目　　次

巻頭言

リソースなくしてリサーチなし

研究をおこなうためには研究材料が必須である。「リソースなくしてリサーチなし」と言われるゆえんである。ライフサイエンスは、生物系統や細胞、遺伝子、などの生物研究材料（バイオリソース）を研究者間で共有することにより大きく発展してきた。バイオリソースは一度絶えたら二度と復活はできないし、遺伝的な変化もするという特別の性質を持っている（ライフサイエンス委員会バイオリソース整備戦略作業部会より抜粋）。バイオリソースの維持には特別な配慮を必要とする。1921（大正 10）年の九州大学農学部開設当初から歴代の諸教授によって、農業ならびに学術研究上、重要なバイオリソースの収集が行われ、学問の発展に寄与してきた。中でも、カイコ・イネ・発酵微生物等に関しては系統数の増加・多様化が顕著で、保有系統の維持・管理業務のために、さらに高度な遺伝情報の解明と新規系統の開発が必要とされた。バイオリソースの維持・評価・開発・提供を専門として取り扱うために、遺伝子資源研究センターが 1987 年に設置された。設立当初は 10 年時限の施設であったが、10 年時限の到来を機会に遺伝子資源開発研究センターとして恒久的な施設に変身した。伊都キャンパスに移転とともに、カイコ及びイネは新設した各バイオリソース施設においてリソースの維持・評価などを行っている。さらに、イネとカイコについては 2002 年より国家プロジェクトとして発足したナショナルバイオリソースプロジェクト(NBRP)に当初より参画し、バイオリソースの開発、評価、維持、提供事業に益々拍車がかかることとなった。

本センターが有するバイオリソースは国内外の研究者等に提供されており、ライフサイエンスに関する数多くの優れた研究成果を生み出している。詳しくは遺伝子資源開発研究センターの HP を参照されたい。

「バイオリソースは一度失うと復元が難しい」。バイオリソース関係者の間では、2011 年の東日本大震災後にこの言葉が現実味を増して感じられるようになった。同震災でのバイオリソースの被害は比較的少なくて済んだといわれているが、東北大学では実験中のマウスリソース全てが犠牲になった。こうした現実を踏まえ、震災を含めた自然災害や火災などの事故に備えた取り組みが必要である。イネについては国立遺伝学研究所（遺伝研）とリソース種子を交換してそれぞれバックアップとして保管しており、遺伝研所有の種子稔実率が低い野生イネの一部を本学指宿試験地で栽培している。カイコについては卵を長野県にある天然の冷蔵庫である風穴(ふうけつ)にバックアップ保存している。風穴は昭和初期までは全国で蚕の保存に

用いられていた。前近代的と思われるかもしれないが停電の心配は無用で、この 10 年着実にバックアップ機能を果たしている。微生物は遺伝研の IBBP プロジェクトに参画し、微生物資源の消失リスクの軽減化を行なっている。また、九州が誇る地熱地域からの微生物分離や九州が地理的・環境的にも近い東南アジアの微生物資源の探索も行い、世界規模での微生物資源の維持・管理に務めている。こうした自然災害に加え、2020 年に発生し世界を震撼させているコロナ渦においてもリソースの維持は継続しなければならない。少人数で効率よく安全にリソースを次代へ継承する方策もまた我々の喫緊の宿題となっている。

こうした様々な難問が尽きないバイオリソース事業であるが、そのためには後進となる人材の育成が重要である。バイオリソース業務は直接の論文にほとんど結びつかない。当センター教員は、研究者として自身の研究を遂行することが重要であることはもちろんであるが、決して少ないといえないエフォートを使って論文にならないバイオリソース業務を遂行していかねばならない。このような人材は一朝一夕に育つものではないにもかかわらず、人材が育たなければバイオリソースの維持が困難となることは明らかである。

遺伝子資源開発研究センターでは「リソースなくしてリサーチなし、リサーチなくしてリソースなし」を肝に銘じながら、教職員一丸となって粛々と事業を進めており、今後も事業の発展を期しながら事業を進めていく。現在、全分野の教授は前身となる遺伝子資源研究センター設立以来 4 代目もしくは 3 代目である。今後、初代教授より受け継いできた、加えて九州大学農学部創立以来の歴代の諸教授より受け継いできたバイオリソースを継承していくことは当然として、新奇バイオリソース開発、バイオリソース維持、評価のための新奇技術開発等に真摯に取り組み、社会的責務を果たしていく決意を九州大学農学部創立 100 年という歴史の節目に立ち、新たにしている。

（九州大学農学部創立百周年記念誌より）

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　伴野　豊

家蚕遺伝子開発分野

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	福森　寿善	技術職員	山本　和典

植物遺伝子開発分野

教授	熊丸　敏博	テクニカルスタッフ	原田　良子
准教授	久保　貴彦	技術補佐員	池田　恵利佳
学振特別研究員	福田　真子	技術補佐員	松尾　由起恵
海外学振特別研究員	Elakhdar Ammar	技術補佐員	中條　裕子

微生物遺伝子開発分野

教授	土居　克実	研究支援推進員	山口　幸子
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
・イネの生殖発生と進化に関わる機構の解明
・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

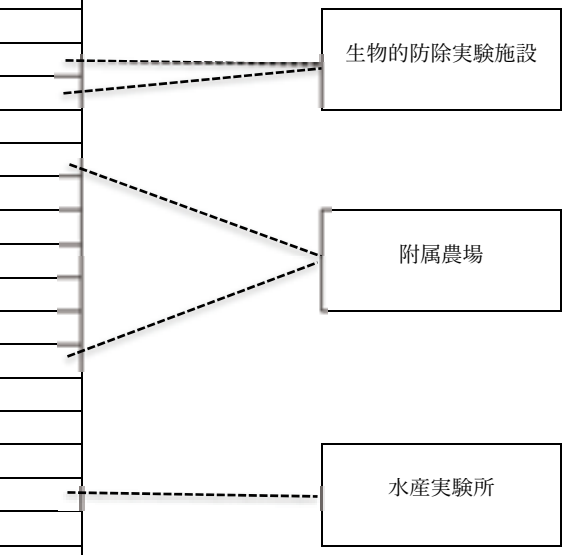
各分野とも、所定の許可を得た本学部内外の学生や研究者等に対し、研究の場や遺伝子資源材料を提供し、さらに研究指導と教育を行っている。

5. 遺伝子資源開発研究センター運営委員会委員　（令和3年3月31日現在）

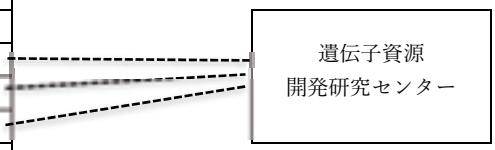
委員長	伴野　豊	（遺伝子資源開発研究センター）
教授	熊丸　敏博	（遺伝子資源開発研究センター）
教授	土居　克実	（遺伝子資源開発研究センター）
准教授	久保　貴彦	（遺伝子資源開発研究センター）
教授	片倉　喜範	（生命機能科学部門）
教授	日下部　宜宏	（資源生物科学部門）
准教授	小名　俊博	（環境農学部門）
教授	磯田　　宏	（農業資源経済学部門）
教授	青木　智佐	（生物的防除研究施設）
教授	穴井　豊昭	（附属農場）
教授	古賀　信也	（附属演習林）

6. 組織図

部門	講座	研究分野
資源生物科学	農業生物科学	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
		昆虫ゲノム科学
		昆虫産業創生学
		衛生昆虫学
		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	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



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) 山本 和典・藤井 告・西川 和弘・田村 圭・木村 友祐・福森 寿善・伴野 豊,
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Journal of Insect Biotechnology and Sericology 2021 Jul 9; **90**:33-40.

3) Fujii T, Kakino K, Fukumori H, Hino M, Lee JM, Kusakabe T, Banno Y.
Non-molting dwarf (nm-d) as a mutant of *Bombyx mori* with a defect in purine synthesis.
Insect Biochem Mol Biol. 2021 Nov;**138**:103636.
doi: 10.1016/j.ibmb.2021.103636.

4) Tanaka M, Fujii T, Mon H, Lee JM, Kakino K, Fukumori H, Ebihara T, Nagasato T, Hino M, Tonooka Y, Moriyama T, Fujita R, Banno Y, Kusakabe T.
Silkworm FoxL21 plays important roles as a regulator of ovarian development in both oogenesis and ovariole development.
Insect Biochem Mol Biol. 2022 Apr;**143**:103737.
doi: 10.1016/j.ibmb.2022.103737.

5) Yamamoto K, Endo S,
Bombyx mori-derived aldo-keto reductase AKR2E8 detoxifies aldehydes present in mulberry leaves,
Chemico-Biological Interactions, **351**, 109717 (2021)

6) Yamamoto K, Yamaguchi M,
Characterization of a novel superoxide dismutase in *Nilaparvata lugens*,
Archives of Insect Biochemistry & Physiology, **e21862**, (2020)

7) Hirowatari A, Yamamoto K,
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The Protein Journal, **40 (1)**, 63-67 (2021)

B. 著書・総説

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昆虫と自然 2021, Jun.30 ;**56**（7）: 2－4.

