

## Accelerator Applications Research Division Ion Beam Breeding Group

### 1. Abstract

The ion beam breeding group studies the various biological effects of fast heavy ions and develops new techniques for breeding plants and microbes using heavy-ion irradiations. Heavy ions can produce dense and localized ionizations in matters along their tracks, in contrast to photons (X-rays and gamma rays), which produce randomly distributed isolated ionizations. These localized and dense ionizations can cause double-strand breaks of DNA, which are not easily repaired and result in mutations more effectively than single-strand breaks. Heavy ion beams produced at the RIKEN Ring Cyclotron (RRC) are high energy and can penetrate long ranges, allowing them to irradiate biological samples in the air. This group utilizes a dedicated beam line (E5B) of the RRC to irradiate microbes, plants and animal cells with beams ranging from C to Fe ions. Its research subjects cover the study of ion-beam radiation mutagenesis, genome-wide analyses of mutations, and the development of new plants and microbial varieties by heavy-ion irradiation. Forty-three new varieties have already been brought to the market. The group also provides researchers with mutagenesis technology to discover new genes and develop improved new varieties, contributing to solving societal issues such as food, energy, the environmental problems.

### 2. Major Research Subjects

- (1) Study on the biological effects by heavy-ion irradiation
- (2) Research and development of the breeding technology using heavy-ion irradiation
- (3) Innovative applications of heavy-ion irradiation

### 3. Summary of Research Activity

RIKEN Nishina Center promotes research on applications of heavy ions from the RRC using 135 MeV/nucleon C, N, Ne ions, 95 MeV/nucleon Ar ions, 90 MeV/nucleon Fe ions, and from the IRC using 160 MeV/nucleon Ar ions to biology and agriculture. We also develop breeding technology for microbes and plants. The main subjects are:

#### (1) Study on the biological effects by heavy-ion irradiation

Linear energy transfer (LET) is an important parameter in determining biological effectiveness. LET is the energy that an ionizing particle deposits in a target per unit path length. The optimal LET for mutagenesis in dry seeds (LET<sub>max</sub>) was found to be 30 keV/μm in Arabidopsis, 23–39 keV/μm in buckwheat, 23–50 keV/μm in rice, and 50–70 keV/μm in wheat. By contrast, irradiation with 290 keV/μm has a low mutation rate but high lethality in plants. A whole-genome analysis using high-throughput sequencing is a powerful tool for characterizing the nature of induced mutations. In addition, we developed a new pipeline, the Automated Mutation Analysis Pipeline (AMAP), for the rapid detection of whole-genome mutations. Using a whole-genome mutational analysis in Arabidopsis and rice, we comprehensively characterized the mutation effects of ion beams of C (30 keV/μm) and Ar (290 keV/μm). C-ion beams mainly induced single-nucleotide variants (SNVs). Ar-ion beam showed a mutation spectrum different from that at C ion, SNVs and small deletions decreased, and the proportion of large deletions (>100 bp) and chromosomal rearrangements increased. We propose and develop tailor-made ion beam breeding techniques based on these results.

#### (2) Research and development of breeding technology using heavy-ion irradiation

We propose the tailor-made ion beam breeding technique. When a user wishes to obtain mutants at high efficiency, we recommend choosing LET<sub>max</sub> (30–50 keV/μm) irradiation. LET<sub>max</sub> is effective for breeding due to its extremely high mutation frequency. The most common mutations, SNVs and small deletions, are sufficient to disrupt a single gene. Since 2001, using C and nitrogen ion irradiations, we have developed 43 new varieties that have been introduced to the market in Japan, the USA and the European Union. Thus, irradiation with these ions can efficiently produce knockout mutants of a target gene and can be applied to mutagenomics research, such as discovering novel genes using mutants. If a user requires mutations with large deletions or chromosomal rearrangements, Ar-ion irradiation (290 keV/μm) is suitable. It is a promising new mutagen suitable for functional analyses of tandem duplicated genes. We have found that ions with higher LETs, such as Ar and Fe, are effective for the mutation breeding of microbes. Over 14 breweries in Japan use two new yeast strains created by Fe-ion irradiation to produce high-quality sake. We built a new beamline, ‘Wide AppliCable to Mutagenesis Experiment (WACAME),’ to increase the availability of nuclides with higher LET and longer ranges. The ion beams are accelerated by three cyclotrons, AVF, RRC and IRC, to 160 MeV/nucleon and sent to the E5B beam line. The new Ar ion (184 keV/μm) showed a higher mutation rate than the 290 keV/μm Ar ion. In the future, we plan to develop a unique technology for breeding using these heavier ion beams.

