Accelerator Applications Research Division
Beam Mutagenesis Group
Ion Beam Breeding Team

1. Abstract

Ion beam breeding team studies various biological effects of fast heavy ions. It also develops new technique to breed plants and microbes by heavy-ion irradiations. Fast 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 ionization can cause double-strand breaks of DNA which are not easily repaired and result in mutation more effectively than single-strand breaks. A unique feature of our experimental facility at the RIKEN Ring Cyclotron (RRC) is that we can irradiate living tissues in atmosphere since the delivered heavy-ion beams have energies high enough to penetrate deep in matter. This team utilizes a dedicated beam line (E5B) of the RRC to irradiate microbes, plants and animals with beams ranging from carbon to iron. Its research subjects cover study of ion-beam radiation mutagenesis, genome-wide analyses of mutation, and development of new plants and microbial varieties by heavy-ion irradiation. Thirty-nine new varieties have already been brought to the market.

2. Major Research Subjects

(1) Study on the biological effects by heavy-ion irradiation
(2) Study on the molecular nature of DNA alterations induced by heavy-ion irradiation
(3) Innovative applications of heavy-ion irradiation

3. Summary of Research Activity

We study biological effects of fast 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. We also develop breeding technology of microbes and plants. Main subjects are:

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

Heavy-ion beam deposits a concentrated amount of dose at just before stop with severely changing the linear energy transfer (LET). The peak of LET is achieved at the stopping point and known at the Bragg peak (BP). Adjusting the BP to target malignant cells is well known to be effective for cancer therapy. On the other hand, a uniform dose distribution is a key to the systematic study for heavy-ion mutagenesis, thus to the improvement of the mutation efficiency. Plants and microbes therefore, are irradiated using ions with stable LET. We investigated the effect of LET ranging from 23 to 640 keV/μm, on mutation induction using dry seeds of the model plants Arabidopsis thaliana and rice (*Oryza sativa* L.). The most effective LET (LETmax) was 30 keV/μm in *Arabidopsis*. LETmax irradiations showed the same mutation rate as that by chemical mutagens, which typically cause high mutation rate. The LETmax was 23–39 keV/μm in buckwheat, 23–50 keV/μm in rice and 50–70 keV/μm in wheat. By contrast, when LET was 290 keV/μm, the mutation rate was low and the survival rate was greatly reduced in plants. In the case of microbe, filamentous fungus (*Neurospora crassa*), the Ar ions at 290 keV/μm demonstrated higher mutagenic activity than the Fe-ions at 640 keV/μm. Thus, the LET is an important factor to be considered in heavy-ion mutagenesis.

(2) Study on the molecular nature of DNA alterations induced by heavy-ion irradiation

A whole-genome analysis with high-throughput sequencing is a powerful tool used to characterize the nature of induced mutations. We have been using whole genome sequencing to analyze DNA mutations in *Arabidopsis* and rice genomes. C ions with LETmax mainly induced single nucleotide variants (SNVs) and small insertions and deletions (InDels), while the number of large deletions and chromosomal rearrangements was low in *Arabidopsis*. However, 290 keV/μm Ar ions showed a different mutation spectrum: SNVs and number of small InDels was low, while the number of large deletions ($\geq$100 bp) and chromosomal rearrangements was high. Number of mutated gene induced by C-ion and Ar-ion irradiation is less than 10, relatively small, and often only 1 mutation is found near the mapped location. Thus, irradiation with these ions can efficiently generate knockout mutants of a target gene and can be applied to reverse genetics. Rice mutants of the causative gene induced by ion-beam irradiation were compared at 23–50 keV/μm and 290 keV/μm with typical LETs. The most mutations irradiated with C ion at 23–50 keV/μm were small deletions (<100 bp). Irradiation with 290 keV/μm Ar-ion resulted in the highest number of large deletions and decreased small deletions. In rice, as in *Arabidopsis*, the LETmax with high mutagenic effects was dominated by SNVs and small deletion mutations, while large deletions and chromosomal rearrangement mutations dominated LETs with high lethal effects.