2) 福森寿善,
カイコの凍結保存技術の開発と有効性－昆虫種の新たな保全対策技術としての期待－,
昆虫と自然 2021, Jun.30 ;**56**（7）, 16－20.

C. 学会発表

1) 福森寿善・田中大介・伴野豊,
カイコ精巣のガラス化法を用いた長期保存の検討,
CRYOPRESERVATION CONFERENCE 2021, 2021 年 11 月 11－12 日(オンライン開催).

2) 松尾育代・藤井告・伴野豊・小林淳,
クワコとカイコの雑種系統における蛹期間調節遺伝子の探索,
日本蚕糸学会第 92 回大会, 2022 年 3 月 14 日, 山口大学 (オンライン開催).

3) 福森寿善・田中大介・伴野豊,
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NBRP におけるカイコ・野蚕リソース提供事業の現状と展望,
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第 66 回日本応用動物昆虫学会大会, 2022 年 3 月 21 日, 明治大学 (オンライン開催).

6) 山本幸治, 山田直隆, 黒木勝久, 榊原陽一, 水光正仁
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7) 山本幸治, 遠藤智史,
カイコ AKR を介した桑香気成分（揮発性アルデヒド）の代謝,
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第 48 回日本毒性学会学術年会, 2021 年 07 月 09 日, on-line.

D. 特許出願

なし

【植物遺伝子開発分野】

A. 原著論文

1) Kitajima R, O Matsuda, T Kumamaru, A Kume.
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Doi: org/10.3389/fpls.2022.866404

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Genetic background negates improvements in rice flour characteristics and food processing properties caused by a mutant allele of the PDIL1-1 seed storage protein gene.
Rice **15**, 13, March 2022. Doi: 10.1186/s12284-022-00560-w

4) Nagamatsu S, T Wada, R Matsushima, N Fujita, S Miura, N Crofts, Y Hosaka, O Yamaguchi, T Kumamaru.
Mutation in BEIIb mitigates the negative effect of the mutation in ISA1 on grain filling and amyloplast formation in rice.
Plant Molecular Biology **108**, 497-512, March 2022.
Doi: 10.1007/s11103-022-01242-3

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D. 特許出願

なし

E. データベース等

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【微生物遺伝子開発分野】

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第 58 回化学関連支部合同九州大会, 2021.07

D. 特許出願

なし

2. 原著論文要旨

家蚕遺伝子開発分野

蚕糸・昆虫バイオテック 90（1），41～44（2021）
SANSHI-KONCHU BIOTEC

イチジクカサン *Trilocha varians* の繭色の性差と温度が繭色と成虫体色に及ぼす影響

山本　和典・藤井　　告・西川　和弘・田村　　圭・
木村　友祐・福森　寿善・伴野　　豊*
国立大学法人　九州大学大学院　農学研究院
（2021年1月14日受付；2021年3月9日受理）

Kazunori YAMAMOTO, Tsuguru FUJII, Kazuhiro NISHIKAWA, Kei TAMURA,
Yusuke KIMURA, Hisayoshi FUKUSHORI and Yutaka BANNO*:
Sexual difference of cocoon color and influence of temperature to the cocoon color and the adult body color in *Trilocha varians*

Trilocha varians, a bombycid moth, inhabits mainly in South and Southeast Asia and was first identified in Okinawa, Japan, in 2001. We reared *T. varians* in the laboratory at 25°C and 20°C and evaluated some phenotypes. Most traits were the same as the published report, but a few new characteristics were observed. The female yellow cocoon color was darker than that of the males at both temperatures. The larvae reared at 20°C produced cocoons lighter in color than those reared at 25°C. We also observed that the adult body color kept at 20°C was darker than those maintained at 25°C during the pupal stage. *Graduate School of Bio Resources and Bioenvironmental Science, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 319-0395, Japan*
Key words: phenotypes, *Trilocha varians*, cocoon color, adult body color, characteristics

緒　言

イチジクカサン *Trilocha varians* は，鱗翅目 Lepidoptera カイコガ科 Bombycidae *Trilocha* 属に分類され，主にインド，ネパール，ベトナム，タイ，ミャンマー，中国南部，台湾，スマトラ，ジャワなどの南アジア，東南アジアに生息している（Zolotuhin and Witt, 2009）。日本では，2001年に本種の成虫が沖縄で初めて記録された（Kishida, 2002）。幼虫はガジュマル *Ficus microcarpa*，アコウ *F. superba*，イヌビワ *F. erecta* などのイチジク属の植物を食べ，生息地であるマレーシアやフィリピンでは街路樹であるベンジャミン *F. benjamina* の害虫として知られている（Basari *et al.*, 2019; Navasero and Navasero, 2014）。

イチジクカサンは，カイコガ科に属する。本科には日本では本種の他に5属6種（カイコ *Bombyx mori*，クワコ *B. mandarina*，オオクワコモドキ *Oberthueria falcigera*，カギバモドキ *Pseudandraca gracilis*，スカシサン *Prismosticta hyalinata*，テンオビシロカサン *Emolatia moorei* が確認されているので本種を加えると6属7種となる（岸田，2011）。新規に日本で確認されたイチジクカサンは家畜化されたカイコとの比較生物学研究に有効で，文部科学省が進めるNBRP（National BioResource Project）のカイコリソースにも加えられている（NBRP参照 https://nbrp.jp/）。

著者らの所属する九州大学はNBRP事業として学習院大学嶋田研究室から2019年にイチジクカサンの寄託を受け継代・分譲することになった。寄託直後は本種を25℃の温度条件で飼育を行った。しかし，本種は非休眠性で年間通しての飼育が必要であり継代に多大な労力が必要であった。そこで，継代の労力の軽減を図る目的で，1世代を長くするため20℃での飼育を行った。25℃と20℃で飼育を行い，その間の形質評価を行ったところ，これまでに知られていなかったイチジクカサンの形質特性を認めたので報告する。

材料と方法

1）イチジクカサン（*T. varians*）

本実験は，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で行った。使用したイチジクカサンは2009年当時東京大学大学院農学生命科学研究科昆虫遺伝研究室大門高明博士（現在京都大学大学院農学研究科教授）によって沖縄本島で採集され，東京大学で維持，2018年からは学習院大学にて維持され，2019年に九州

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家蚕遺伝子開発分野

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Development of interspecific semiconsomic strains between the domesticated silkworm, *Bombyx mori* and the wild silkworm, *B. mandarina*

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We constructed interspecific chromosome substituted strains using the wild silkworm, *Bombyx mandarina* and the domesticated silkworm, *B. mori*. In each developed strain, the entire diploid genome, except one targeted chromosome of *B. mandarina*, was substituted with *B. mori* chromosomes. We named these semiconsomic strains as T02 to T28, in which each strain number corresponds to the targeted chromosome number. In each of these strains, the target chromosome is heterozygous to that of *B. mori*. There are many phenotypic differences between these two species. Comparing the phenotypes of each strain enabled us to identify those chromosomes of *B. mandarina* that influenced some of its traits. We found five *B. mandarina* chromosomes that were correlated with the dominant phenotypes. The sibcrossing of the T02-T28 strains resulted in the identification of four *B. mandarina* chromosomes corresponding to the recessive phenotypes.

Key words: *Bombyx mori*, *Bombyx mandarina*, chromosome, consomic strain, semiconsomic strain, phenome

INTRODUCTION

The silkworm, *B. mori* is considered to be domesticated from the wild silkworm *B. mandarina* by human selection. Although the two species are closely related, they have many phenotypic differences, including egg color, larval body color, and flying ability (Ohmura, 1950; Kawarabata, 1998; Banno, 2010). Using the available genome data of the two species (The International Silkworm Genome Consortium, 2008; Xia *et al.*, 2009; Xiang *et al.*, 2018; Zhu *et al.*, 2019), comparative genomic studies of the two species have led to the identification of genes that may be involved in the domestication process (Xiang *et al.*, 2018). Recently, Wang *et al.* (2020) successfully identified candidate genes for two behavioral traits, namely, climbing and mimicry. However, the genetic basis of the phenotypic differences between the two species remains to be elucidated.

To accelerate the genetic study of the different phenotypes, we developed one of consomic strain, which we named semiconsomic strains, between *B. mori* and *B. mandarina*. The consomic and related strains, also known as chromosome substituted strains, are powerful tools for identifying chromosomes harboring genes that influence specific phenotypes (Lagrange and Fournie, 2009). Consomic strains have been primarily constructed in plants; this approach has also been applied to animals, such as mice (Takada and Shiroishi, 2012) and the teleost medaka, *Oryzas latips* (Kirchmaier *et al.*, 2015). In the semiconsomic strains developed in this study, the entire diploid genome, except for one targeted chromosome of *B. mandarina* in half of the individuals of each strain, was substituted with *B. mori* chromosomes so that only one autosome of *B. mandarina* was introgressed into the *B. mori* genetic background, although the females possessed the W chromosome of *B. mandarina*. Therefore, if a novel phenotype were segregated in a strain, we could identify the *B. mandarina* chromosome responsible for the novel phenotypes. By observing the phenotypes of the novel strains, we identified five dominant phenotypes and their causative *B. mandarin* chromosomes. We also identified four recessive phenotypes and their causative *B. mandarina* chromosomes by evaluating the phenotypes of the progenies from the sibcrossing of the semiconsomic strains. In addition, we described the breeding process and observed phenotypes.