#### (3) Innovative application of heavy-ion irradiation

In 1998, we formed a consortium for ion-beam breeding consisting of 24 groups. In 2024, the consortium grew to 188 groups from Japan and 24 from overseas. Previously, ion-beam breeding procedures were carried out mainly using flowers and ornamental plants. We have recently put new varieties on crops. Due to climate change, the harvest time of the Satsuma mandarin has trends to be earlier; it is desirable to breed new varieties suitable for long-term storage. A new variety called ‘Harushizuka’ is harvested one month later due to the later coloring of the fruit. It exhibits less rind puffing and a high citric acid content, making it suitable for long-term storage. Additionally, it can be shipped from March to April when the product is in short supply. Shizuoka Prefecture has sold 1,900 seedlings, aiming to start selling the fruit in 2027. We plan to utilize whole genome mutation analysis data to develop a DNA marker

for variety identification. Demand for small chrysanthemums increases during the Bon Festival. Using the same cultivation method, the farmer aims to produce three colors of chrysanthemums: red-purple, white, and yellow. The red-purple cultivar, which has a wide range of flower color mutations, was selected as the original species, and the white and yellow flower color mutants were selected. In addition, since C-ion irradiation did not produce a monochromatic yellow mutant this time, a yellow mutant was obtained from a population re-irradiated with Ar ions (283 keV/ $\mu\text{m}$ ) using the red mutant as an intermediate mother plant. The three-color small chrysanthemum has almost the same morphology and cultivation characteristics as the original species with only the flower color mutation, and it blooms during the Bon Festival so that it can be shipped during this season. Furthermore, they have also succeeded in developing new colors of petunia, a strain of kelp that can tolerate high water temperatures, and a dwarf variety of early-maturing durum wheat.

## Members

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## List of Publications & Presentations

### Publications

#### [Original Papers]

- T. Hirano, M. Murata, Y. Watarikawa, Y. Hoshino, T. Abe, and H. Kunitake, “Distinctive development of embryo and endosperm caused by male gametes irradiated with carbon-ion beam,” *Plant Reprod.* **37**, 355–363 (2024).
- K. Ishii, Y. Kazama, T. Hirano, J. A. Fawcett, M. Sato, M. Y. Hirai, F. Sakai, Y. Shirakawa, S. Ohbu, and T. Abe, “Genomic view of heavy-ion-induced deletions associated with distribution of essential genes in *Arabidopsis thaliana*,” *Front. Plant Sci.* **15**, 1352564 (2024).
- M. Shii, Y. Kajiya, M. Murata, T. Abe, H. Kunitake, and T. Hirano, “Effects of fertilization of male gametes with heavy-ion beam irradiation on embryo and endosperm development in *Cyrtanthus mackenii*,” *Cytologia* **89**, 133–139 (2024).
- D. Qin, K. Toyokura, M. Nakano, T. Abe, Y. Higuchi, P. Arens, K. Taniguchi, M. Shibata, and M. Kusaba, “Structural complexity of the white flower locus in *Chrysanthemum*,” *J. Hortic. Sci. Biotechnol.* **100**, 82–91 (2024).
- M. T. Fujiwara, Y. Yoshioka, Y. Kazama, T. Hirano, Y. Niwa, T. Moriyama, N. Sato, T. Abe, S. Yoshida, and R. D. Itoh, “Principles of amyloplast replication in the ovule integuments of *Arabidopsis thaliana*,” *Plant Physiol.* **196**, 137–152 (2024).
- K. Furukawa, Y. Ono, T. Shimokawa, H. Kitamura, T. Abe, T. Ebisuzaki, N. Saito, S. Wada, S. Sato, S. Tabata, and T. Matsuyama, “Intracultivar DNA identification in *Cymbidium* using DNA polymorphisms generated using simple genome scanning with random primers and heavy-ion beam irradiation,” *Scientia Horticulturae* **337**, 113598 (2024).
- Y. Ukai, H. Taoka, M. Kamada, Y. Wakui, F. Goto, K. Kitazaki, T. Abe, A. Hokura, T. Yoshihara, and H. Shimada, “*Athyrium yokoscense*, a cadmium-hypertolerant fern, exhibits two cadmium stress mitigation strategies in its roots and aerial parts,” *J. Plant. Res.* **137**, 1019–1031 (2024).
- A. Sanjaya, R. Nishijima, Y. Fujii, M. Asano, K. Ishii, Y. Kazama, T. Abe, and M. T. Fujiwara, “Rare occurrence of cryptic 5’ splice sites by downstream 3’ splice site/exon boundary mutations in a heavy-ion-induced *egy1-4* allele of *Arabidopsis thaliana*,” *Front. Plant Sci.* **15**, 1388040 (2024).
- H. Murata, H. Ichida, T. Abe, and Y. Hayashi, “<sup>40</sup>Ar<sup>17+</sup> beam-induced mutant of the plant symbiotic fungus *Tricholoma matsutake* has higher extracellular  $\beta$ -1, 4-endoglucanase activity than the  $\gamma$ -ray-induced mutant G1 and exhibits morphological changes in substrate cultivation in vitro,” *Bulletin of FFPRI* **23**, 89–92 (2024).