(3) Innovative application of heavy-ion irradiation

In 1999, we formed a consortium for ion-beam breeding consisting of 24 groups. In 2022, the consortium grew to 184 groups from Japan and 22 from overseas. Previously, the ion-beam breeding procedures were carried out using mainly flowers and ornamental plants. We have recently put a new non-pungent and tearless onion, 'Smile Ball,' on the market along with 'Kiku Meigetsu,' an edible late flowering chrysanthemum. In addition, a new project was launched to expand the cultivation area of this variety of chrysanthemum in Yamagata prefecture. Beneficial variants have been grown for various plant species, such as high yield sea weeds, lipids hyperaccumulating unicellular alga, medicinal plant with high productivity of medicinal ingredient, peanuts without major allergens, oranges with delayed coloring and one-month late harvest, and lettuce with a low browning property as a cut vegetable. As

a result of a collaborative study with Shizuoka prefecture, we have created a new variety of 'Haru Shizuka,' which is late coloring. The harvest time of the Satsuma mandarin has tended to come earlier due to global warming, 'Haru Shizuka' is harvested about a month later than the original variety, but the fruits are sour when harvested. 'Haru Shizuka' is suitable for long-term storage and the fruits turn into good quality sweet oranges during storage till April. By broadening the target of heavy-ion breeding extending from flowers to crops, the technology will contribute to solving the global problems of food shortage and environmental destruction.

Members

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List of Publications & Presentations

Publications

[Original Papers]

A. Deguchi, F. Tatsuzawa, K. Ishii, T. Abe, and K. Miyoshi, "Localized repression of two bHLH genes is involved in the formation of white margins and white abaxial surfaces in carnation petals by inducing the absence of anthocyanin synthesis," Hortic. Sci. **91**, 68 (2022).

橋口太亮, 飯山光太郎, 長井純一, 藤川和博, 石井公太郎, 阿部知子, 「サツマイモ『シロユタカ』と外観識別可能な『こなみずき』欠刻葉系統の作出」, 日本作物学会九州支部会報 **88**, 22 (2022).

H. Park, Y. Narasako, T. Abe, H. Kunitake, and T. Hirano, "Comprehensive effects of heavy-ion beam irradiation on sweet potato (*Ipomoea batatas [L.] Lam.*)," Plant Biotechnol (Tokyo) **39**, 311 (2022).

Y. Kazama, M. Kitoh, T. Kobayashi, K. Ishii, M. Krasovec, Y. Yasui, T. Abe, S. Kawano, and D. A. Filatov, "A CLAVATA3-like gene acts as a gynoecium suppression function in white campion," Mol. Biol. Evol. **39**, 10 (2022).

阿部知子, 林依子, 大野豊, 畑下昌範, 高城啓一, 「イオンビーム育種技術の開発に取り組む加速器施設」, アグリバイオ **6**, 8 (2022).

平野智也, 「イオンビームを用いた花き植物の品種改良」, アグリバイオ **6**, 14 (2022).

中村茂和, 阿部知子, 「長期貯蔵に適した温州みかん新品種『春しずか』の育成」, アグリバイオ **6**, 24 (2022).

風間裕介, 畑下昌範, 木元久, 櫻井明彦, 「イオンビームを用いた微生物の品種改良」, アグリバイオ **6**, 29 (2022).

H. J. Kim, K. Nakammura, Y. Sakakura, K. Suga, K. Tsuneizumi, T. Abe, M. Yamada, M. Kawada, T. Katayama, N. Tezuka, T. Kobayashi, M. Koiso, and A. Hagiwara, "Effects of dietary microalgal species and hormone treatment on the lorica size and reproductivity of heavy-ion beam irradiated rotifers," Fish. Sci. **89**, 61 (2023).

[Review Articles]

T. Hirano, Y. Kazama, H. Kunitake, and T. Abe, "Mutagenic effects of heavy-ion beam irradiation to plant genome," Cytologia **87**, 3 (2022).

阿部知子, 「イオンビーム育種技術の開発—農学と加速器物理学のシナジー効果」, アグリバイオ **6**, 6 (2022).