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MATERIALS AND METHODS

Animals

B. mandarina, used in this study were originally collected in Sakado-city, Saitama Prefecture, Japan, in 1982.

家蚕遺伝子開発分野

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Non-molting dwarf (*nm-d*) as a mutant of *Bombyx mori* with a defect in purine synthesis

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Silkworm
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BmATIC
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ABSTRACT

There are several known non-molting mutations of the silkworm, *Bombyx mori*, including non-molting dwarf (*nm-d*). Larvae with this mutation hatch normally and start eating leaves, but die before the completion of the first ecdysis. Genetic analysis of the *nm-d* mutation would contribute to the isolation of essential genes for the larval development of lepidopteran insects. To identify the causative gene of the *nm-d* locus, we conducted RNA-seq based rough mapping. Using two sets of RNA-seq data, one from a pooled sample of normal larvae, and one from a pooled sample of *nm-d* larvae, the *nm-d* locus was narrowed to a 500 kb region. Among the genes located in this region, a *nm-d*-specific exon loss was identified in the *Bombyx* homolog of the ATIC (5-aminoimidazole-4-carboxamide ribonucleotide transformylase/inosine 5′-monophosphate cyclohydrolase) (*Bm*ATIC) gene, which catalyzes the final two steps of the *de novo* purine biosynthetic pathway in mammals. PCR and subsequent sequencing analysis revealed that a region containing exon 9 of the *BmATIC* gene is deleted in the *nm-d* larvae. A knockout allele of the *BmATIC* gene (*BmATIC*^{4Cr}), that was generated using the CRISPR/Cas9 system, revealed that first instar knockout larvae died while exhibiting the dark brown larval body that is a typical feature of mutants that lack uric acid in the integument. Lethal larvae resulted from crosses between +/*BmATIC*^{4Cr} moths. The uric acid content in the whole-body of the first instar was drastically reduced in the *nm-d* larvae compared to normal larvae. These results indicated that the *BmATIC* gene is responsible for the *nm-d* phenotype, and that *nm-d* larvae have a defect in purine biosynthesis, including uric acid. We also discuss the possibility that the *BmATIC* mRNA is maternally transmitted to eggs. Our results indicated that RNA-seq based mapping using pooled samples is a practical method for the identification of the causative genes of lethal mutations.

1. Introduction

Bombyx mori is a model lepidopteran insect, widely used in genetic and physiological research. About 260 loci of phenotypic mutations have been reported in *B. mori* (Banno et al., 2005). In Japan, resources for mutants of *B. mori* are centralized at Kyushu university, with the aid of the National Bioresource Project, which was started in 2002. Researchers can access data about 560 silkworm strains, covering most of the known morphological mutations (SilkwormBase, https://shigen.nig.ac.jp/silkwormbase/top.jsp). Since 2008, when updated genome data was released, positional cloning of Mendelian mutants was undertaken in many laboratories, mainly in Japan and China (The International Silkworm Genome Consortium, 2008). Currently, about 60 loci of phenotypic mutations have been cloned. However, most of the cloned mutants are viable and fertile. Infertile or lethal mutations tend to be avoided by researchers undertaking positional cloning, because experiments involving the crossing of these mutants are time- and labor-consuming, compared to those involving viable and fertile mutants.

Molting in insects involves the replacement of an old larval cuticle

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Silkworm FoxL21 plays important roles as a regulator of ovarian development in both oogenesis and ovariole development

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Keywords:
Forkhead box transcription factor L2
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Genome editing
Bombyx mori

ABSTRACT

The ovary is an important organ in reproduction. In insects, especially lepidopteran insects, the oocytes and reproductive organs develop rapidly during the pupal stage. Despite their drastic morphological changes, the molecular mechanisms of ovary development are not fully understood. In this study, it is found that forkhead box transcription factor L2, member 1 (*FoxL21*), which is known to be involved in ovarian differentiation and maintenance in vertebrates, is required for the development of the ovary in the silkworm, *Bombyx mori*. *FoxL21* was expressed in the ovary and ovariole during the larval and pupal stage, respectively. In silkworms in which *FoxL21* was knocked out by genome editing, multiple ovarian dysfunctions, such as, abnormal egg formation, thinning of the ovariole sheaths, and defective connection of the oviductus germinus with the ovariole were observed. Finally, ovarian transplantation experiments using the knockout silkworms revealed that FoxL21 functions in the ovariole, but not in the oviductus germinus.

1. Introduction

Fox family proteins are transcription factors characterized by the presence of a forkhead box DNA binding domain (FKH domain) and are divided into 19 subfamilies. Fox transcription factors have been identified in many organisms, from humans to yeast, and it have been reported to have 50, 44, and 17 *FOX* genes in humans, mice, and fruit fly, respectively (Jackson et al., 2010; Kaestner et al., 2000; Lee and French, 2004). Many of the Fox family proteins are involved in the regulation of development, differentiation, and metabolism (Carlsson and Mahlapuu, 2002). Eighteen genes, including two *FoxL2* genes, have been reported in silkworms, *Bombyx mori*, while only one *FoxL2* gene has been identified in humans and mice. Two members of the *FoxL2* subfamily is also highly conserved in insects and have been found in *Bombyx mori*, *Danaus plexippus*, *Heliconius melpomene* and in seven bee species (Nie et al., 2018; Song et al., 2015). The two *FoxL2*s of *Bombyx mori*, *FoxL21* and *FoxL22*, belong to evolutionarily distinct clusters and are located on different chromosomes (Song et al., 2015). These differences between FoxL21 and FoxL22 suggest that they may have different roles in biological processes. However, since the functions of these two *FoxL2* genes have not been investigated in the silkworm, we first focused on *FoxL21*, a homolog of human *FoxL2*.

FoxL2, in particular, has been reported to play an important role in the differentiation and maintenance of the mammalian ovary (Georges et al., 2013). Loss of FoxL2 function results in poor follicle formation in mice (Schmidt et al., 2004; Uda et al., 2004) and sex reversal in goats (Boulanger et al., 2014). Knockdown of *FoxL2* causes abnormal chorion formation in *Nilaparvata lugens* and suppresses vitellogenin expression in *Aedes aegypti*, resulting in reduced egg implantation (Hansen et al., 2007; Ye et al., 2017) indicating that even in insects, FoxL2 play important roles in egg formation. Thus, although the role of FoxL2 in ovarian development has been reported in several insects, the function

Abbreviation: FoxL21, forkhead box transcription factor L2, member 1.

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Bombyx mori-derived aldo-keto reductase AKR2E8 detoxifies aldehydes present in mulberry leaves

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NADPH

ABSTRACT

Lepidopterans are agricultural pests. Since the silkworm is a model for lepidopterans, analysis of the enzymes produced by silkworms is of great interest for developing methods of pest control. The aldo-keto reductase (AKR) superfamily catalyzes the reduction of aldehydes by converting a carbonyl group to an alcohol group. Here, we characterized a new AKR present in the silkworm *Bombyx mori*, which has been designated as AKR2E8. Amino acid sequence and phylogenetic analyses showed that AKR2E8 is similar to human AKR1B1 and AKR1B10. Three amino acid residues in the active site were identical among AKR2E8, AKR1B1, and AKR1B10. Recombinant AKR2E8 overexpressed in *Escherichia coli* used nicotinamide adenine dinucleotide phosphate as a coenzyme to reduce the aldehydes present in mulberry (*Morus alba*) leaves. AKR2E8 was found to reduce hexanaldehyde, hexanal, heptanal, nonanal, *trans*-2-nonenal, and citral. No nicotinamide adenine dinucleotide-dependent activity was detected. *Akr2e8* mRNA was detected in the testes, ovaries, and fat body; the highest expression was found in the midgut. The substrate specificity and highest observed expression of AKR2E8 in the midgut suggests that AKR2E8 may play a major role in aldehyde detoxification in silkworms. The findings of this study may assist in the development of pest control methods for controlling the population of lepidopterans, such as silkworms, that damage crops.

1. Introduction

A large number of agricultural pests include lepidopterans. For example, the diamond backmoth (*Plutella xylostella*), tobacco cutworm (*Spodoptera litura*), and Heliothine moths (*Helicoverpa zea* and *H. virescens*) are known to be serious pests of agricultural crops [1–3]. As the silkworm is a model for lepidopteran insects, the metabolic analysis of insecticides and other compounds in silkworms is of great interest. Detoxification enzymes, including cytochrome P450, carboxylesterase, and glutathione transferase, have been studied in view of insecticide detoxification [3]. The aldo-keto reductase (AKR) superfamily comprises approximately 190 proteins that catalyze nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reactions and participate in various physiological processes [4]. Based on the diversity in amino acid sequences and substrate specificities [5], the AKR superfamily is divided into families, and each family is further divided into subfamilies, including hydroxysteroid dehydrogenases, prostaglandin synthases, detoxification enzymes of xenobiotics, aldose reductases, and aldehyde reductases [5]. Among the subfamilies, the AKR1B subfamily, which contains aldose reductases, has been extensively studied [6] because it is involved in the development of cancer and diabetes [6,7].