#### [Review Article]

阿部知子, 「花見に行ってみたら, 新しい技術が生まれた」, *ISOTOPE NEWS* **796**, 1 (2024).

### Presentations

#### [International Conferences/Workshops]

- A. Takatsuka (poster), T. Kazama, H. Mireau, Y. Iwai, H. Ichida, T. Abe, and K. Toriyama (poster), “Identification of fertility restorer of Tadukan-type cytoplasmic male sterile rice,” 13th International Conference for Plant Mitochondrial Biology, Saint-Malo, France, May 26–30, 2024.
- A. D. Nagalla (poster), R. Morita, H. Ichida, Y. Hayashi, Y. Shirakawa, T. Sato, Y. Fukuta, K. Toriyama, H. Saito, Y. Okumoto, and T. Abe, “Isolation of an early-heading mutant induced by carbon-ion irradiation in Indonesian native rice cultivar, ‘Gemjah Beton,’” 13th International Congress on Plant Molecular Biology, Cairns, Australia, June 24–28, 2024.
- K. Ishii (poster), Y. Kazama, T. Hirano, J. A. Fawcett, F. Sakai, Y. Shirakawa, S. Ohbu, and T. Abe, “Genome-wide analysis of heavy-ion induced deletions associated with distribution of essential genes in *Arabidopsis thaliana*,” *ibid.*
- Y. Kazama (poster), T. Kobayashi, M. Kitoh, K. Ishii, M. Krasovec, Y. Yasui, T. Abe, S. Kawano, and D. A. Firatov, “Unveiling the sex-determining system regulating gynoeceum development in the dioecious plant *Silene latifolia*,” *ibid.*
- A. Takatsuka (poster), T. Kazama, S. Arimura, H. Mireau, Y. Iwai, H. Ichida, T. Abe, and K. Toriyama, “Identification and mechanistic analysis of the disruption of pollen release caused by the conflict between mitochondrial and nuclear genes in rice cultivar Tadukan,” Gordon Research Seminar on Mitochondria and Chloroplasts, Barcelona, Spain, July 6–7, 2024.
- T. Abe (invited), “Trends in mutation breeding in Japan,” Mutation Breeding Network Workshop, Vienna, Austria, July 22–26, 2024.
- J. Duan (oral), M. Terai, J. Shimada, T. Abe, Y. Hayasi, and N. Yotsukura, “Breeding of high-water temperature cultivars of *Saccharina japonica* (Phaeophyceae, Laminariales) by heavy ion beam irradiation,” Wuhan, China, November 11–15, 2024.

#### [Domestic Conferences/Workshops]

- 阿部知子 (招待講演), 「世界に一つだけの花を育ててみよう—イオンビームで拓く未来の農業, 新たな世界」, 北陸原子力懇談会 エネルギー講演会, 富山市 (富山電気ビルディング), 2024年1月19日.
- 田村匠平 (口頭発表), 保倉明子, 白川侑希, 阿部知子, 高橋一聡, 小椋康光, 「イネ *Oryza sativa* L. cv. Nipponbare のカルスにおける可溶性および不溶性セレン化合物」, 第7回日本セレン研究会, 目黒区 (東京工業大学), 2024年3月2–3日.
- 風間裕介 (口頭発表), 生駒拓也, 西嶋遼, 池田美穂, A. D. Nagalla, 阿部知子, 「シロイヌナズナの染色体で遺伝子量補償は起こるのか」, 一般社団法人日本育種学会第145回講演会, 文京区 (東京大学), 2024年3月16–17日.
- 黛隆宏 (ポスター発表), 畑下昌範, 高城啓一, 石井公太郎, 阿部知子, 風間裕介, 「トレンニア新規変異体 Frilly Petal Undulation 1 (fpu1) の原因遺伝子の探索」, 同上.
- 田中朋之 (口頭発表), 小林麻子, 阿部知子, 「温感受性イネ突然変異体 flo11-2 を用いた白未熟粒発生機構の解析第2報: 二重変異体の単離」, 日本作物学会第257回講演会, 津市 (三重大学), 2024年3月28–29日.

