcus* 属, *Pediococcus* 属, *Pseudomonus* 属, *Enterococcus* 属及び *Thermus* 属
43 種 1104 株

(C) 変異株　*Bacillus* 属, *Geobacillus* 属, *Lactobacillus* 属および *Thermus* 属
23 種 185 株

II. 放線菌

(A) 基準株　*Micromonospora* 属, *Nocardia* 属, *Rodococcus* 属, *Streptomyces* 属および *Streptoverticillium* 属
155 種 171 株

(B) 分離株　*Streptomyces* 属
5 種 5 株

(C) 変異株　*Streptomyces* 属
10 種 311 株

III. プラスミド

(A) 導入プラスミドベクター　大腸菌、枯草菌（含む納豆菌）、乳酸菌、放線菌および酵母系統
165 種類

(B) 分離プラスミド　枯草菌（含む納豆菌）、乳酸菌および放線菌系統
127 種類

(C) 変異・構築プラスミド　4300 種類以上

IV. ファージ

(A) 導入ファージ・ファージベクター　大腸菌、乳酸菌、放線菌系統
35 種類

(B) 分離ファージ　乳酸菌および放線菌、アーキア系統
207 種類

(C) 変異・構築ファージ　大腸菌、乳酸菌および放線菌系統
85 種類

V. 糸状菌

(A) 基準株　*Aspergillus* 属, *Mucor* 属および *Penicillium* 属
3 種 25 株

VI. 酵母

(A) 基準株　*Saccharomyces* 属および *Candida* 属
3 種 3 株

VII. 昆虫培養細胞

Bombyx 属, *Spodoptera* 属及び *Trichoplolsia* 属
7 種 11 株

VIII. 昆虫ウイルス及び組換え体

(A) 昆虫ウイルス　5 種類
(B) 組換え体ウイルス　6 種類

上記以外の有用微生物資源については、現在、発酵学教室及び微生物工学教室においてそれぞれ保存・管理されている。

V. センター規程

九州大学農学研究院附属遺伝子資源開発研究センター規程

（趣旨）

第一条　この規程は、九州大学農学部附属遺伝子資源研究センター（以下「センター」という。）の組織及び運営に関し必要な事項を定める。

（センターの目的）

第二条　センターは、遺伝子の保存、開発及び利用に関する研究を行うことを目的とする。

（分野）

第三条　センターに、次の分野を置く。

一　家蚕遺伝子開発分野

二　植物遺伝子開発分野

三　微生物遺伝子開発分野

（センターの長）

第四条　センターに長を置き、農学部の責任及び兼任の教授のうちから教授会の議を経て選定する。

2 センターの長は、センターの管理及び運営を総括する。

3 センターの長の任期は、二年とする。

4 センター長は、再任されることができる。

（運営委員会）

第五条　センターの管理運営に関する重要な事項を審議するため、遺伝子資源開発センター運営委員会（以下「運営委員会」という。）を置く。

第六条　運営委員会は、委員長及び次の各号に掲げる委員をもって組織する。

一　センターの専任の教官のうちから選ばれた者三人

二　農学科、農芸化学科、林学科及び食糧化学工学科の専任の教授及び助教授のうちから選ばれた者各一人

三　前二号に掲げる者以外の農学科の専任の教授、助教授及び講師のうちから選ばれた者一人

四　農学部附属農場及び演習林の専任の教授及び助教授のうちから選ばれた者各一人

五　農学研究科遺伝子資源工学専攻の専任の教授及び助教授のうちから選ばれた者一人

2 委員の任期は、二年とする。ただし、委員に欠員が生じた場合の後任者の任期は、前任者の残任期間とする。

3 委員は、再任されることができる。

4 委員は、農学部長が委嘱する。

第七条　委員長は、センターの長をもって充てる。

2　委員長は、運営委員会を召集し、その議長となる。

3　委員長に事故等があるときは、あらかじめ委員長の指名する委員がその職務を代行する。

第八条　運営委員会は、委員の過半数の出席がなければ、議事を開き、議決をすることができない。

2　運営委員会の議事は、出席した委員の過半数をもって決し、可否同数のときは、議長の決するところによる。

（雑則）

第九条　この規程に定めるもののほか、センターの管理運営に関し必要な事項は、運営委員会の議を経て、センターの長が定める。

附則

1　この規程は、平成九年四月一日から施行し、平成九年四月十六日から適用する。

2　九州大学農学部附属遺伝子資源研究センター規程（昭和六十二年五月二十九日施行）は、廃止する。

VI. 英文摘要

INSTITUTE OF GENETIC RESOURCES

The institute of Genetic Resources had been established in May, 1987, and was then reorganized in April, 1997, within the Faculty of Agriculture, Kyushu University. The Institute is devoted to basic and applied studies on genetics with special interest in the stock maintenance of agriculturally important organisms. Silkworm, rice and fermentative microorganisms are chosen as the main materials from the viewpoint that their scientific researches have been carried out and developed chiefly in Japan. Emphasis has also been placed on studies at molecular level to contribute to the development of biotechnology and to establish gene libraries of these biological resources.

Silkworm Genetics Division

BANNO, Yutaka	Ph. D.	Professor
YAMAMOTO, Koji	Ph. D.	Assistant Professor

a) Linkage analysis of silkworm
b) Mutagenesis and teratogenesis in silkworm
c) Analysis of gene expression
d) Maintenance of the mutant stocks
e) Construction of a genetic linkage map of silkworm genome
f) Cytological studies of the deficient and translocated chromosomes

Plant Genetic Division

KUMAMARU, Toshihiro	Ph. D.	Professor
KUBO, Takahiko	Ph. D.	Associate Professor

a) Resolution of the mechanism controlling the transport and the accumulation of the seed storage proteins in rice.
b) Identification and functional analysis of genes involved in reproductive development and evolution of rice.
c) Construction of the rice mutation pool.
d) Conservation and evaluation of rice genetic resources.

Microbial Genetics Division

DOI, Katsumi	Ph. D.	Professor
FUJINO, Yasuhiro	Ph. D.	Assistant Professor

a) Survey, development and preservation of microbial genetic resources
b) Genetics and breeding of industrial bacteria: *Streptomyces*, *Lactobacillus*, *Bacillus*, *Thermus*, etc.
c) Functional analysis and application of novel and useful genes found in industrial bacteria
d) Isolation and characterization of bacterial and archaeal viruses
e) Investigation of biomineralization in geothermal environment

VII. センター研究棟配置図

家蚕遺伝子開発分野

(AG22棟：カイコバイオリソース研究施設)

Tel.& Fax. 092-802-4820, 4819, 4816, 4822

植物遺伝子開発分野

(アグリ・バイオ研究施設棟)

Tel. & Fax. 092-802-4842, 4844, 4843

微生物遺伝子開発分野

(アグリ・バイオ研究施設棟)

Tel. & Fax. 092-802-4845, 4846

編集後記

九州大学農学研究院遺伝子資源開発研究センター年報第21号を発刊致しました。平成29年度は、遺伝子資源開発研究センター長として引き続き熊丸敏博先生が就任され、センターの運営にご尽力いただいております。

平成29年4月、微生物遺伝子開発分野に藤野泰寛助教が赴任され、本センターの教員数は6名となりました。全員の力を合わせ、更なるセンターの発展に邁進する所存であります。今後とも、本センターの活動にご支援とご教示いただきますよう、お願いいたします。

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