# 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 mutation 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 carbon to iron. Its research subjects cover the study of ion-beam radiation mutagenesis, genome-wide analyses of mutation, and the development of new plants and microbial varieties by heavy-ion irradiation. Forty-two new varieties have already been brought to the market. The group also provides researchers with the mutagenesis technology to discover new genes and improve new varieties, contributing to solving societal issues such as food, energy, the environment, and climate change.

## 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. Main subjects are:

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

Linear Energy Transfer (LET) is an important parameter in biological effects. LET is the energy an ionizing particle gives the target per unit path length along its track. We found the optimal LET for mutation induction, designed as LETmax, was 30 keV/$\mu$m in *Arabidopsis*, 23–39 keV/$\mu$m in buckwheat, 23–50 keV/$\mu$m in rice and 50–70 keV/$\mu$m in wheat. By contrast, irradiation with 290 keV/$\mu$m has a low mutation rate but high lethality in plants. A whole-genome analysis with high-throughput sequencing is a powerful tool used to characterize 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/$\mu$m) and Ar (290 keV/$\mu$m). 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 LETmax (30–50 keV/$\mu$m) irradiation. LETmax is effective for breeding because of its very 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 produced 32 new varieties that have appeared on 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/$\mu$m) 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 available nuclides with higher LETs 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/$\mu$m) showed a higher mutation rate than the 290 keV/$\mu$m 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 2023, the consortium grew to 188 groups from Japan and 23 from overseas. Previously, the ion-beam breeding procedures were carried out using mainly 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 is suitable for long-term storage because it has less rind puffing and is resistant to rotting, and 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 use whole genome mutation analysis data to create a DNA marker for variety identification. Demand for small chrysanthemums increases during the Bon Festival. Using the same cultivation method, the farmer hopes to have 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/$\mu$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. In addition, the users have succeeded in breeding new colors of hibiscus, cherry blossoms flowering without long periods of low temperatures, and a dwarf with early maturing durum wheat.

## Members

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# List of Publications & Presentations

## Publications

### [Original Papers]

H. Park, T. Abe, H. Kunitake, and T. Hirano, "Characterization of a novel mutant with inhibition of storage root formation in sweet potato," Breed. Sci. **73**, 212–218 (2023).

H. Ichida, T. Kazama, S. Arimura, and K. Toriyama, "The mitochondrial and plastid genomes of *Oryza sativa* L. cv. Taichund 65," Plant Biotechnol (Tokyo) **40**, 109–112 (2023).

H. Ichida, H. Murata, S. Hatakeyama, A. Yamada, and A. Ohta, "Near-complete de novo assembly of *Tricholoma bakamatsutake* chromosomes revealed the structural divergence and differentiation of *Tricholoma* genomes," G3: Genes, Genomes, Genetics **13**, jkad198 (2023).

M. Ogawa, K. Tsuneizumi, T. Abe, and M. Nozawa, "Testing immediate dosage compensation in *Drosophila miranda* via irradiation with heavy-ion beams," Genes Genet. Syst. **98**, 201–206 (2023).

### [Review Articles]

阿部知子, 風間祐介, 平野智也, 「重イオンビームによる育種技術の開発」, 育種学研究 **25**, 170–176 (2023).

阿部知子, 林依子, 森田竜平, 池田時浩, 高城啓一, 畑下昌範, 「ゲノム解析を用いたイオンビーム育種技術の高度化」, JATAFF ジャーナル **11**, 25–30 (2023).

### [Book]

阿部知子, 「イオンビーム育種技術の開発と応用」, 量子ビーム科学の基礎と応用, 45–50 (2023).

## Presentations

### [International Conferences/Workshops]

T. Toyama (Oral), H. Shirahama, Y. Kazama, R. Nishijima, K. Ishii, T. Abe, H. Kunitake, and T. Hirano, "Analysis of genetic mutation in large flower mutant ohbana2 of Arabidopsis thaliana," 15th JKTC International Student Seminar, Miyazaki, Japan, September 19–20, 2023.

M. Shii (Oral), Y. Kajiya, T. Abe, H. Kunitake, and T. Hirano, "Effects of fertilization of male gametes with argon-ion irradiation on embryo and endosperm development in Cyrtanthus mackenii," ibid.