Compared to mammalian AKRs, insect AKRs have not been extensively studied. While studying insecticide metabolism in the silkworm, *Bombyx mori*, we identified a new member of the AKR superfamily, AKR2E8. Three AKRs (AKR2E4, AKR2E5, and bmALD1) have previously been identified in *B. mori* [8–10]. The specific substrates for these enzymes have been found to be 3-dehydroecdysone for AKR2E4 and bombykal for AKR2E5. 3-Dehydroecdysone is an inactive molting hormone [8], whereas bombykal is an aldehyde form of bombykol, a sex pheromone [9]. bmALD1 can reduce glucose and 2-nonenal, suggesting that it may participate in glucose metabolism and antioxidant reactions [10].

AKR2E8 recognizes aldehydes as substrates. In general, aldehydes are highly reactive compounds. The high expression of AKR2E8 in the

Abbreviations: AKR, aldo-keto reductase; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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RESEARCH ARTICLE

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Characterization of a novel superoxide dismutase in *Nilaparvata lugens*

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Abstract

The brown planthopper (*Nilaparvata lugens*) is a major agricultural pest of rice crops. Analysis of the enzymes produced by *N. lugens* is important to develop pest-control methods. Superoxide dismutase (SOD) is a detoxification enzyme that catalyzes the conversion of superoxide anions (reactive oxygen species) into oxygen and hydrogen peroxide. As there have been no reports on SOD in *N. lugens*, in this study, we characterized a new SOD in the brown planthopper, nlSOD1. Amino acid sequence and phylogenetic analyses revealed that nlSOD1 is a member of the Cu/Zn-SOD family. Recombinant nlSOD1, when overexpressed in *Escherichia coli*, catalyzes the dismutation of superoxide radicals into molecular O_2 and H_2O_2. Exposure to various insecticides induced nlSOD1 messenger RNA expression. These results indicate that nlSOD1 may contribute to the insecticide resistance of *N. lugens*. The findings of this study may assist in the development of novel methods to control the population of *N. lugens*.

KEYWORDS
antioxidant, brown planthopper, *Nilaparvata lugens*, superoxide dismutase

1 | INTRODUCTION

Aerobic organisms constantly consume oxygen, which is utilized by the mitochondria to produce the energy required to sustain life. Some of these oxygen molecules may be converted into reactive oxygen species (ROS) during metabolic processes. Exposure of tissues and cells to radiation (ultraviolet rays) and chemical agents also results in the production of ROS (Atalay et al., 2020; Lee et al., 2019; Qureshi et al., 2021). ROS, including

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Investigation of the Substrate-Binding Site of a Prostaglandin E Synthase in *Bombyx mori*

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Abstract
Prostaglandin E synthase (PGES) catalyzes the conversion of prostaglandin H_2 to prostaglandin E_2 in the presence of glutathione (GSH) in mammals. Amid the limited knowledge on prostaglandin and its related enzymes in insects, we recently identified PGES from the silkworm *Bombyx mori* (bmPGES) and determined its crystal structure complexed with GSH. In the current study, we investigated the substrate-binding site of bmPGES by site-directed mutagenesis and X-ray crystallography. We found that the residues Tyr107, Val155, Met159, and Glu203 are located in the catalytic pockets of bmPGES, and mutagenesis of each residue reduced the bmPGES activity. Our results suggest that these four residues contribute to the catalytic activity of bmPGES. Overall, this structure-function study holds implications in controlling pests by designing rational and efficient pesticides.

Keywords Prostaglandin · Prostaglandin E synthase · lepidoptera · Site-directed mutagenesis

Abbreviations

bmPGES	Prostaglandin E synthase
BSPT	2-(2-benzothiazolyl)-5-styryl-3-(4-phthalhydrazidyl) tetrazolium chloride
CDNB	1-chloro-2,4-dinitrobenzene
GSH	Glutathione
GST	Glutathione transferase
PGs	Prostaglandins
HPGDS	Hematopoietic prostaglandin D synthase
HQL79	4-benzhydryloxy-1-[3-(1H-tetrazol-5-yl)-propyl]- piperidine

1 Introduction

Glutathione transferases (GSTs, EC 2.5.1.18) are ubiquitously expressed and responsible for the intracellular detoxification of various xenobiotic and endogenous substances, such as unsaturated aldehydes and prostaglandins (PGs) by catalyzing their conjugation with glutathione (GSH) [1, 2]. Various GST classes (delta, epsilon, omega, sigma, and zeta), as well as unclassified GSTs, have been identified in *Bombyx mori* [3–10]. Recently, X-ray structures of the delta-class, sigma-class, omega-class, unclassified, and unclassified 2 GSTs from *B. mori* have been determined [5, 6, 8, 9, 11]. A series of PG isomers are present in *B. mori*, including PGH_2, PGD_2, and PGF_2 [12]. We previously identified and structurally characterized prostaglandin E synthase (PGES) from *Bombyx mori* (bmPGES), an enzyme that catalyzes the isomerization of PGH_2 to PGE_2 in the silkworm [6]. Both mammalian PGES and bmPGES belong to the sigma-class GSTs [6, 13, 14]. Disruption in the bmPGES-encoding gene affected PGE_2 content, expression of chorion genes, and egg formation in silkworms. These results indicated a probable role of bmPGES in reproduction of *B. mori*. Using crystal structure analysis of bmPGES, we identified the amino acid residues involved in the GSH-binding site and electron-sharing network [15]. For a better understanding of the molecular basis of bmPGES catalysis, we examined the structure and catalytic function of bmPGES, with major focus on its substrate-binding site in the present study.

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Dark panicle color and high panicle position increase spikelet temperature of rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) quality and yield are degraded by high temperature, especially at the ripening stage after the heading of panicles. The effect is lethal when the panicle temperature (T_p) is excessively high; therefore, maintaining a low T_p is important to avoid deleterious impacts on the grains. Microclimatic factors and plant physiological elements determine the T_p. One determining factor is the color (or reflectance) of spikelets that constitute the panicle because it determines the absorption of shortwave radiation energy. An additional factor is the panicle position because it influences heat exchange by the wind and input energy from downward shortwave radiation. In this study, inter-strain differences in spikelet color and panicle height at heading were assessed. The T_p of strains differing in panicle color and panicle height were measured with thermocouples. In addition, to estimate the effect of each trait, we adopted a micrometeorological model. Panicle color was quantified using a hyperspectral sensor. Combining the spectral reflectance and spectral radiation, we assessed the effect of panicle color on T_p. The differences in panicle color and panicle position significantly affected T_p. The strain with a dark panicle had a maximum measured T_p about 1.8 ℃ higher than that of the strain with a light-colored panicle. The T_p of a strain with panicles at higher positions was up to 2.0 ℃ higher than that of a strain with panicles at lower positions. These relationships were consistent with the model estimates. When shortwave radiation was strong, the difference in T_p between strains showed a positive correlation, suggesting that the temperature difference was associated with shortwave radiation. Therefore, we concluded that rice strains with a brighter panicle color and low panicle position are less prone to deleterious impacts of high temperature because net radiation is reduced.

Key words: High temperature deterioration, Micrometeorology, Panicle height, Panicle temperature, Spikelet reflectance

1. Introduction

The Intergovernmental Panel on Climate Change (IPCC) predicts an increase in global temperature of 4.4 ℃ under the Shared Socio-economic Pathway 5 set at 8.5 W m^{-2} of radiative forcing (SSP5-8.5) by 2100 (IPCC, 2021). Thus, an adaptive solution to global warming for rice (*Oryza sativa* L.) production is required. Rice is sensitive to high air temperature (T_a), which results in low quality and yield of grains (Arshad *et al.*, 2017; Xiong *et al.*, 2017). In particular, high T_a in the heading and ripening stages causes marked deterioration in grain quality and yield. Two types of quality and yield loss are induced: development of chalky rice grains (CRGs) and heat-induced spikelet sterility (HISS). In Japan, CRGs incidence is predicted to increase in all regions, especially in western Japan (Takimoto *et al.*, 2019). Because CRGs are inferior to normal grains in palatability (Wakamatsu *et al.*, 2007), an economic loss of US$404.1 million y^{-1} in the 2040s is predicted under RCP 8.5 (Masutomi *et al.*, 2019). Thus, adaptation to global warming is important not only for rice production but also for economic well-being.