- 保倉明子 (口頭発表), 田村匠平, 熊谷和博, 白川侑希, 阿部知子, 高橋一聡, 小椋康光, 「セレンのオキソアニオンを添加したイネのカルスにおけるセレン化合物の化学形態分析」, 第 84 回分析化学討論会, 京都市 (京都工芸繊維大学), 2024 年 5 月 18–19 日.
- 阿部知子 (招待講演), 「重イオンビームを用いて新品種を創る」, 理研軽金属工業株式会社科学講演会 2024 in 静岡, 静岡市 (しづきんホールユーフォニア), 2024 年 6 月 15 日.
- 高塚歩 (口頭発表), 風間智彦, Hakim Mireau, 岩井裕子, 市田裕之, 阿部知子, 鳥山欽哉, 「イネ品種 Tadukan に由来する細胞質雄性不稔に対する回復遺伝子の分子遺伝学的解析」, イネ遺伝学・分子生物学ワークショップ 2024, 文京区 (東京大学), 2024 年 7 月 4–5 日.
- 西嶋遼 (口頭発表), J. Fawcett, 坂本卓也, 生駒拓也, 杉田和陽, 鶴飼優葉, 兵渡友誉, 乾弥生, 田中裕之, 伊藤武彦, 阿部知子, 松永幸大, 風間裕介, 「シロイヌナズナにおける染色体部分重複と欠失が遺伝子発現に与える影響」, 日本遺伝学会第 96 回大会, 高知市 (高知工科大学), 2024 年 9 月 4–6 日.
- 小林壮生 (口頭発表), 鬼頭萌, 西嶋遼, 池田美穂, D. A. Filatov, 風間裕介, 「マンテマ属の雌雄異株化における CLV3 様遺伝子の機能分化」, 同上.
- 鶴飼優葉 (ポスター発表), 西嶋遼, J. Fawcett, 坂本卓也, 杉田和陽, 生駒拓也, 兵渡友誉, 田中裕之, 伊藤武彦, 阿部知子, 松永幸大, 風間裕介, 「シロイヌナズナ染色体部分重複変異体のエピゲノム変化」, 同上.
- 小林壮生 (ポスター発表), 畑中悠那, 池田美穂, 風間裕介, 「雌雄異株植物ヒロハノマンテマの X 染色体は雌ざい発達に関与するの」, 日本植物携帯学会第 36 回総会・大会, 宇都宮市 (宇都宮大学), 2024 年 9 月 13 日.
- 黛隆宏 (ポスター発表), 石井公太郎, 畑下昌範, 高城啓一, 東山哲也, 阿部知子, 風間裕介, 「トレニア新規変異体 frilly petal unduration1 (fpu1) の花卉の形態変化と原因遺伝子同定」, 同上.
- 黛隆宏 (口頭発表), 石井公太郎, 畑下昌範, 高城啓一, 阿部知子, 風間裕介, 「トレニア新規変異体 Frilly Petal Undulation 1 (fpu 1) の原因遺伝子の探索」, 日本植物学会第 88 回大会, 宇都宮市 (宇都宮大学), 2024 年 9 月 14–16 日.
- 常泉和秀 (ポスター発表), 山田美恵子, 林依子, 最上谷美穂, 佐藤陽一, 阿部知子, 「重イオンビーム照射による有用ミリンソウ系統の選抜」, 同上.
- 植村亮介 (ポスター発表), N. V. Quoc, 阿部知子, 國武久登, 平野智也, 「花器官サイズ調節遺伝子が花卉形態に及ぼす影響」, 同上.
- 西嶋遼 (口頭発表), J. Fawcett, 坂本卓也, 鶴飼優葉, 兵渡友誉, 杉田和陽, 生駒拓也, 乾弥生, 田中裕之, 伊藤武彦, 阿部知子, 松永幸大, 風間裕介, 「染色体部分重複がシロイヌナズナゲノムに与える影響」, 一般社団法人日本育種学会第 146 回講演会, 広島市 (広島大学), 2024 年 9 月 19–20 日.
- 森田竜平 (ポスター発表), 市田裕之, 林依子, 白川侑希, 一瀬勝紀, 佐藤雅志, 鳥山欽哉, 阿部知子, 「アルゴンイオンビーム照射で作出した顕性の早生形質を示すイネ変異体は複雑な染色体構造変異を有する」, 同上.
- A. D. Nagalla (poster), R. Morita, H. Ichida, Y. Hayashi, Y. Shirakawa, T. Sato, Y. Fukuta, K. Toriyama, H. Saito, Y. Okumoto, and T. Abe, 「Identification of an early-flowering mutant in Indonesian native rice cultivar: 'Gemdjah Beton」, 同上.