### [Domestic Conferences/Workshops]

A. Takatsuka (口頭発表), T. Kazama, H. Ichida, T. Abe, K. Toriyama, "Exploration for a restorer of fertility gene for cytoplasmic male sterility derived from Oryza sativa cv. Tadukan," 第 17 回東北育種研究集会, 仙台市 (東北大学), 2022 年 11 月 26 日.

岩井裕子 (口頭発表), 市田裕之, 風間智彦, 鳥山欽哉, 「W1112 型細胞質雄性不稔性イネの稔性回復遺伝子候補 PPR791 の解析」, 同上.

藤原誠 (口頭発表), 原拓也, 大坪昴平, 小林永実, 金澤美加子, 篠塚舞, 植竹祥子, 大谷友佑, 熊田翔吾, 阿部知子, 伊藤竜一, 「オオカナダモの異型細胞形成の研究: 葉面における分布パターンについて」, 日本生物教育学会 107 回全国大会, 高崎市 (高崎健康福祉大学), 2023 年 3 月 4–5 日.

髙野真尋 (口頭発表), 寺﨑香菜子, 宮﨑朝花, 玉谷亘, 川口眞理, 臼杵豊展, 笹川展幸, 平野智也, 風間裕介, 阿部知子, 藤原誠, 「オオカナダモの異型細胞形成の研究: プロトプラスト単離法の開発について」, 同上.

藤原誠 (口頭発表), 矢野由磨, 浅野優衣, 宮﨑梨菜, 篠原万由子, 村松亮輔, サンジャヤ アルビン, 小池菜奈, 吉野彩花, 安澤愛, 名護しほ, 風間裕介, 阿部知子, 伊藤竜一, 「シロイヌナズナ葉緑体分裂異常変異体における孔辺細胞葉緑体の増殖・形態解析」, 日本農芸化学会 2023 年度大会, オンライン, 広島市 (広島コンベンションホール), 2023 年 3 月 14–17 日.

曽根悠介 (口頭発表), 太田知宏, 加藤光弘, 中嶌輝子, 中村茂和, 阿部知子, 「ウンシュウミカン新品種 '春しずか' の特性と育成経過」, 園芸学会令和 5 年度春季大会, 大津市 (龍谷大学), 2023 年 3 月 15–22 日.

阿部知子 (口頭発表), 風間裕介, 平野智也, 「重イオンビームによる育種技術の開発」, 一般社団法人日本育種学会 第 143 回講演会, 静岡市 (静岡大学), 2023 年 3 月 17–18 日.

風間裕介 (口頭発表), 鬼頭萌, 小林壮生, 石井公太郎, M. Krasovic, 安井康夫, 阿部知子, 河野重行, D. A. Filatov, 「雌雄異株植物ヒロハノマンテマの雌蕊抑制に関わる性決定遺伝子 GSFY の同定」, 同上.

村井耕二 (口頭発表), 野村文希, 多田博子, 苗木麗奈, 荒井里実, 阿部知子, 「重イオンビーム照射による日長反応性遺伝子 Ppd-D1 を欠失したコムギ変異体の作出」, 同上.

阿部知子 (口頭発表, 招待講演), 「ゲノム解析を用いたイオンビーム育種技術の高度化」, ガンマーフィールドシンポジウム Final, 文京区 (東京大学), 2023 年 3 月 25 日.

藤井優紀 (ポスター発表), A. Sanjaya, 風間裕介, 阿部知子, 藤原誠, 「シロイヌナズナの表皮葉緑体核様体の簡易観察法」, 日本農芸化学会関東支部大会, 川崎市 (明治大学), 2023 年 8 月 25 日.

宮﨑梨菜 (ポスター発表), 窪園雅人, 鈴木麻央, 佐藤志保, 石川浩樹, 佐々木駿, 風間裕介, 阿部知子, 伊藤竜一, 藤原誠, 「シロイヌナズナの孔辺細胞形成時における葉緑体の増殖と分配」, 同上.

黛隆宏 (ポスター発表), 畑下昌範, 高城啓一, 阿部知子, 風間裕介, 「重イオンビーム照射で得られたトレニア新規フリル変異体の花弁の形態変化」, 植物学会第 87 回大会, 札幌市 (北海道大学), 2023 年 9 月 4 日 (オンラインポスター), 7–9 日 (オンサイト).