Deterioration of rice quality and yield is closely associated with the panicle temperature (T_p) in addition to T_a. Sato and Inaba (1973) revealed that high T_p is a more important factor for development of CRGs than high leaf temperature. This finding indicates that one reason for occurrence of CRGs is decline in the panicle sink strength for starch accumulation. Proteomic and genomic studies have revealed that high temperature upregulates starch degradation enzymes, such as α-amylase (Kaneko *et al.*, 2016; Yamakawa *et al.*, 2007), which is considered to be an important factor because α-amylase degrades starch in the spikelet, resulting in loosely packed starch grains and, ultimately, opaque grains. In addition, high T_p increases the risk of HISS, which arises at T_a of approximately 35 ℃ in the flowering stage (Matsui *et al.*, 1997, 2007). However, HISS is not a serious problem in paddies in Australia, where T_a in a paddy field may attain ~40 ℃. The stability in yield under such high T_a was associated with the extremely dry atmosphere and strong wind above the canopy. Such conditions make it possible to maintain a low T_p despite the high T_a (Matsui *et al.*, 2007). Therefore,

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植物遺伝子開発分野

frontiers in Plant Science

ORIGINAL RESEARCH
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Loss of OsEAF6, a Subunit of the Histone Acetyltransferase Complex, Causes Hybrid Breakdown in Intersubspecific Rice Crosses

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Gene duplication plays an important role in genetic diversification, adaptive evolution, and speciation. Understanding the mechanisms and effects of postzygotic isolation genes is important for further studies of speciation and crop breeding. The duplicate recessive genes *hwe1* and *hwe2* cause hybrid breakdown, characterized by poor vegetative growth and reproductive dysgenesis in intersubspecific crosses between *Oryza sativa* ssp. *indica* and *japonica*. Using a map-based cloning strategy, we found that *HWE1* and *HWE2* encode the Esa1-associated factor 6 (EAF6) protein, a component of histone acetyltransferase complexes. The *indica hwe1* and *japonica hwe2* alleles lacked functional *EAF6*, demonstrating that the double recessive homozygote causes hybrid breakdown. Morphological and physiological observations showed that weak plants with double recessive homozygotes had serious morphological defects with a wide range of effects on development and organs, leading to leaves with reduced chlorophyll content, flower and pistil malformation, and anomalies of gametogenesis. These findings suggest that EAF6 plays a pivotal role in the transcriptional regulation of essential genes during the vegetative and reproductive development of rice.

Keywords: hybrid breakdown, histone acetyltransferase, rice, duplicate recessive gene, speciation

INTRODUCTION

In eukaryotic cells, histone acetylation regulates the chromatin structure, affecting gene transcription, DNA replication, and DNA damage repair. Nucleosome acetyltransferase of histone 4 (NuA4), a histone acetyltransferase (HAT) complex, is composed of multiple proteins and preferentially acetylates histones H4 and H2A on the nucleosome. The components of NuA4 are highly conserved in yeast and human (Doyon et al., 2004). Yeast NuA4 consists of 13 subunits, with two independent NuA4 sub-complexes, namely, piccolo-NuA4, composed of Esa1, Epl1, Yng2, and Eaf6, and the TINTIN triad of Eaf5/7/3 (Wang X. et al., 2018). Piccolo-NuA4, which is thought to also exist alone, contains the catalytic subunit protein essential Sas2-related acetyltransferase-1 (Esa1) (Ohba et al., 1999; Boudreault et al., 2003). Esa1 alone can acetylate free histones but cannot acetylate nucleosomal histones (Doyon et al., 2004). This protein also plays a crucial role in cell cycle progression and DNA double-strand break repair (Clarke et al., 1999;

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Rice

RESEARCH Open Access

Genetic Background Negates Improvements in Rice Flour Characteristics and Food Processing Properties Caused by a Mutant Allele of the *PDIL1-1* Seed Storage Protein Gene

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Abstract

Phenotypic differences among breeding lines that introduce the same superior gene allele can be a barrier to effective development of cultivars with desirable traits in some crop species. For example, a deficient mutation of the *Protein Disulfide Isomerase Like 1–1* (*PDIL1-1*) gene can cause accumulation of glutelin seed storage protein precursors in rice endosperm, and improves rice flour characteristics and food processing properties. However, the gene must be expressed to be useful. A deficient mutant allele of *PDIL1-1* was introduced into two rice cultivars with different genetic backgrounds (Koshihikari and Oonari). The grain components, agronomic traits, and rice flour and food processing properties of the resulting lines were evaluated. The two breeding lines had similar seed storage protein accumulation, amylose content, and low-molecular-weight metabolites. However, only the Koshihikari breeding line had high flour quality and was highly suitable for rice bread, noodles, and sponge cake, evidence of the formation of high-molecular-weight protein complexes in the endosperm. Transcriptome analysis revealed that mRNA levels of fourteen *PDI*, *Ero1*, and *BiP* genes were increased in the Koshihikari breeding line, whereas this change was not observed in the Oonari breeding line. We elucidated part of the molecular basis of the phenotypic differences between two breeding lines possessing the same mutant allele in different genetic backgrounds. The results suggest that certain genetic backgrounds can negate the beneficial effect of the *PDIL1-1* mutant allele. Better understanding of the molecular basis for such interactions may accelerate future breeding of novel rice cultivars to meet the strong demand for gluten-free foods.

Keywords: Rice (*Oryza sativa* L.), Seed storage protein mutation, Protein disulfide isomerase, Rice flour characteristics, Food processing suitability, Gene expression, Grain component, Agronomic trait, Genetic background

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Background

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. Consumer preference causes strong demand for high-quality grain in rice cultivars, although increasing crop yield has long been an important requirement in many rice breeding programs (Champagne et al. 1999; Fitzgerald et al. 2009; Hori and Yano 2013; Custodio et al. 2019). Rice is mainly cooked

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Mutation in *BEIIb* mitigates the negative effect of the mutation in *ISA1* on grain filling and amyloplast formation in rice

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Abstract

Key message **Mutation of the *BEIIb* gene in an *isa1* mutant background mitigates the negative effect of the *ISA1* mutation on grain filling, and facilitates recovery of amyloplast formation in rice endosperm.**

Abstract In this study, the effect of branching enzyme IIb and isoamylase 1 deficiency on starch properties was demonstrated using high resistant starch rice lines, Chikushi-kona 85 and EM129. Both lines harbored a mutation in the *BEIIb* and *ISA1* genes and showed no BEIIb and ISA1 activity, implying that both lines are *beIIb isa1* double mutants. The amylopectin long chain and apparent amylose content of both mutant lines were higher than those of the wild-type. While both mutants contained loosely packed, round starch grains, a trait specific to *beIIb* mutants, they also showed collapsed starch grains at the center of the endosperm, a property specific to *isa1* mutants. Furthermore, *beIIb isa1* double mutant F_2 lines derived from a cross between Chikushi-kona 85 and Nishihomare (wild-type cultivar) showed significantly heavier seed weight than the *beIIb* and *isa1* single mutant lines. These results suggest that co-occurrence of *beIIb* and *isa1* mutant alleles in a single genetic background mitigates the negative effect of the *isa1* allele on grain filling, and contributes to recovery of the amyloplast formation defect in the *isa1* single mutant.

Keywords *Oryza sativa* L. · Resistant starch · Amylopectin · Branching enzyme · Isoamylase · Mutation

Introduction

Starch stored in tubers, pulses, and seeds usually comprises two kinds of α-glucans (amylose and amylopectin), and the ratio of amylose to amylopectin affects starch properties and its end usage in the food industry. Amylose is synthesized via the collaborative action of ADP-Glc pyrophosphorylase (AGPase) and granule-bound starch synthase I (GBSSI), whereas amylopectin is synthesized by AGPase, soluble starch synthase (SS), starch-branching enzyme (BE), and starch-debranching enzyme (DBE) (Tetlow 2011; Fujita 2014). BE isoforms are involved in creation of the

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Serine hydroxymethyltransferase participates in the synthesis of cysteine-rich storage proteins in rice seed

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ABSTRACT

The *low level of cysteine-rich proteins* (*lcrp*) mutation indicates a decrease in cysteine-rich (CysR) prolamines, α-globulin, and glutelin. To identify the causing factor of *lcrp* mutation, to elucidate its function, and to elucidate the role of CysR proteins in the formation of protein bodies (PBs), *lcrp* mutant was analyzed. A linkage map of the *LCRP* gene was constructed and genomic DNA sequencing of a predicted gene within the mapped region demonstrated that *LCRP* encodes a serine hydroxymethyltransferase, which participates in glycine-serine interconversion of one-carbon metabolism in the sulfur assimilation pathway. The levels of L-Ser, Gly, and Met in the sulfur assimilation pathway in the *lcrp* seeds increased significantly compared to that in the wildtype (WT). As the *lcrp* mutation influences the growth of shoot and root, the effects of the addition to the medium of amino acids and other compounds on the sulfur assimilation pathway were studied. Electron-lucent PBs surrounded by ribosome-attached membranes were observed accumulating cysteine-poor prolamines in the *lcrp* seeds. Additionally, glutelin-containing PBs were smaller and distorted in the *lcrp* seeds compared to those in the WT. These analyses of PBs in the *lcrp* seeds suggest that cysteine-rich proteins play an important role in the formation of PBs in rice.