- 石上高大 (ポスター発表), 奥村和哉, 阿部知子, 田中朋之, 「イネ子実デンプンの新奇低温糊化性因子の探索」, 日本作物学会第 258 回講演会, 岡山市 (岡山大学), 2024 年 9 月 26–27 日.
- 大久保貴博 (口頭発表), 渡村加奈子, 加藤光弘, 白川侑希, 石井公太郎, 阿部知子, 「DNA マーカーを用いたウンシュウミカン新品種 '春しずか' の品種識別技術の確立」, 一般社団法人園芸学会令和 6 年度秋季大会, 西原町 (琉球大学), 2024 年 11 月 3–5 日.
- 黛隆宏 (口頭発表), 石井公太郎, 畑下昌範, 高城啓一, 小嶋美紀子, 竹林裕美子, 榊原均, 東山哲也, 阿部知子, 風間裕介, 「花卉がフリル化したトレニア新規変異体の原因遺伝子の探索」, 北陸植物学会第 14 回大会, 富山市 (富山大学), 2024 年 11 月 10 日.
- 小林壮生 (口頭発表), 畑中悠那, 落合詩月, 池田美穂, 西嶋遼, 風間裕介, 「雌雄異株植物ヒロハノマンテマの X 染色体の雌ざい発達の検証」, 同上.
- 鶴飼優葉 (口頭発表), 西嶋遼, J. Fawcett, 坂本卓也, 杉田和陽, 生駒拓也, 兵渡友誉, 田中裕之, 伊藤武彦, 阿部知子, 松永幸大, 風間裕介, 「重イオンビームで誘発したシロイヌナズナ染色体部分重複変異体 Ar55-as5-6 で見られたエピゲノム変化」, 同上.
- 岡本充智 (口頭発表), 橘卓三, 阿部知子, 「サトイモ '愛媛農試 V2 号' 生存率に及ぼす重イオンビーム照射線量の影響」, 日本育種学会四国談話会第 88 回講演会, 松山市 (愛媛大学), 2024 年 11 月 29 日.
- 植村亮介 (ポスター発表), V. Q. Nhat, 阿部知子, 國武久登, 平野智也, 「花器官サイズ調節遺伝子による花卉形態制御」, 育種学会九州支部会第 19 回九州育種談話会, 宮崎市 (宮崎大学), 2024 年 12 月 9 日.
- N. Wattanakasemsakun (poster), M. Shii, T. Abe, H. Kunitake, T. Hirano, 「In vitro culture of embryo sacs derived from fertilization of male gametes irradiated with heavy-ion beams」, 同上.
- 大胡海翔 (ポスター発表), 田村匠平, 保倉明子, 熊谷和博, 白川侑希, 阿部知子, 「X 線吸収分光法によるイネのカルスにおけるセレンの化学形態分析」, 令和 6 年度 分析イノベーション交流会, 八王子市 (東京たま未来メッセ展示場), 2024 年 12 月 23–24 日.

## Awards

- 阿部知子, 「テラーメイド変異誘発法の開発と食品産業への貢献」, 食創会 第 28 回安藤百福賞 優秀賞, 2024 年 3 月 12 日.
- 佐藤陽一, 「大型海藻の生理生態学的研究と種苗生産など社会実装への貢献」, 第 10 回 CYTOLOGIA 奨励賞, 2024 年 6 月 15 日.
- Y. Kazama, M. Kitoh, T. Kobayashi, K. Ishii, M. Krasovec, Y. Yasui, T. Abe, S. Kawano, D. A. Filatov, 「A CLAVATA 3-like gene acts as a gynoceium suppression function in white champion」, 日本植物形態学会 平瀬賞, 2024 年 9 月 13 日.

## Outreach Activity

We established the "Asagao (morning glory) club" to deepen the understanding of our technology of mutation breeding. The club distributes the morning glory seeds irradiated with C-ion on request, and collects and compiles the observation reports of their growth.