Presentations

[International Conferences/Workshops]

T. Abe (invited), Y. Hayashi, R. Morita, Y. Shirakawa, and H. Ichida, "Ion-beam mutagenesis for creation of new varieties and discovery of genes," Bangabandhu International Conference on Sustainable Agriculture through Nuclear and Frontier Research (Webinar), Online, January 19–21, 2022.

Y. Kazama (oral) and T. Abe, "Effect of linier energy transfer in the heavy-ion mutagenesis and breeding," The 32nd Annual Meeting of MRS-J, Yokohama, Japan, December 5–7, 2022.

T. Mayuzumi (poster), A. Matsuta, M. Hatashita, K. Takagi, T. Abe, K. Murai, and Y. Kazama, "Heavy-ion beams with high linear energy transfer frequently produces morphological mutants in the M1 generation of an ornamental plant *Torenia fournieri*," The 32nd Annual Meeting of MRS-J, Yokohama, Japan, December 5–7, 2022.

[Domestic Conferences/Workshops]

石井公太郎 (口頭発表), 風間裕介, 浅野円花, 竹下毅, 阿部知子, 河野重行, 「クロレラの内部倍数性と Ar・Fe イオンビーム照射による染色体の分断化と再構成」, 日本藻類学会 第 45 回大会, オンライン, 2022 年 3 月 16–17 日.

石井公太郎 (口頭発表), 風間裕介, 平野智也, J. A. Fawcett, 酒井富士子, 白川侑希, 大部澄江, 阿部知子, 「重イオンビームで誘発される欠失変異と必須遺伝子のシロイヌナズナゲノム上での分布に関する俯瞰的解析」, 日本育種学会 第 141 回講演会, オンライン, 2022 年 3 月 20–21 日.

杉田和陽 (口頭発表), サンジャヤ アルビン, 西嶋遼, 村井耕二, 阿部知子, 風間裕介, 「シロイヌナズナの新規染色体再編成変異体で見られたダイナミックな形態変化」, 同上.

黛隆宏 (口頭発表), 松田彩花, 畑下昌範, 高城啓一, 阿部知子, 村井耕二, 風間裕介, 「トレニアの新規フリル変異体で見られた花弁の細胞サイズの変化」, 同上.

H. Park (口頭発表), 奈良迫洋介, 阿部知子, 國武久登, 平野智也,「重イオンビーム照射がサツマイモの塊根形成に及ぼす影響」, 同上.

小林壮生 (ポスター発表), 高橋真佐子, 杉山立志, 石井公太郎, 河野重行, 風間裕介, 「根端分裂組織の細胞周期の同調に着目したヒロハノマンテマの染色体標本作製方法」, 同上.

椎槙子 (ポスター発表), 加治屋優希, 阿部知子, 星野洋一郎, 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機構解析」, 園芸学会令和 4 年度秋季大会, 山形市 (山形大学), 2022 年 9 月 7–13 日.

武田信哉 (口頭発表), 市田裕之, 阿部知子, 有村慎一, 風間智彦, 陳孫禄, 金岡義高, 貴島祐治, 鳥山欽哉, 「台中 65 号の細胞質およびアフリカイネの核を持つ TG-CMS の原因遺伝子解析とその稔性回復様式の調査」, 第 39 回日本植物バイオテクノロジー学会, 堺市 (大阪公立大学), 2022 年 9 月 11–13 日.

生駒拓也 (ポスター発表), サンジャヤ アルビン, 池田美穂, 西嶋遼, 村井耕二, 阿部知子, 風間裕介, 「シロイヌナズナ染色体における遺伝子量補正の調査」, 日本遺伝学会第 94 回大会, 札幌市 (北海道大学), 2022 年 9 月 14–17 日.

杉田和陽 (ポスター発表), サンジャヤアルビン, 西嶋遼, 田中裕之, 伊藤武彦, 村井耕二, 阿部知子, 風間裕介, 「シロイヌナズナの新規染色体部分的重複変異体における遺伝子発現変動とクロマチン動態」, 同上.