1. Introduction

Seed storage proteins are classified into globulins, prolamines, and glutelins, which are salt-, alcohol-, and acid- and/or alkali-soluble, respectively. Although rice storage proteins are translated on the endoplasmic reticulum (ER), the translation and accumulation sites vary depending on the type of protein. Two types of ER subdomains, protein body-ER (PB-ER) and cisternal ER (cis-ER), and two types of accumulation sites, PB-I and PB-II, exist in rice endosperm [1,2]. Prolamines translated on PB-ER directly accumulate in the ER and form PB-I within the PB-ER, while glutelins are translated as precursors on cis-ER and transported via the Golgi apparatus to the protein storage vacuole (PSV) where the precursors are cleaved into acidic and basic subunits and are accumulated as PB-II [3–7]. Globulins translated on PB-ER are transported with proglutelin precursors to the PSV via the Golgi apparatus and accumulated in PB-II [8].

Rice prolamines, encoded by a multigene family, are divided into cysteine-rich (CysR) and cysteine-poor (CysP) prolamines, which contain 10, 14, and 16 kDa- and 13 kDa molecular polypeptides, respectively [3]. CysR prolamines contain 10–12 cysteine residues, whereas CysP prolamines have no cysteine residues [9,10]. Rice α-globulin (26 kDa), encoded by a single gene, contains 8 cysteine residues [11], and glutelins, encoded by a multigene family, contain 7–10 cysteine residues [12,13].

Some rice CysR prolamines contain conserved sequences at 3 distinct regions, termed as A, B, and C, with the consensus motifs LxxC, CCxQL, and PxxC, respectively [9,14–16]. Rice α-globulin, which accumulates mostly on the surface of PB-II [17], also contains the conserved ABC regions of storage proteins [11], which are essential for protein localization in the PBs [18].

Matsusaka et al. (2003) reported a seedling-lethal recessive mutation in rice that resulted in a decrease in CysR prolamins (10, 14, and 16 kDa), 26 kDa-α-globulin, and acidic subunit (40 kDa) of glutelin, which can only be sustained as a heterozygous mutant line [19]. This mutation was termed *low level of CysR proteins* (*lcrp*). The rough ER-derived H_2O_2 levels and sulfhydryl groups markedly decreased in the homozygous mutant seeds compared to the WT seeds [20], suggesting that the mutation may have altered the function of a factor involved in cysteine

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α-globulin-rich rice cultivar, low glutelin content-1 (LGC-1), decreases serum cholesterol concentration in exogenously hypercholesterolemic rats

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Abstract

BACKGROUND: Rice α-globulin has been reported to have serum cholesterol-lowering activity in rats. However, it is still unclear whether α-globulin exerts this effect when taken as one of the dietary components. In the present study, we investigated the effect of two cultivars of rice, low glutelin content (LGC)-1 and LGC-Jun, on reducing serum cholesterol in exogenously hypercholesterolemic (ExHC) rats. LGC-1 is enriched in α-globulin (10.6 mg g^{-1} rice flour, which is an approximately 1.5 times higher α-globulin content than in Koshihikari a predominant rice cultivar in Japan), whereas LGC-Jun is a globulin-negative cultivar.

METHODS: ExHC rats, the model strain of diet-induced hypercholesterolemia, were fed 50% LGC-1 or LGC-Jun and 0.5% cholesterol-containing diets for 2 weeks, followed by measurement of cholesterol metabolism parameters in serum and tissues.

RESULTS: Serum cholesterol and non-high-density lipoprotein cholesterol levels were significantly lower in the LGC-1 group compared to the LGC-Jun group. Cholesterol intestinal absorption markers, hepatic and serum levels of campesterol and β-sitosterol, and lymphatic cholesterol transport were not different between the two groups. Levels of 7α-hydroxycholesterol, an intermediate of bile acid synthesis, showed a downward trend in the livers of rats that were fed LGC-1 ($P = 0.098$). There was a significant decrease in the hepatic mRNA expression of *Cyp7a1* (a synthetic enzyme for 7α-hydroxycholesterol) in the LGC-1 group compared to the LGC-Jun group.

CONCLUSION: Dietary LGC-1 significantly decreased serum cholesterol levels in ExHC rats. The possible mechanism for the cholesterol-lowering activity of LGC-1 is partial inhibition of bile acid and cholesterol synthesis in the liver.

Keywords: rice α-globulin; LGC-1; cholesterol; hypercholesterolemic rats; *Cyp7a1*; bile acid synthesis inhibition

INTRODUCTION

Cholesterol is biosynthesized in all animal cells and serves as an essential structural component of animal cell membranes and also as a precursor for the biosynthesis of steroid hormones and bile acid.[1,2] It has been reported that excessive cholesterol intake increases the risk of hypercholesterolemia, which leads to atherosclerosis.[3] Risk factors for atherosclerosis include hypercholesterolemia, hypertension and oxidative stress, amongst others.[4] An epidemiological study has revealed that lowering serum cholesterol levels is a key factor in the prevention of atherosclerosis.[5] There is a significant value in understanding ways to reduce cholesterol levels using diet or supplements to help prevent and treat atherosclerosis.

Several nutrients have been confirmed to have cholesterol-lowering activity, such as unsaturated fatty acids and proteins.[6] Plant protein intake shows higher cholesterol-lowering activity

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植物遺伝子開発分野

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Invited Review

Collection, preservation and distribution of *Oryza* genetic resources by the National Bioresource Project RICE (NBRP-RICE)

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Biological resources are the basic infrastructure of bioscience research. Rice (*Oryza sativa* L.) is a good experimental model for research in cereal crops and monocots and includes important genetic materials used in breeding. The availability of genetic materials, including mutants, is important for rice research. In addition, *Oryza* species are attractive to researchers for both finding useful genes for breeding and for understanding the mechanism of genome evolution that enables wild plants to adapt to their own habitats. NBRP-RICE contributes to rice research by promoting the usage of genetic materials, especially wild *Oryza* accessions and mutant lines. Our activity includes collection, preservation and distribution of those materials and the provision of basic information on them, such as morphological and physiological traits and genomic information. In this review paper, we introduce the activities of NBRP-RICE and our database, Oryzabase, which facilitates the access to NBRP-RICE resources and their genomic sequences as well as the current situation of wild *Oryza* genome sequencing efforts by NBRP-RICE and other institutes.

Key Words: National Bioresource Project (NBRP), wild *Oryza*, *Oryza sativa*, mutants, methyl nitrosourea (MNU), chromosomal segment substitution lines (CSSLs), nearly isogenic lines (NILs).

Introduction

The National Bioresource Project (NBRP) of Japan is a set of government-supported programs run to collect, preserve and distribute bioresources that are essential to the life sciences (Kurata *et al.* 2010). One NBRP program is NBRP-RICE. Rice is an indispensable staple food crop that accompanies with human beings under the pressure of selection and breeding. Genetic resources of rice, such as cultivars, landraces, wild *Oryza* species, mutants and other experimental strains, are essential to both breeding and basic science. It is thought that there are over 350,000 cultivated lines of rice in the world (FAO 2010); many of them are stored and maintained in large-scale genetic resource centers such as the International Rice Research Institute (IRRI Philippines), the National Agriculture and Food Research Organization (NARO Japan) and other centers in rice-producing Asian countries. On the other hand, experimental strains and other genetic resources, such as mutants, wild species and other research materials, are maintained in relatively small institutes or universities (Eamens *et al.* 2004, Hsing *et al.* 2007, Jeon *et al.* 2000, Kim *et al.* 2004, Kolesnik *et al.* 2004, Kurata and Yamazaki 2006, Li *et al.* 2017, Miyao *et al.* 2003, Sallaud *et al.* 2004, van Enckevort *et al.* 2005). One such example is NBRP-RICE, operated by the National Institute of Genetics (NIG) and Kyushu University, Japan. NIG has charge of collecting, preserving and providing *Oryza* genetic resources. Kyushu University collects, preserves and provides experimental lines such as mutant strains, chromosomal segment substitution lines (CSSLs) and aneuploids derived from wild *Oryza*. In Japan, the Genebank project in NARO extensively collects landraces and cultivars and, thus, the collections of NBRP-RICE and NARO Genebank complement together to meet the request of resources used for both basic science and breeding.

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微生物遺伝子開発分野

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Genomic characterisation of *Lacticaseibacillus paracasei* phage ΦT25 and preliminary analysis of its derived endolysin

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ABSTRACT

Bacteriophage infection is a serious problem in milk fermentation where it causes a delay in the fermentation process and reduces product quality. This study provides the first molecular characterisation of a lactic acid bacteriophage from spoiled fermented milk tanks in the dairy industry in Thailand. Bacteriophage ΦT25 is virulent, and shows strong lytic activity against *Lacticaseibacillus paracasei* LPC. Genome analysis demonstrated that phage ΦT25 possesses a linear, double-stranded DNA genome of 38,421 bp. To assay endolysin activity of phage ΦT25 (ORF35), Lysin vector pET21a was constructed by cloning the lysinT25 gene into plasmid pET21a. The size of lysinT25 endolysin was predicted to be ~41 kDa. lysinT25 was able to lyse a wide range of lactic acid bacteria and other Gram-positive bacteria, such as *Staphylococcus aureus* and *Listeria monocytogenes*. In addition, this endolysin has a synergistic inhibitory effect against *Escherichia coli* when combined with chloroform as an outer-membrane permeabiliser.