小林壮生 (ポスター発表), 鬼頭萌, 石井公太郎, M. Krasovec, 安井康夫, 阿部知子, 河野重行, D. A. Filatov, 風間裕介, 「雌雄異株植物ヒロハノマンテマの性決定遺伝子 GSFY はどのように誕生したのか」, 同上.

外山大夢 (ポスター発表), 白濱瞳, 風間裕介, 西嶋遼, 石井公太郎, 阿部知子. 國武久登, 平野智也, 「シロイヌナズナ大輪変異体 ohbana2 における遺伝子変異解析」, 同上.

椎槙子 (ポスター発表), 加治屋優希, 阿部知子, 國武久登, 平野智也, 「アルゴンイオンビーム照射雄性配偶子の受精が胚および胚乳発達に及ぼす影響」, 同上.

鵜飼優子 (口頭発表), 田岡裕規, 鎌田まなか, 涌井裕子, 後藤文之, 北﨑一義, 阿部知子, 保倉明子, 吉原利一, 島田浩章, 「カドミウム耐性のあるシダ植物 Athyrium yokoscense は, 根と地上部で 2 つのストレス軽減戦略を示す」, 同上.

小林壮生 (ポスター発表), 鬼頭萌, 石井公太郎, M. Krasovec, 安井康夫, 阿部知子, D. A. Filaotv, 河野重行, 風間裕介, 「CLV3 様遺伝子をコードする GSFY は雌雄異株植物ヒロハノマンテマの雌ずいの発達を抑制する」, 日本植物形態学会第 35 回総会・大会, 札幌市 (北海道大学), 2023 年 9 月 6 日.

生駒拓也 (ポスター発表), 西嶋遼, 池田美穂, 阿部知子, 風間裕介, 「シロイヌナズナの染色体で遺伝子量補正は起きるのか」, 日本遺伝学会第 95 回大会, 熊本市, 2023 年 9 月 6–8 日.

風間裕介 (口頭発表), 鬼頭萌, 小林壮生, 石井公太郎, M. Krasovec, 安井康夫, 阿部知子, 河野重行, D. A. Filatov, 「雌雄異株植物ヒロハノマンテマの雌ずい発達を抑制する性決定遺伝子 GSFY の同定」, 同上.

黛隆宏 (ポスター発表), 畑下昌範, 高城啓一, 阿部知子, 風間裕介, 「トレニア新規フリル変異体に対するサイトカイニン分解阻害剤 (CPPU) 処理の影響」, 一般社団法人日本育種学会 第 144 回講演会, 神戸市 (神戸大学), 2023 年 9 月 16–18 日.

石川詩絵里 (ポスター発表), 根本花奈美, 川上空里子, 森田竜平, 阿部知子, 中野絢菜, 相井城太郎, 「重イオンビーム照射による自殖性フツウソバ突然変異集団の作出」, 同上.

阿部知子 (招待講演), 「重イオンビームを用いた変異誘発法の開発—物理学者との楽しく実りある研究生活—」, メンデル講演会, 第 10 回和田賞・キトロギア奨励賞受賞講演会, 文京区 (東京大学), 2023 年 10 月 21 日.

## Awards

R. Tabassum, T. Dosaka, R. Morita, H. Ichida, Y. Ding, T. Abe, T. Katsube-Tanaka, “The conditional chalky grain mutant ‘flo11-2’ of rice (Oryza sativa L.) is sensitive to high temperature and useful for studies on chalkiness,” 第 20 回日本作物学会論文賞, 2022 年 10 月.

重イオン育種技術開発グループ（代表: 阿部知子, 風間裕介, 平野智也) 「重イオンビームによる育種技術の開発」, 日本育種学会学会賞, 2023 年 3 月.

## Patent

平野智也, 國武久登, Park Hyung Jun, 阿部知子, 奈良迫洋介, 「塊根の収量が増大した塊根植物の製造方法」, 特許出願番号 2022-169984, 出願日 2022 年 10 月 24 日.

## Outreach Activities

阿部知子, 「理研が開発, 『仁科蔵王』が見頃？」, J-COM つながるニュース, 2023 年 4 月 11 日.

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.