小川雅文 (ポスター発表), 常泉和秀, 阿部知子, 野澤昌文, 「重粒子線照射によるショウジョウバエ Y 染色体の部分破壊: 遺伝子量補償の即時性の検証に向けて」, 同上.

池田公季 (ポスター発表), 旭置桐哉, 風間裕介, 石井公太郎, 阿部知子, 國武久登, 平野智也, 「シロイヌナズナ petal compensation exhibiting 変異体における花器官細胞サイズ制御機構の解析」, 日本植物学会第 86 回大会, 京都市 (京都府立大), 2022 年 9 月 15–19 日.

石井公太郎 (口頭発表), 浅野円花, 阿部知子, 河野重行, 「クロレラゲノム配列の更新と重イオンビーム誘導変異体の染色体再編成の解析」, 同上.

田中朋之 (口頭発表), 小林麻子, 阿部知子, 「高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 254 回日本作物学会講演会, 福島市 (福島大学), 2022 年 9 月 20–21 日.

市田裕之 (口頭発表), Ni Lei, 森田竜平, 阿部知子, 「機械学習による変異バリデーション法の開発と検証」, 日本育種学会 第 142 回講演会, 帯広市 (帯広畜産大学), 2022 年 9 月 23–25 日.

上田純平 (口頭発表), 風間裕介, 阿部知子, 村井耕二, 「時計遺伝子 WPCL1 の欠失による一粒系コムギ早生変異体の早生性を抑制するイオンビーム変異体 late-heading1 の解析」, 同上.

杉田和陽 (口頭発表), サンジャヤアルビン, 西嶋遼, 田中裕之, 伊藤武彦, 村井耕二, 阿部知子, 風間裕介, 「染色体再編成が植物ゲノムに及ぼす影響」, 北陸植物学会第 12 回大会, 富山市 (富山大学), 2022 年 11 月 13 日.

生駒拓也 (口頭発表), サンジャヤアルビン, 池田美穂, 西嶋遼, 村井耕二, 阿部知子, 風間裕介, 「シロイヌナズナで遺伝子量補正は起きるのか」, 同上.

黛隆宏 (口頭発表), 松田彩花, 畑下昌範, 高城啓一, 阿部知子, 村井耕二, 風間裕介, 「重イオンビームを用いた園芸植物トレニアの花形変異体の作出」, 同上.

鬼頭萌 (口頭発表), 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. Filaotv, 風間裕介, 「雌雄異株植物ヒロハノマンテマの決定候補遺伝子 GSFY の同定」, 同上.

小林壮生 (口頭発表), 鬼頭萌, D. A. Filaotv, 風間裕介, 「ヒロハノマンテマ性決定遺伝子の X 染色体連鎖パラログ GSFX の機能解析」, 同上.

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Award

阿部知子, 第 10 回和田賞, 公益財団法人日本メンデル協会, 4 月, 2022.

Press Releases

「春先に出荷可能な温州みかんの作出に成功」, 2021 年 12 月 8 日.

“Accelerators accelerate many fields,” T. Abe, RIKEN People, December 20, 2021.

研究最前線「重イオンビームで温州みかんの品種改良」, RIKEN News 481, 18 (2022).

「巨大 Y 染色体発見から 99 年目の快挙—ヒロハノマンテマの性決定遺伝子の同定に成功—」, 2022 年 9 月 28 日.

Patent

蝶野真喜子, 神山紀子, 阿部知子, 「種子の白色性を向上させたコムギ」, 特許出願番号 2021-166243, 出願日 2021 年 10 月 8 日.

Outreach Activities

阿部知子, 「重イオンビームを用いて新しい品種を創る」, 第 9 回 理研イノベーションセミナー, オンライン, 2022 年 1 月 21 日.

阿部知子, 「重イオンビームを用いて新しい品種を創る」, 理研と未来を創る会第 28 回講演会・見学会, 和光市, 2022 年 9 月 6 日.

阿部知子,「加速器を用いた重イオンビーム育種の発展」, シンポジウム「理化学研究所仁科研究室のキセキ」, 和光市, 2022 年 10 月 28 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.