1. Introduction

Members of *Lacticaseibacillus* (formerly known as the *Lactobacillus casei* group) are commonly used as starter cultures in the dairy fermentation process; however, the increasing use of these strains can lead to bacteriophage (phage) infection in dairy processing plant environments (Holzapfel, Haberer, Geisen, Björkroth, & Schillinger, 2001). In the release of phage progeny, all double-stranded (ds) DNA viruses follow a lysin-mediated breakdown of peptidoglycan (PG) with endolysin (Lood et al., 2015; Pohane & Jain, 2015; Young, 2014). Endolysins are PG hydrolases produced by phages, which lyse the host bacterial cell. In the past decade, interest in endolysin has increased as an alternative preservative and antimicrobial to destroy pathogens in food and medical processes (Schmelcher, Donovan, & Loessner, 2012c; Young, Wang, & Roof, 2000).

Endolysins are considered as a new class of antibiotics as they can destroy the PG of Gram-positive bacterial cell walls. Endolysins targeting Gram-positive bacteria are separated into two distinct types of functional domains termed cell wall binding domains (CBDs) and enzymatically active domains (EADs) (Schmelcher et al., 2012a). The N-terminus generally contains the EAD that confers the catalytic activity of the endolysin, while the C-terminal CBD enables the endolysin to selectively and specifically bind to the target bacterial cell wall (Fenton, Ross, McAuliffe, O'Mahony, & Coffey, 2011; Schmelcher, Powell, Becker, Camp, & Donovan, 2012). Based on the specific PG bond attacked by the EAD, endolysins can be classified into at least five different groups: muramidase (lysozyme), transglycosylase, glucosaminidase [digests N-acetylmuramic acids (NAM) and N-acetylglucosamine (NAG)], amidase (digests NAM and peptides), and endopeptidase (digests within the peptide chain of PG) (Borysowski, Weber-Dąbrowska, & Górski, 2006). Advances in the bioinformatic analysis of whole genome sequencing data have identified multiple endolysins from the genomes of isolated phages (Fernández-Ruiz, Coutinho, & Rodríguez-Valera, 2018). An endolysin of phage PL-1 of *L. casei* (Kashige,

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微生物遺伝子開発分野

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequence of *Bacillus cereus* Strain HT18, Isolated from Forest Soil

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ABSTRACT The genome sequence of *Bacillus cereus* strain HT18, isolated from forest soil, was 5,333,415 bp long. The genome included 5,825 putative coding sequences and 35.2% GC content; the strain had 5 plasmids. Average nucleotide identity based on BLAST+ (ANIb) and digital DNA-DNA hybridization (dDDH) results showed that HT18 was 98.78% and 90.70% homologous, respectively, to *B. cereus* ATCC 14579[T].

The *Bacillus cereus* group (phylum *Firmicutes*) comprises Gram-positive, spore-forming, facultative, anaerobic, rod-shaped bacteria with low-GC-content genomes (1). It includes eight closely related species with high genomic homology and 16S rRNA gene sequence similarity—*B. anthracis*, *B. cereus*, *B. cytotoxicus*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, *B. toyonensis*, and *B. weihenstephanensis* (2). Phenotypic features, such as motility and hemolysis, used to classify species within this group can differ within and among species, leading to the use of the average nucleotide identity based on BLAST+ (ANIb) and digital DNA-DNA hybridization (dDDH) as classification indices (3, 4).

Strain HT18 was isolated from forest soil in Hashimoto, Wakayama, Japan. The soil samples were flooded, filtered, and incubated overnight at 37°C on LB agar (Nacalai Tesque). After colony isolation, the cells were cultured in LB broth at 37°C for 24 h. Then, genomic DNA was isolated using Marmur's method (5).

Sequencing was a combination of short and long reads. Short-read sequencing libraries were constructed using the NEBNext Ultra II FS DNA library prep kit (New England Biolabs [NEB]) and decoded on a MiSeq instrument (Illumina). A total of 1,488,012 reads with 431,000,004 bases were decoded with an average insert size of 641 bp and spot length of 602 bp with 2 × 300-bp paired ends. Low-quality bases (Q scores of <15) were trimmed, and short reads (<25 bp) were removed using Platanus trim version 1.0.7 (http://platanus.bio.titech.ac.jp/pltanus_trim) (6). Long-read sequence libraries were constructed using the rapid barcoding kit (SQK-RBK004), and sequencing was performed on a MinION device (Oxford Nanopore Technology [ONT]) using an R9.4.1 flow cell. No size selection was performed before library preparation. A total of 48,894 reads with 455,539,372 bases were decoded using Guppy version 4.0.15. The recovered data with NanoFilter technology (quality, ≥10; length, ≥2 kb; head crop, 100 bp) revealed 23,033 ONT reads with a mean read length of 17,050 bp and an N_{50} value of 27,780 bp. *De novo* assembly was performed using Unicycler version 0.4.8 (https://github.com/rrwick/Unicycler) (7). The genome annotation and rotation of the chromosome to bring *dnaA* first was performed with DFAST version 1.4.0 (https://dfast.ddbj.nig.ac.jp) (8). ANIb and dDDH values were calculated from the *JSpeciesWS* online service version 3.8.5 (http://jspecies.ribohost.com/jspeciesws/) and the Genome-to-Genome Distance Calculator version 3.0 (http://ggdc.dsmz.de/ggdc.php) (9) using the

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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（博士課程）

Wazifa Afrin	Study on glutathione 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

大学院生物資源環境科学府（修士課程）

佛坂　優虎	カイコの初期発生における遺伝的変異体の研究

【植物遺伝子開発分野】

大学院生物資源環境科学府（修士課程）

Rahma Siti Nur Azizah Fauziyati	Genetic and evolutionary study of *INCENTIVE FOR KILLING POLLEN (INK)* gene responsible for hybrid male sterility in rice

大学院生物資源環境科学府（博士課程）

Thein Lin	Identification of genes related to starch synthesis in rice endosperm

【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）

東郷　舜	乳酸球菌における寿命調節遺伝子の同定と長寿メカニズムの解明
森　絵美香	SARS-CoV-2 感染予防を目指すファージ療法の開発
Kwon soyong	*Thermus* 属細菌における Fur Family 遺伝子の機能解析

大学院生物資源環境科学府（修士課程）

Yang pui yung	乳酸菌における D-アミノ酸生成機構の解明
森田　大幹	*Thermus* 属細菌を宿主とする新奇異種タンパク発現系の開発
松中　南	シイタケ軟腐病菌を宿主とするバクテリオファージの特性解析
堀江　裕介	ファージ由来 holin タンパク質を利用したがん治療法の基礎研究
権藤　万弥	乳酸菌プロファージの誘導メカニズム解明
長岡　未久	好熱性ファージϕOH3 由来 integrase の機能解析
桑原　芽美	乳酸菌のプラズマローゲン生成機構の解明
中村　太一	ファージ由来 holin タンパク質を利用したがん治療法の基礎研
永吉　正汰	*Geobacillus kaustophilus* を宿主とするファージの特性解析

大学院生物資源環境科学府（博士課程）

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
下元　仁美	浴室汚染菌の性状・ゲノム解析

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」

基盤研究(B)　研究代表　伴野　豊
「染色体のパッケージングによるカイコとクワコの形質差に関与する遺伝子のトラッピング」

JASRI 研究助成金
研究代表　山本幸治
「殺虫剤代謝酵素の構造解析」

【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)」

基盤研究(B)　研究代表　熊丸敏博
「イネ種子の細胞内物質貯蔵における小胞体機能タンパク質の機能解明とその育種的利用」

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)　研究代表　土居克実
「好熱性繊維状ファージの生活環と宿主応答から辿る極限環境での遺伝子水平伝播と進化」

共同研究（（株）レオロジー機能食品研究所）研究代表　土居克実
「プロズマローゲン生産性細菌の探索と遺伝子解析」

共同研究（（株）レオロジー機能食品研究所）研究代表　土居克実
「遺伝子組み換え乳酸菌が作出するプロズマローゲンによる非アルコール性脂肪性肝疾患(NAFLD)疾病リスク低減のための特定保健用食品の開発」

柿原科学技術研究財団　研究助成金　研究代表　土居克実
「新型コロナウィルス感染防止・治療を目指すファージ療法の開発」

共同研究（（株）ダスキン）研究代表　土居克実
「新型コロナウイルス（SARS-CoV-2）株不活性化資材の探索」

3.　講演会・セミナー・講習会

【家蚕遺伝子開発分野】

伴野　豊
文部科学省ナショナルバイオリソースプロジェクトオンラインセミナー
「バイオリソースの先端技術まるわかり」において「実用段階に達したカイコの凍結保存技術とその有用性」公演　2021 年 8 月

JST 主催サイエンスアゴラ　「カイコ・シルク～過去・現在・未来をつむぐ」
群馬県樹徳高校企画によるオンライン講演会にゲスト講演　2021 年 11 月

第 8 回全国風穴サミット　「風穴の歴史と利用、自然環境」と題してオンライン講演

【植物遺伝子開発分野】

熊丸敏博
福岡県立明善高等学校　総合的な学習時間「大学セミナー」:農学部の特色と生物資源について, 2021 年 7 月

4. 海外渡航

【家蚕遺伝子開発分野】

なし

【植物遺伝子開発分野】

なし

【微生物遺伝子開発分野】

なし

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】

なし

【微生物遺伝子開発分野】

なし

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。突然変異体系統に加え、最近は大型、雌雄鑑別が容易な系統の利用が広がっている。理学、農学系、薬学系からの依頼が中心となっている。

		2017	2018	2019	2020	2021
生物体での分譲	国内	763 件	862 件	806 件	449 件	652 件
	国外	163 件	188 件	202 件	97 件	98 件
DNA での分譲	国内	0 件	0 件	0 件	40 件	0 件
	国外	0 件	0 件	0 件	0 件	60 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）

文部科学省のナショナルバイオリソースプロジェクト NBRP 採択による経費、文部省の系統保存費の補助金を受け、本分野のメイン事業として行っている。ここでは、NBRP 活動を中心に抜粋し、報告する。本年度の NBRP 事業は、先端科学に対応し得る高品質なカイコバイオリソースを収集・保存・提供する基盤を構築することを目的に、九州大学（中核機関)、学習院大学（分担機関)、信州大学（分担機関）と共同で事業を行った。

・中核機関である本分野のコアリソース約 500 種類は長野県松本市の風穴、信州大学の野蚕系統は九州大学でバックアップ保存した。コア系統の 90%は凍結保存によるバックアップ体制もあり、2 重の備えとなっている。遺伝子組換え系統の大半（約 150 系統）は凍結保存のみにより維持し、クワコ染色体をカイコ染色体で置換して系統であるコンソミック系統の凍結保存バックアップも進めている。
・カイコで開発した卵巣の凍結保存方法により分担機関（信州大学）が保有するエリサンの凍結保存を行った。
・ニュースレター“おかいこさま”を 2 回（4 月、8 月、12 月）発行し、研究コミュニティに配布した。
・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。

・運営委員会（2022 年 2 月 24 日）を開催するとともに日本蚕糸学会とは連携を密に行い、ユーザーの意見を収集し事業に反映させた。

＜個別の事業概要＞

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　10 系統、ENU 系統を 2 系統収集した。p20, p50 系統で置換したクワコのコンソミック系統の高度化を進めた。

・薬物評価、病態モデル、有用タンパク質の発現に適した 3 系統のカイコ系統の配布は好評である。

・保有系統のうち 600 系統に関し、卵、幼虫、蛹時期に形質の評価を行い、高品質化をはかった。

② カイコバイオリソースの保存・提供

・新規に 50 系統の凍結保存を行った。

・致死遺伝子 4 系統について、分子マーカーによる管理を可能とした。

・飼育室環境の管理、良質桑を確保するための桑園管理（福岡市 3 ヶ所、指宿市 1 ヶ所）を行った。

・第 1 期でコア系統を中心に採卵し、それを基本に、年間計 6 回の飼育により、卵から成虫の各ステージでの提供事業を行い、提供件数は 811 件であった。2020 年度はコロナ感染拡大による影響で 546 件であったが、回復傾向となった。

③ 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った。カイコの飼育は 5 月から 6 月の第 1 期の飼育が最も多い。コロナ影響拡大で、アルバイトによるクワ摘みが課題であったが、今年度は 3 齢期からの桑は外部委託を行った。天候に大きく左右される作業であり、雨が続き業者との連絡を密にすることで何とか桑が確保できた。カイコの飼育、経過は順調であった。

◎2021 年度（2021.4～2022.3、R2/R1）の桑園移管理業務の主なものは以下の通りである。

2021 年 4 月

第 3 工区 2 圃場（2014 年 3 月、2015 年 3 月植付）、4 工区 1、2、3 圃場（2016 年 3 月植付）、4、5、6 圃場（2017 年 3 月植付）、カイコバイオリソース研究施設建物前圃場（2018 年 3 月植付）、以上総面積約 2.6ha の管理がスタートする。

5 月

例年この時期の幼虫飼育期間中は約 6 トンの桑を必要とする。昨年は職員のみで桑摘作業を行ったが、飼育作業との同時進行は負担が大きく、今年は 3 齢以降の桑については造園業者に委託した。また、20 系統は長野県上田市の上田蚕種株式会社へ飼育委託した。1 期の収穫圃場は 3－1、3―2、4－1 とした。1 期桑葉収穫圃場の条切、条粉砕を行った。

6 月

1 期桑葉収穫圃場丸桑 3 号肥料（日本肥料）散布、トラクタ耕運。

8 月

5 期飼育用桑葉確保のため 4－2 中間伐採、条粉砕。丸桑 3 号肥料（日本肥料）散布、トラクタ耕運。

9 月

カイコバイオリソース研究施設前のパイプハウスおよび 4－5 圃場のパイプハウス内のシマグワの中間伐採。丸桑 3 号肥料（日本肥料）散布。

10 月

全桑園除草。

11 月

パイプハウスのビニール覆いかけを行う。（沖縄桑の冬季使用のため）

12 月

矮小枝の剪定&粉砕

2022 年 1 月

来年度 2 期飼育用桑葉確保のため、4 工区 17、20、21、22 圃場の春刈、条粉砕。指宿試験地より NBRP 依頼の桑葉発送。

2 月

全桑園堆肥散布&耕運。伊都桑園シマグワ挿木。指宿試験地においてもシマグワ挿木。指宿試験地より NBRP 依頼の桑葉発送。

3 月

全桑園にカミキリ殺虫剤注入。丸桑 3 号肥料（日本肥料）散布&耕運。

家蚕遺伝子開発分野の航空写真：空から見た本分野の全景。2020年7月の大雨で発生した北側西斜面の崩落跡が映る。

篤松時代の家蚕遺伝子開発分野の建物。2020年に取り壊された。右写真の下の隅には蚕具を洗った水槽、右上には箱崎中学との間にあった松の木が残る。この松は昔の「千代の松原」の名残で、昔、九大が海岸線に沿っていたことを物語っている。

指宿試験地の管理棟。この中に蚕飼育室、蚕室がある。右は桑畑。南国で亜熱帯桑を植え、冬季を中心に飼育に活用されている。建物は来年度改築予定。

【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/

japonica 型栽培品種に由来するゼブラ葉変異体（左）と病斑葉変異体（右）

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
2017	402			18-3,855	2-2
2018	474			14-145	2-17
2019	470			12-542	2-14
2020	863			15-2018	0-0
2021	408			12-525	0-0

【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内規

（趣旨）

第 1 条　この内規は、九州大学大学院農学研究院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 2 条　センターは、遺伝子資源の保存、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 3 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 4 条　センターに長（以下「センター長」という。）を置き、農学研究院の専任の教授をもって充てる。センター長は、センターの管理及び運営を総括する。

2　センター長は、次の各号に掲げる事項に該当する場合に選考する。

一　　センター長の任期が満了するとき。

二　　センター長が辞任を申し出て、研究院教授会の承認を得たとき。

三　　センター長が欠員となったとき。

3　センターの長の任期は、4 月 1 日から翌々年の 3 月 31 日までの 2 年とし、再任を妨げない。ただし、引き続き二期を超えて在任することはできない。

4　第 2 項第二号及び第三号の場合における後任者の任期は、前項本文の規定にかかわらず、前任者の残任期間とする。ただし、この残任期間が 1 年以上の場合は、これを一期とみなす。

5　センター長の選考は、研究院教授会において行い、有効投票の過半数の獲得によりセンター長候補者を決定する。

（運営委員会）

第5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6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　センターに勤務を命じられた農学研究院の専任の教員のうちから選ばれたもの3人

二　各部門 (農学部附属農場、農学部附属演習林、生物的防除研究施設及びセンターに勤務を命じられた農学研究院の専任の教員を除く。)の専任の教授及び准教授のうちから選ばれた者各1人

三　農学部附属農場、農学部附属演習林及び生物的防除研究施設に勤務を命じられた農学研究院の専任の教授及び准教授のうちから選ばれた者各1人

2　委員の任期は2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、研究院長が委嘱する。

第7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8条　運営委員会は、委員の過半数が出席しなければ、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9条　この内規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、センター長が定める。

附　則

1　この規程は、平成12年4月1日から施行する。

2　この規定施行後、最初に任命されるセンター長は、この規定に基づき選考された者とみなし、その任期は第4条第3項の規定にかかわらず、平成13年3月31日までとする。

3　農学部附属遺伝子資源開発研究センター規定(平成9年2月12日施行)は廃止する。

附　則

この内規は、平成 12 年 11 月 8 日から施行する。

附　則

この内規は、平成 19 年 4 月 1 日から施行する。

附　則

この内規は、平成 22 年 4 月 1 日から施行する。

附　則

この内規は、平成 25 年 4 月 1 日から施行する。

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 Laboratory

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 Laboratory

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 Laboratory

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